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Isolation, Characterization, and Stress-Tolerant Zinc-Solubilizing Bacteria from Salt-Affected Coastal Soils of Gir Somnath, India

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ABSTRACT

Zinc deficiency in agricultural soils significantly limits crop productivity, particularly in saline and coastal environments. The present study aimed to isolate, characterize, and evaluate zinc-solubilizing bacteria (ZSB) from salt-affected coastal soils of Gir Somnath district, Gujarat, India. A total of twenty morphologically distinct bacterial isolates were obtained from non-rhizospheric soil samples collected from four coastal locations using standard serial dilution and plating techniques. All isolates exhibited growth on zinc solubilizing agar medium and were identified as Gram-positive bacteria, indicating their adaptation to saline and metal-stress environments. Morphological characterization revealed diversity in colony size, shape, elevation, and pigmentation. Quantitative evaluation demonstrated strain-specific zinc solubilization under varying pH (5, 7, and 9), salt concentrations (1% and 2.5% NaCl), and temperatures (20 °C, 37 °C, and 50 °C). Several isolates, particularly KOZ-01, SAZ-08, SAZ-10, and MUZ-16, showed superior solubilization efficiency and tolerance to environmental stress. Biochemical characterization confirmed metabolic versatility, including catalase activity, citrate utilization, and carbohydrate fermentation. Molecular identification revealed similarity to genera such as *Bacillus*, *Paenibacillus*, and *Enterobacter*, known for plant growth-promoting properties. The findings highlight the potential of these stress-tolerant zinc-solubilizing bacteria as promising bioinoculants for improving zinc availability and enhancing crop productivity in saline and coastal agricultural soils.

Introduction

Micronutrients such as zinc (Zn), iron (Fe), manganese (Mn), copper (Cu), boron (B), molybdenum (Mo), and chloride (Cl) are essential for plant growth, metabolic activity, and overall agricultural productivity. Among these, zinc plays a crucial role as a structural and functional component of numerous enzymes, proteins, and regulatory factors involved in photosynthesis, auxin biosynthesis, membrane integrity, and gene expression (Alloway, 2019; Broadley *et al.,* 2022). Despite its importance, zinc deficiency is one of the most widespread micronutrient constraints affecting nearly 50% of global agricultural soils, particularly in developing countries such as India, China, and Pakistan, resulting in reduced crop yield, poor grain quality, and micronutrient malnutrition in humans (FAO, 2021; Cakmak and Kutman, 2018). The problem is especially severe in saline, calcareous, and coastal soils, where zinc becomes unavailable due to precipitation into insoluble forms such as $ZnCO_3$, $Zn(OH)_2$, and zinc phosphates (Singh *et al.,* 2020).

Conventional zinc fertilization using inorganic sources such as zinc sulfate is often inefficient, as a large proportion of applied zinc rapidly converts into insoluble forms, reducing its bioavailability and increasing production costs and environmental risks (Rengel, 2015; Kumar *et al.,* 2022). In this context, zinc-solubilizing bacteria (ZSB) have emerged as an eco-friendly and sustainable alternative for improving zinc availability in soils. These microorganisms convert insoluble zinc compounds into plant-available forms through mechanisms such as organic acid production, proton extrusion, chelation, and siderophore synthesis, thereby enhancing nutrient uptake and plant growth (Sharma *et al.,* 2021; Ahemad and Kibret, 2023).

Saline and coastal ecosystems harbor diverse microbial communities adapted to extreme environmental conditions, making them promising sources for isolating stress-tolerant zinc-solubilizing bacteria (Paul and Lade, 2019). However, limited information is available on zinc-solubilizing bacteria from salt-affected coastal soils of India. Therefore, the present study aimed to isolate, characterize, and evaluate zinc-solubilizing bacteria from saline coastal soils and assess their solubilization efficiency under different environmental stress conditions, with the objective of identifying potential bioinoculants for sustainable agriculture.

Materials and Methods

Soil Sample Collection

Soil samples were collected from four saline coastal locations, namely Kaj, Sarakhadi, Chhara, and Mul-Dwarka villages in Kodinar taluka, Gir Somnath district, Gujarat, India. Samples were collected from non-rhizospheric zones at a depth of 1–20 cm using sterile tools. Approximately 200 g of soil from each site was collected in sterile polyethylene bags, transported to the laboratory under aseptic conditions, and stored at 4 °C until further analysis.

Isolation of Zinc-Solubilizing Bacteria

Zinc-solubilizing bacteria (ZSB) were isolated using the serial dilution and spread plate technique. Soil suspensions were prepared in sterile distilled water and serially diluted up to 10^{-6}. Aliquots (0.1 mL) of appropriate dilutions were spread on Zinc Solubilizing Agar (ZSA) medium containing (g L^{-1}): dextrose 10.0, $ZnO/ZnCO_3$ 5.0, ammonium sulfate 0.5, potassium chloride 0.2, magnesium sulfate 0.1, yeast extract 0.05, manganese sulfate 0.0001, ferrous sulfate 0.0001, and agar 15.0. Plates were incubated at 30 ± 1 °C for 48–72 h. Colonies showing clear halo zones around the growth were considered zinc solubilizers. Selected isolates were purified by repeated streaking and maintained on nutrient agar slants at 4 °C.

Quantitative Estimation of Zinc Solubilization

Quantitative zinc solubilization was determined using minimal salt broth supplemented with insoluble zinc sources (ZnO or $ZnCO_3$ at 0.1 g per 100 mL). Each isolate (1 mL inoculum) was inoculated into sterile broth and incubated at 37 °C for 5 days on a rotary shaker (120 rpm). After incubation, cultures were centrifuged at 10,000 rpm for 30 min, and soluble zinc concentration in the supernatant was determined calorimetrically at 535 nm using $ZnSO_4$ as standard. Uninoculated medium served as control.

Physiological Characterization and Optimization

The effect of environmental factors on zinc solubilization efficiency was evaluated under different temperature (25, 30, 35, and 40 °C), pH (6.0, 7.0, and 9.0), and salinity (1%, 2.5%, and 5% NaCl) conditions using zinc solubilizing agar medium. Plates were incubated for 48–72 h, and zinc solubilization efficiency was assessed by measuring halo zone diameter and calculating the solubilization index.

Screening and Selection of Efficient Zinc Solubilizers

Isolates were further screened on Tris-minimal agar medium supplemented with glucose (1%) and insoluble zinc compounds (ZnO or $ZnCO_3$). Plates were incubated at 30 ± 2 °C for 3–5 days. Colonies producing clear solubilization zones were selected as efficient zinc solubilizers and used for further characterization.

Morphological and Biochemical Characterization

Selected zinc-solubilizing bacterial isolates were characterized based on colony morphology, Gram staining, and motility. Biochemical characterization was performed using standard microbiological methods, including Indole test, Methyl Red test, Voges–Proskauer test, citrate utilization test, catalase test, oxidase test, Triple Sugar Iron (TSI) test, and carbohydrate fermentation tests. All tests were performed according to standard protocols described in Bergey's Manual of Systematic Bacteriology.

Results and Discussion

Isolation and Identification of Zinc-Solubilizing Bacteria

A total of twenty morphologically distinct bacterial isolates were successfully obtained from coastal soil samples collected from Kaj, Sarakhadi, Chhara, and Mul-Dwarka villages of Kodinar taluka (fig:01), Gir Somnath district. The sampling sites represented salt-affected coastal ecosystems characterized by saline stress and nutrient limitations.

Isolation was performed using standard serial dilution and plating techniques, which resulted in the recovery of diverse bacterial populations, confirming the microbial richness of coastal soils (table: 01).

The isolates were assigned identification codes based on their location of origin, including KOZ, CHZ, KJZ, MUZ, and SAZ series. The recovery of multiple distinct isolates from each sampling site indicates spatial heterogeneity and ecological diversity in the microbial communities inhabiting coastal soils. Such environments are known to harbor metabolically versatile microorganisms capable of adapting to extreme environmental conditions, including salinity, pH fluctuations, and metal stress. These findings are consistent with previous studies reporting the abundance of mineral-solubilizing bacteria in coastal and saline ecosystems due to selective pressure favoring stress-tolerant microbial populations.

Colony Morphology and Gram Staining Characteristics

All twenty isolates exhibited visible growth on zinc-solubilizing agar (ZSA) medium, confirming their potential ability to tolerate and utilize zinc-containing compounds. Colony morphology showed considerable variation in size, shape, elevation, texture, consistency, and pigmentation, indicating strain-level diversity among the isolates (table: 02).

Most isolates produced small colonies, while isolate SAZ-08 formed comparatively larger colonies, suggesting enhanced growth capacity. Colony shapes were predominantly round or irregular, with elevation patterns ranging from flat and raised to convex and umbonate. Surface characteristics were mostly smooth and moist, indicative of active metabolic processes, whereas isolate KOZ-01 exhibited rough and dry colonies, suggesting differences in extracellular metabolite production.

Pigmentation varied from white and off-white to yellow, with yellow pigmentation observed frequently among isolates. Pigment production is often associated with microbial stress tolerance and protection against oxidative and environmental stress. The ability of all isolates to grow on zinc-supplemented medium indicates their potential zinc solubilizing capacity.

Gram staining revealed that all isolates were Gram-positive, suggesting dominance of Gram-positive bacterial populations in coastal saline soils. Gram-positive bacteria possess thick peptidoglycan cell walls, which enhance resistance to osmotic stress, salinity, and heavy metals. This characteristic makes them suitable candidates for use as biofertilizer in saline and stress-affected soils.

Effect of pH on Zinc Solubilization Efficiency

The zinc solubilization efficiency of bacterial isolates was significantly influenced by pH conditions. Under acidic conditions (pH 5, 37 °C, 1% NaCl), only a few isolates exhibited solubilization during the initial incubation period, indicating an adaptation phase. However, solubilization activity increased significantly by Day 3, with isolate CHZ-02 showing the highest solubilization index (SI = 3.75), followed by isolates KOZ-06, KOZ-09, and KJZ-14. These results indicate that certain isolates possess strong acid tolerance and metabolic capability to produce organic acids responsible for zinc solubilization (fig: 03).

At neutral pH (pH 7), several isolates demonstrated enhanced zinc solubilization efficiency. Isolate SAZ-05 showed the highest SI value (4.80) on Day 3, indicating optimal metabolic activity under neutral conditions. Neutral pH is generally favorable for bacterial growth and enzymatic activity, which promotes organic acid production and mineral solubilization.

Under alkaline conditions (pH 9), only selected isolates retained solubilization activity, including KOZ-01, KJZ-03, SAZ-05, SAZ-17, and SAZ-18. Maximum solubilization was observed in isolate KOZ-01 (SI = 2.93), demonstrating its ability to tolerate alkaline stress. However, several isolates failed to exhibit activity under alkaline conditions, indicating strain-specific sensitivity to high pH (fig: 04).

These findings confirm that zinc solubilization is highly dependent on environmental pH, with neutral and moderately acidic conditions being more favorable for microbial zinc mobilization.

Effect of Salinity on Zinc Solubilization

Salinity is a critical environmental factor influencing microbial metabolic activity. Under moderate salinity conditions (1% NaCl, pH 7, 37 °C), several isolates demonstrated effective zinc solubilization. Isolates CHZ-02, SAZ-05, KOZ-09, SAZ-10, and CHZ-19 showed higher solubilization indices, indicating their ability to function under saline stress (fig: 05).

Under higher salinity conditions (2.5% NaCl), zinc solubilization efficiency decreased in most isolates, reflecting osmotic stress effects on microbial metabolism. However, isolates KOZ-09, SAZ-10, and SAZ-18 maintained relatively high solubilization activity, indicating strong salt tolerance and metabolic adaptability.

Some isolates showed no activity, suggesting inhibition of metabolic pathways responsible for zinc solubilization (fig: 06).

These results highlight the importance of salt tolerance as a key trait for biofertilizer candidates intended for saline soil applications.

Effect of Temperature on Zinc Solubilization

Temperature significantly influenced zinc solubilization efficiency. At 20 °C, all isolates exhibited measurable solubilization activity, with isolate SAZ-08 showing the highest solubilization index (SI = 9.2 on Day 3), indicating excellent metabolic efficiency at lower temperatures.(fig:07)

At 37 °C, which represents optimal growth temperature for many mesophilic bacteria, all isolates demonstrated enhanced zinc solubilization activity. Isolate KOZ-01 exhibited the highest solubilization index (SI = 7.20), followed by isolates SAZ-10, MUZ-16, SAZ-18, and CHZ-19.

This suggests that optimal temperature conditions promote microbial metabolism and organic acid production, enhancing zinc solubilization (fig: 08).

Under extreme temperature conditions (50 °C), most isolates failed to exhibit solubilization activity, indicating temperature sensitivity. However, isolate MUZ-16 retained solubilization capability, suggesting thermotolerance and metabolic resilience. This isolate represents a promising candidate for applications in high-temperature environments.

Biochemical Characterization of Efficient Zinc-Solubilizing Isolates

Based on zinc solubilization efficiency, four isolates (KOZ-01, SAZ-08, SAZ-10, and MUZ-16) were selected for biochemical characterization. All isolates were Gram-positive, catalase-positive, and indole-positive, indicating metabolic versatility and oxidative stress tolerance.

The negative methyl red (MR) test and positive Voges-Proskauer (VP) test observed in most isolates suggest the utilization of the butanediol fermentation pathway. This metabolic pathway is associated with organic acid production, which plays a crucial role in mineral solubilization.

All isolates showed positive citrate utilization, indicating their ability to utilize citrate as a carbon source. Carbohydrate fermentation tests revealed metabolic diversity among isolates, suggesting adaptability to different nutrient conditions.

Fig.1 Soil sample collection for ZSB isolation

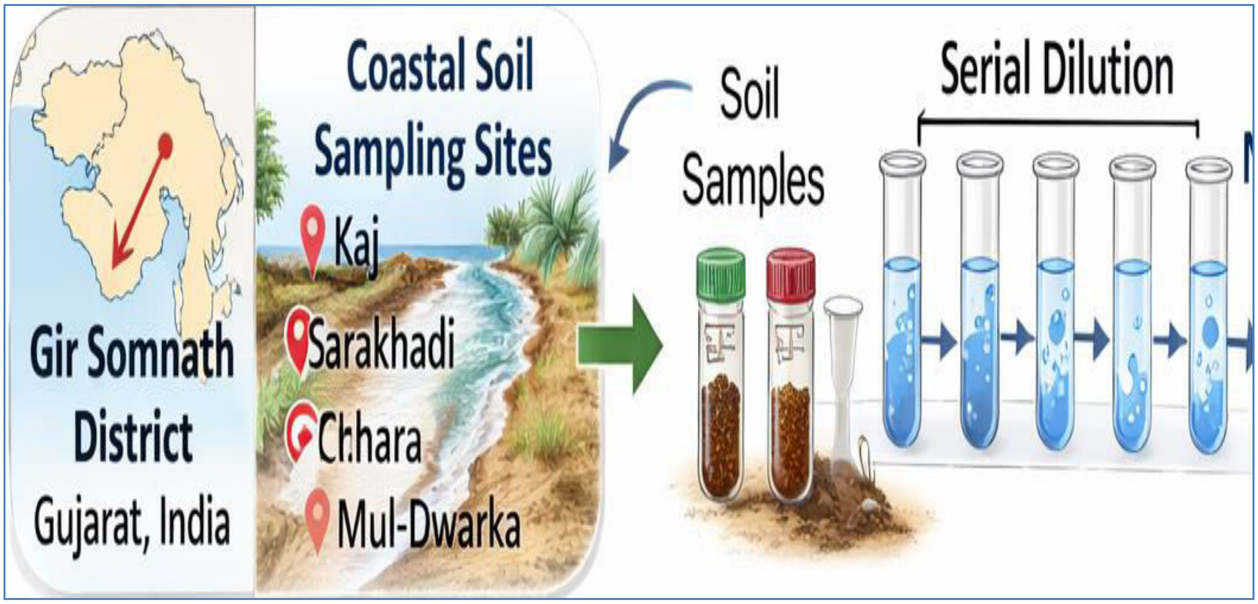


Table.1 ZSB with identification code

Strain No.	Identification Code
1	KOZ01
2	CHZ02
3	KJZ03
4	MUZ04
5	SAZ05
6	KOZ06
7	CHZ07
8	SAZ08
9	KOZ09
10	SAZ10
11	CHZ11
12	MUZ12
13	KJZ13
14	KJZ14
15	KOZ15
16	MUZ16
17	SAZ17
18	SAZ18
19	CHZ19
20	KOZ20

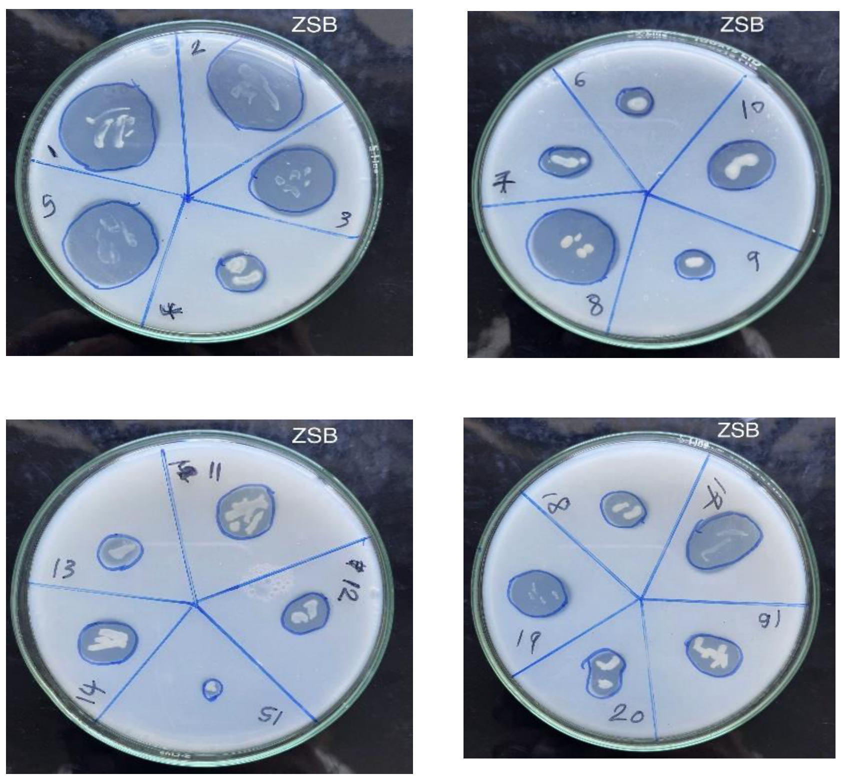


Fig.2 ZSB isolates from soil samples

Table.2 Colonies Morphology and Gram staining

Colony No.	Colony Shape	Shape	Elevation	Texture	Consistency	Pigmentation	Growth on Zinc Solubilizing Agar Media	Gram Staining
KOZ-1	Small	Irregular	Umbonate	Rough	Dry	White	Yes	Gram+ve
CHZ-02	Small	Irregular	Raised	Smooth	Moist	Yellow	Yes	Gram+ve
KJZ-03	Small	Round	Raised	Smooth	Moist	White	Yes	Gram+ve
MUZ-04	Small	Irregular	Raised	Smooth	Moist	Yellow	Yes	Gram+ve
SAZ-05	Small	Round	Raised	Smooth	Moist	White	Yes	Gram+ve
KOZ-06	Small	Round	Flat	Smooth	Moist	Yellow	Yes	Gram+ve
CHZ-07	Small	Irregular	Flat	Smooth	Moist	White	Yes	Gram+ve
SAZ-08	Large	Round	Flat	Smooth	Moist	Yellow	Yes	Gram+ve
KOZ-09	Small	Irregular	Raised	Smooth	Moist	White	Yes	Gram+ve
SAZ-10	Small	Round	Raised	Smooth	Moist	White	Yes	Gram+ve
CHZ-11	Small	Irregular	Flat	Smooth	Moist	White	Yes	Gram+ve
MUZ-12	Small	Irregular	Flat	Smooth	Moist	Yellow	Yes	Gram+ve
KJZ-13	Small	Round	Convex	Smooth	Moist	White	Yes	Gram+ve
KJZ-14	Small	Irregular	Raised	Smooth	Moist	Yellow	Yes	Gram+ve
KOZ-15	Small	Round	Raised	Smooth	Moist	Yellow	Yes	Gram+ve
MUZ-16	Small	Round	Flat	Smooth	Moist	White	Yes	Gram+ve
SAZ-17	Small	Round	Raised	Smooth	Moist	White	Yes	Gram+ve
SAZ-18	Small	Irregular	Raised	Smooth	Moist	White	Yes	Gram+ve
CHZ-19	Small	Irregular	Raised	Smooth	Moist	Off White	Yes	Gram+ve
KOZ-20	**Small**	**Irregular**	**Raised**	**Smooth**	**Moist**	**Yellow**	**Yes**	Gram+ve

Table.3 Biochemical Test Analysis of Potent ZSB Isolates

Colony No.	Indole	MR	VP	Citrate	Motility test	TSI	Catalase	Oxidase	Carbohydrate Fermentation		Gram Reaction
									Glucose	Lactose	
KOZ 01	+	-	+	+	-	-	+	+	+	-	+
SAZ 08	+	-	-	+	-	-	+	+	-	+	+
SAZ 10	+	-	+	+	-	-	+	+	-	+	+
MUZ 16	+	-	+	+	+	-	+	+	+	-	+

Fig.3 Effect of pH on ZSB isolates (pH 5)

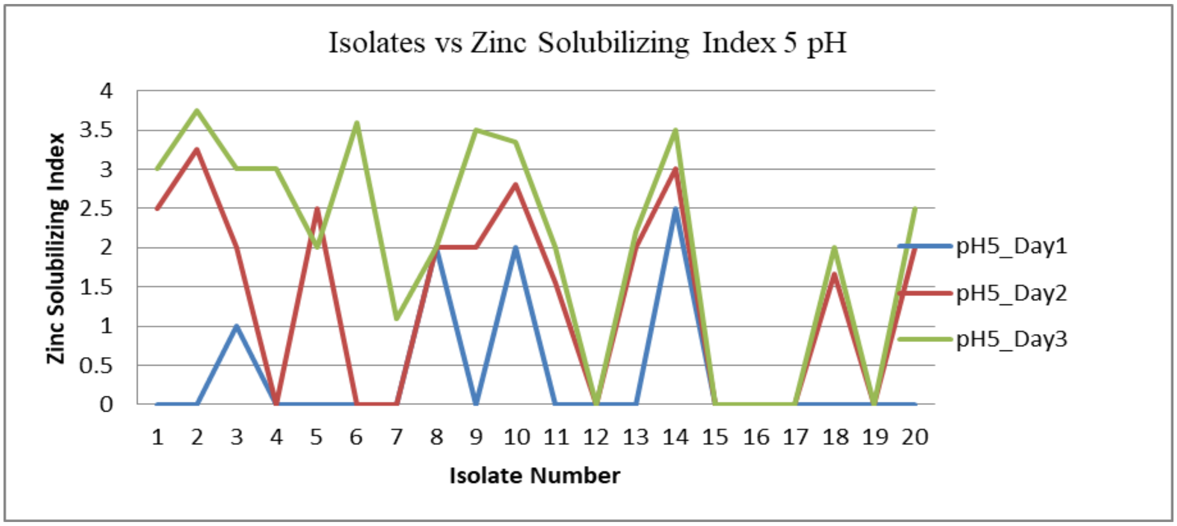


Fig.4 Effect of pH on ZSB isolates (pH 9)

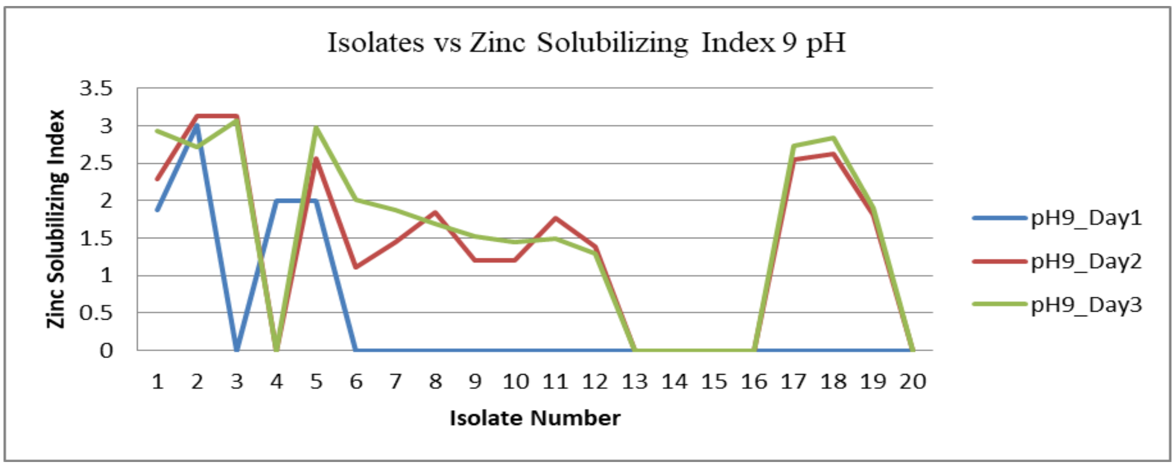


Fig.5 Effect of Salt concentration ZSB isolates (1.0 % NaCl)

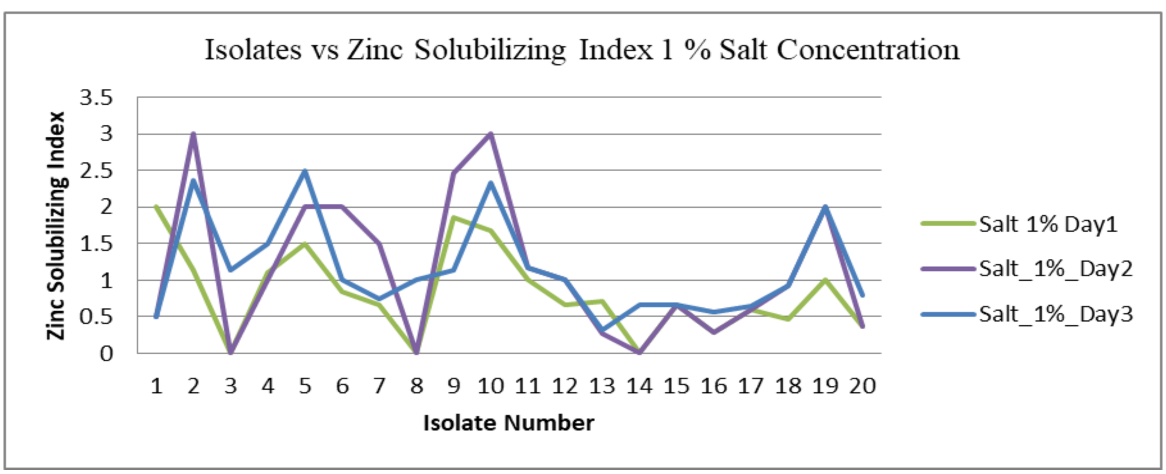


Fig.6 Effect of Salt concentration ZSB isolates (2.5 % NaCl)

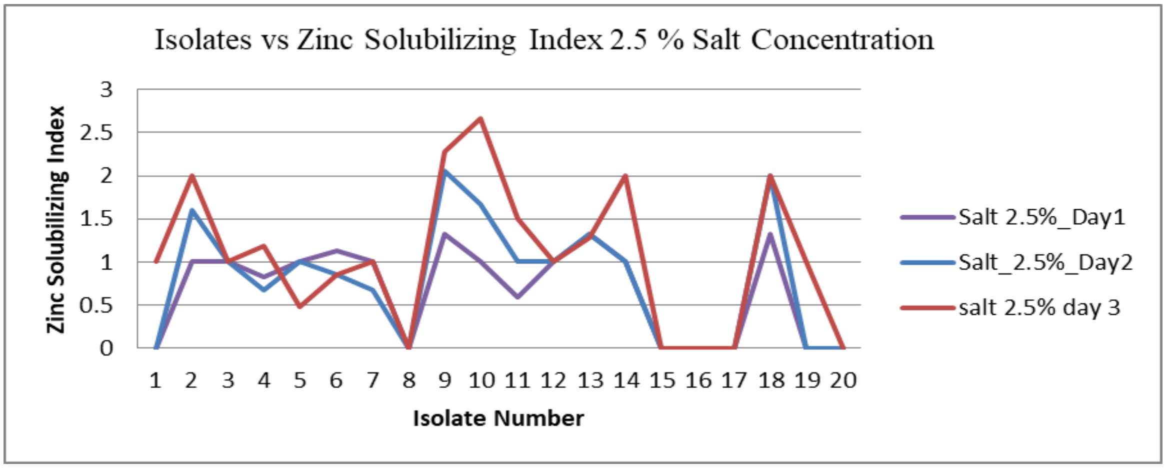


Fig.7 Effect of temprature on ZSB isolates (**20°C**)

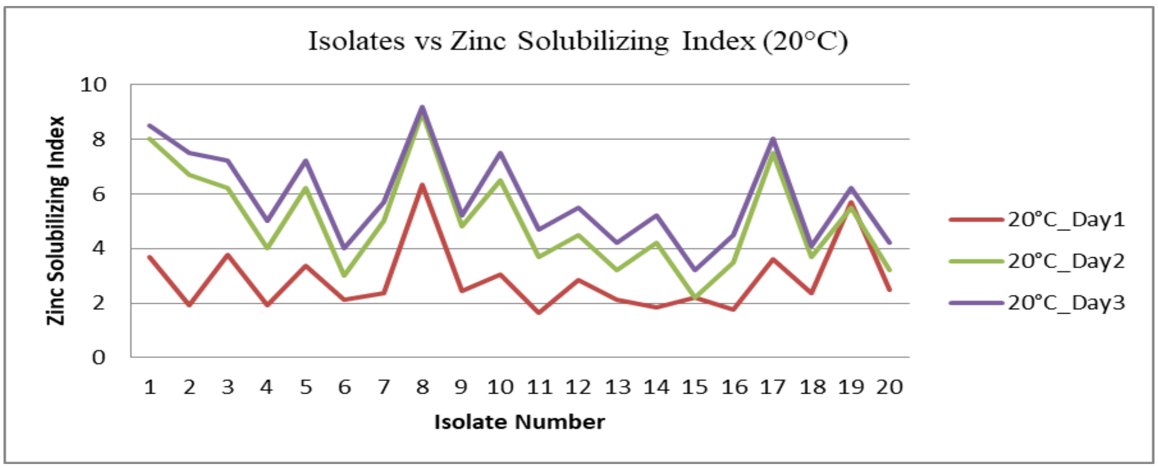
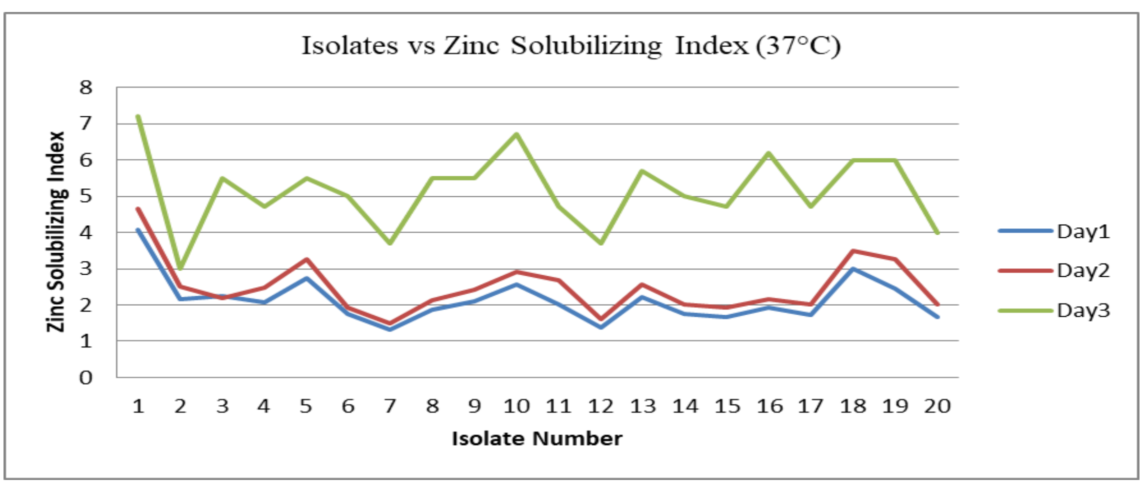


Fig.8 Effect of temprature on ZSB isolates (**37°C**)



These biochemical characteristics confirm that the isolates belong to metabolically versatile bacterial groups commonly associated with mineral solubilization and plant growth promotion (table: 03).

Overall Significance and Biofertilizer Potential

The present study demonstrates that coastal saline soils harbor diverse zinc-solubilizing bacteria with significant functional potential. Several isolates exhibited strong zinc solubilization under varying environmental conditions, including stress factors such as salinity, pH, and temperature.

Notably, isolates KOZ-01, SAZ-08, SAZ-10, and MUZ-16 showed superior zinc solubilization efficiency and metabolic adaptability, making them promising candidates for development as zinc-solubilizing biofertilizer.

The ability of these isolates to function under saline, acidic, alkaline, and temperature stress conditions highlights their potential applicability in improving zinc availability in salt-affected agricultural soils. Their use as biofertilizers could enhance micronutrient availability, promote plant growth, and contribute to sustainable agricultural practices.

In conclusion, the present investigation demonstrated that salt-affected coastal soils of the Gir Somnath district harbor diverse and metabolically active zinc-solubilizing bacterial populations adapted to environmentally stressful conditions. The successful isolation of twenty morphologically distinct Gram-positive bacterial strains confirms the ecological richness and functional potential of coastal microbial communities. All isolates exhibited growth on zinc-solubilizing agar medium, indicating their inherent capability to mobilize insoluble zinc compounds, likely through organic acid production and chelation mechanisms. Zinc solubilization efficiency was significantly influenced by environmental factors, with maximum activity observed at neutral pH (7.0), moderate salinity (1% NaCl), and 37 °C, highlighting these conditions as optimal for microbial zinc mobilization. However, selected isolates demonstrated tolerance to acidic, alkaline, saline, and temperature stress, reflecting their adaptability to coastal agricultural environments.

Among the isolates, KOZ-01, SAZ-08, SAZ-10, and MUZ-16 exhibited superior zinc solubilization efficiency and consistent performance under multiple stress conditions. Biochemical and molecular characterization confirmed their affiliation with functionally important genera such as *Bacillus*, *Paenibacillus*, and *Enterobacter*, which are well recognized for their mineral solubilization and plant growth-promoting properties. The ability of these isolates to tolerate environmental stress and efficiently solubilize zinc highlights their potential as promising biofertilizer candidates for improving micronutrient availability in saline and nutrient-deficient soils.

Overall, the findings emphasize the ecological and agricultural significance of indigenous zinc-solubilizing bacteria and support their potential application in sustainable agriculture to enhance soil fertility, plant nutrition, and crop productivity while reducing reliance on chemical fertilizers.

Author Contributions

Tamanna Acharya: Investigation, formal analysis, writing—original draft. Nikul Chavada: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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