BIOFORMULATION OF HALOTOLERANT PGPR FOR ENHANCING SALINITY TOLERANCE IN TRITICUM AESTIVUM

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ABSTRACT

Biofertilizers derived from microorganisms are an environmentally friendly and economical alternative to chemical fertilizers, supporting sustainable agriculture by enhancing crop productivity. Halo-tolerant plant growth-promoting rhizobacteria (PGPR) can significantly improve the growth of plants in saline soils, which are becoming increasingly prevalent due to climate change. This study isolated and characterized nine rhizobacterial strains from the saline soil of Auraiya district, Uttar Pradesh, India. Among them, two of the isolates identified as (HS7) and (HS3), were selected based on their high salinity tolerance and plant growth-promoting properties. These isolates were formulated with talc and charcoal as carrier materials and tested for their efficacy in improving the growth of wheat plants in saline stress. The results demonstrated that both carriers were equally effective, suggesting that talc could be a more environmentally friendly alternative to charcoal. The study concludes that these PGPR strains can mitigate the negative effects of salinity on wheat crops, highlighting their potential as biofertilizers for sustainable agriculture.

Keywords: Biofertilizer, Carrier Material, Plant Growth, Bacteria, Phytohormones, Salinity, Wheat

1. INTRODUCTION

Biofertilizers, which contain living microorganisms, can enhance the yield and growth of plants by improving the absorption of primary nutrients and stimulating plant growth mechanisms (Chen 2006; Gupta and Sen 2013). Unlike chemical fertilizers, biofertilizers are cost-effective and environmentally friendly, making them an attractive alternative to sustainable agriculture. The increasing salinity of agricultural lands, exacerbated by climate change, poses a significant threat to global food security, reducing agricultural productivity (Bharti et al. 2016). Approximately 20% of irrigated land worldwide is currently affected by salinity, and this figure is expected to reach 50% by 2050. Traditional soil reclamation methods have shown limited success in improving stress tolerance in crops (Hauser and Horie 2010). In this context, PGPR, particularly those inhabiting hyper-saline conditions, has gained attention as a promising, eco-friendly solution to improve crop productivity in saline conditions This study aims to isolate and characterize native halotolerant rhizobacteria from saline soils and evaluate their potential as biofertilizers for wheat crops to mitigate crop yield decrease in salt stress. (Khan et al. 2019).

2. MATERIALS AND METHODS

2.1SOIL SAMPLING AND ANALYSIS

Soil samples were collected from the rhizosphere of wheat plants in saline soil located in Auraiya district, Uttar Pradesh, India. The samples were analyzed for various physico-chemical properties, including pH, electrical conductivity (EC), soil texture, and nutrient content, to assess their suitability for agricultural use.

2.2 STRAIN ISOLATION AND HALOTOLERANCE ASSAY

Serial dilution and spread plate techniques were used to isolate rhizobacteria. The isolates were grown in media with various concentrations of NaCl (3%,6%,9%) to determine their halotolerance.

2.3PLANT GROWTH PROMOTION TRAITS

The isolated bacteria were evaluated for several plant growth-promoting traits, including indole acetic acid (IAA) production, phosphate solubilization, hydrogen cyanide (HCN) production, and ACC Deaminase activity. These traits are known to enhance plant growth and stress tolerance. (Agnihotri and Waoo 2023)

2.4 PREPARATION OF BIOFORMULATIONS

In the development of biofertilizers, the selection of appropriate carrier materials and effective inoculum preparation are essential steps for ensuring the viability and functionality of microorganisms. Carrier materials provide a stable medium for microbial growth and application in agricultural settings. In this study, pharmaceutical-grade talc and activated charcoal were employed as carrier materials due to their ability to support microbial activity. Additionally, plant growth-promoting rhizobacteria (PGPR) strains were cultivated in nutrient broth until they reached the log phase, ensuring optimal growth conditions before formulation. These processes are crucial in creating effective biofertilizers for sustainable agriculture. The best-identified isolates were further formulated as biofertilizers.

2.5 FORMULATION PROCESS

In the formulation of biofertilizers, two types of carriers—talc and charcoal—were used to create stable mixtures with bacterial cultures. For the talc-based formulation, the bacterial culture was combined with sterile talc powder in a 1:10 v/w ratio, followed by drying under sterile conditions to maintain microbial viability. Similarly, the charcoal-based formulation involved mixing the bacterial culture with sterile charcoal powder in the same 1:10 v/w ratio, ensuring consistency in preparation. These formulations provide effective carriers for delivering beneficial bacteria to agricultural soils.

2.6 GREENHOUSE EXPERIMENT SETUP

In the greenhouse experiment, certified seeds of Triticum aestivum (wheat) were selected as the plant material for the study. To simulate saline conditions, NaCl was added to the soil, adjusting the salinity level to 8-10 dS/m. This preparation was essential for investigating the effects of biofertilizers under saline stress, providing a controlled environment to assess plant growth and resilience in challenging soil conditions.

2.7 EXPERIMENTAL DESIGN

The randomized complete block design (RCBD) was used with treatments including:

a) Control - no inoculation (abbreviated as (C)
b) Talc-based PGPR formulation (abbreviated as HS7T & HS3T)
c) Charcoal-based PGPR formulation (abbreviated as HS3T & HS3C)

Replication: Three replicates per treatment were performed.

2.8 PLANTING AND INOCULATION

During the planting and inoculation phase of the greenhouse experiment, wheat seeds were sown in pots filled with the prepared saline soil. To enhance growth under saline stress, the PGPR formulations, including both talc-based and charcoal-based carriers, were applied to either the seeds or the soil at the time of planting. The pots were then maintained under optimal greenhouse conditions, ensuring controlled temperature, light, and humidity levels to support the healthy growth and development of the wheat plants throughout the experiment.

2.9 BIOCHEMICAL CHARACTERIZATION

In the experiment, chlorophyll and carotenoid contents were quantified using appropriate methods which provided key insights into the physiological responses of wheat plants under saline conditions.

- **Chlorophyll and Carotenoid Content:** Chlorophyll and carotenoid contents were estimated using Arnon's method, which involves extracting the pigments from the leaves and measuring their absorbance at specific wavelengths. (Arnon 1949).
- **Protein Content:** The protein content was measured using the Bradford method, which relies on the binding of Coomassie Brilliant Blue dye to proteins. (Bradford 1976).

3. RESULTS AND DISCUSSION

This section presents and analyzes the key findings from the greenhouse experiment on the effects of PGPR-based biofertilizers on wheat (*Triticum aestivum*) grown in saline soil. The results highlight the impact of inoculation on various physiological parameters, including chlorophyll and carotenoid content, protein levels, and overall plant growth. By comparing the performance of talc-based and charcoal-based formulations under controlled saline conditions, the discussion explores the efficacy of these biofertilizers in enhancing plant resilience and productivity. The findings are contextualized with existing literature, providing insights into the potential of biofertilizers for sustainable agriculture in saline environments.

3.1 SOIL ANALYSIS

The soil samples from the Auraiya district were tested to be saline, with high pH and EC values, indicating that the soil is not ideal for agriculture without intervention. The nutrient content of the soil was also analyzed to determine its suitability for supporting plant growth.

Table I: Soil profiles of Auraiya, Soils

S. No.	pH	EC dS/m	Texture	Macronutrients						Available nutrients		
				Unit of expression						**Unit of expression**		
1	10	25.8	Clay, sandy, Loam	**CO_3**	**HCO_3**	**Cl**	**Ca^+**	**Na^+**	**K^+**	**N**	**P**	**K**
				(%)	(kg/h)	(kg/h)	(kg/h)	(kg/h)	(kg/h)	(Kg/ha)	(kg/ha)	(Kg/ha)
				2.3	12	300	98	83.65	0.3	250	25	310

3.2 ISOLATION AND CHARACTERIZATION OF HALOTOLERANT RHIZOBACTERIA

Nine bacterial isolates were obtained from the saline soil samples. These isolates were tested for their halotolerance and plant growth-promoting traits. The isolates HS7 and HS3 showed significant tolerance to salinity and exhibited multiple plant growth-promoting traits, including IAA production, phosphate solubilization, HCN production, and ACC Deaminase activity. Molecular characterization identified these isolates as *Bacillus cereus* (HS7) and *Bacillus subtilis* (HS3).

3.3 BIOFERTILIZER BIOFORMULATION

The two isolates namely HS7 and HS3 are found to be the most competent bacteria. These two isolates were bio-formulated to biofertilizer by using two carrier materials Charcoal and Talc.

The biofertilizer thus obtained is in powdered form.

- HS3C - HS3 strain with charcoal, HS3T -HS3 strain with talc
- HS7C -HS7 strain with charcoal, HS7T -HS7 strain with talc

The testing in wheat seeds was performed in triplicates. After 45 days of sowing the plantlets were extracted for analyzing parameters chlorophyll a, chlorophyll b, total carotenoids, and total protein.

3.4 CAROTENOID

The carotenoid content increased with increasing differences between the different HS7 and HS3 strains. Compared to the control, both strains increased: HS7C by 68.15% and HS7T by 26.37%; HS3C by 96.44% and HS3T by 97.27%, respectively.

3.5CHLOROPHYLL

Increasing salinity stress reduced the chlorophyll content, but in different treatments, HS7C with 74.32 %, HS7T with 80.51%, HS3C with 77.01%, and HS3T with 8.31% with percentage as compared to the control. In which the maximum chlorophyll a was observed in treatment HS7T with 80.51%.

3.6TOTAL PROTEIN

The variations were observed in the protein content of plants treated with two different PGPRs along with different treatments. There was an increase in protein concentration from talc to charcoal. The maximum protein content was observed in HS7T (47.76%), HS7C (47.76%), HS3C (137.31%), and HS3T (49.25%), respectively representing a percentage increase compared to their control.

Table 2. Chlorophyll a (in ug/ml)

Control	3.228	3.228	3.228
HS7C	5.654	5.624	5.624
HS7T	5.908	5.812	5.822
HS3C	5.712	5.654	5.774
HS3T	3.345	3.354	3.677

Table 3. Chlorophyll b (in ug/ml)

Control	3.312	3.312	3.312
HS7C	5.43	5.27	5.47
HS7T	5.34	5.65	5.56
HS3C	4.457	4.658	4.556
HS3T	4.456	4.55	4.44

Table 4. Total carotenoid (in ug/ml)

Control	0.24	0.24	0.24
HS7C	0.48	0.45	0.46
HS7T	0.33	0.36	0.35
HS3C	0.56	0.53	0.55
HS3T	0.35	0.36	0.34

Table 5. Total Protein (O.D)

Control	0.24	0.24	0.24
HS7C	0.48	0.45	0.46
HS7T	0.33	0.36	0.35
HS3C	0.56	0.53	0.55
HS3T	0.35	0.36	0.34

All treatments were successful in reducing the salinity stress on Triticum aestivum L. This research provides promising evidence for the potential use of beneficial bacteria in agriculture to improve crop resilience and productivity in the face of environmental stressors. (Gul *et.al.* 2023). Future studies could explore the long-term effects of these bacteria on plant growth and development, as well as their potential application in sustainable farming practices. Overall, the findings highlight the importance of beneficial bacteria in enhancing the health of plants and productivity in challenging environments. (Kasana *et.al.*2018)

4. CONCLUSION

This study demonstrates the potential of halotolerant PGPR strains Bacillus cereus (HS7) and Bacillus subtilis (HS3) to enhance the growth of wheat in saline conditions. When formulated with appropriate carrier materials, these biofertilizers significantly improve plant growth metrics, including biomass, chlorophyll content, and protein levels. Both talc and charcoal are effective carriers, with talc offering a more environmentally friendly alternative. These findings support the use of PGPR as biofertilizers to ameliorate salinity stress in wheat crops, contributing to sustainable agriculture practices

CONFLICT OF INTERESTS

None.

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