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Isolation and Characterization of Halotolerant Bacteria and Their Effects on Wheat Plants as Plant Growth-Promoting Rhizobacteria.

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ABSTRACT:
Halotolerant bacteria, adept at thriving in high-salinity environments, offer promising solutions for agricultural challenges posed by soil salinity. This research focuses on the isolation and characterization of halotolerant bacteria from saline environments and investigates their potential as plant growth-promoting rhizobacteria (PGPR) for enhancing wheat plant growth under saline conditions. Through extensive isolation and screening processes, numerous halotolerant bacterial strains were identified and characterized for their salt tolerance and plant growth-promoting traits, including indole-3-acetic acid (IAA) production, phosphate solubilization, and siderophore production.

Subsequent greenhouse experiments revealed the efficacy of selected halotolerant bacterial strains in promoting wheat plant growth under saline conditions, with significant improvements observed in plant growth parameters such as shoot length, root length, biomass accumulation, and chlorophyll content. Furthermore, physiological and biochemical analyses demonstrated the salt tolerance enhancement conferred by halotolerant bacteria, as evidenced by improved membrane stability, osmotic stress tolerance, and antioxidant defense mechanisms in inoculated plants. These findings highlight the potential of halotolerant bacteria as effective bioinoculants for sustainable agriculture in saline environments, offering a promising avenue for mitigating the adverse effects of salinity stress on crop production.

The diversity and plant growth-promoting traits exhibited by isolated halotolerant bacteria underscore their adaptability to saline environments and their potential for enhancing crop productivity in salt-affected agricultural lands. By elucidating the mechanisms underlying the beneficial effects of halotolerant bacteria on plant growth and salt tolerance, this research contributes to our understanding of plant-microbe interactions and provides valuable insights for the development of strategies to improve agricultural sustainability in saline environments. Integration of halotolerant bacteria as bioinoculants with agronomic practices such as crop rotation, soil amendment, and water management holds promise for enhancing soil fertility, nutrient availability, and crop resilience in salt-affected agricultural lands, ultimately contributing to food security and environmental sustainability on a global scale.

Future research directions may focus on optimizing bacterial inoculation strategies, elucidating the molecular mechanisms underlying plant-microbe interactions, and exploring the potential synergistic effects of microbial consortia in saline soil management, further advancing our ability to harness the benefits of halotolerant bacteria for sustainable agriculture.

Keywords:

Halotolerant bacteria, Plant growth-promoting rhizobacteria, Wheat plants, Salinity stress, Isolation, Characterization.

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I.Introduction

Salinity stress poses a significant challenge to agricultural productivity worldwide, affecting approximately 20% of irrigated lands and limiting crop growth and yield. Soil salinity, arising from natural processes such as weathering of rocks, irrigation practices, and improper drainage, leads to the accumulation of soluble salts, predominantly sodium chloride (NaCl), in the root zone, disrupting plant water uptake and nutrient absorption. The resultant osmotic stress and ion toxicity severely hinder plant growth and development, ultimately reducing crop yields and economic returns for farmers [1].

In the quest for sustainable solutions to mitigate the adverse effects of salinity stress on crop production, plant growth-promoting rhizobacteria (PGPR) have emerged as promising bioinoculants. PGPR are beneficial soil bacteria that colonize the rhizosphere—the soil region influenced by root exudates—and establish mutualistic relationships with host plants, conferring various benefits such as enhanced nutrient uptake, phytohormone production, disease suppression, and stress tolerance [2].

By harnessing the beneficial interactions between plants and PGPR, agricultural practices can be optimized to improve crop performance, particularly under challenging environmental conditions like salinity stress.

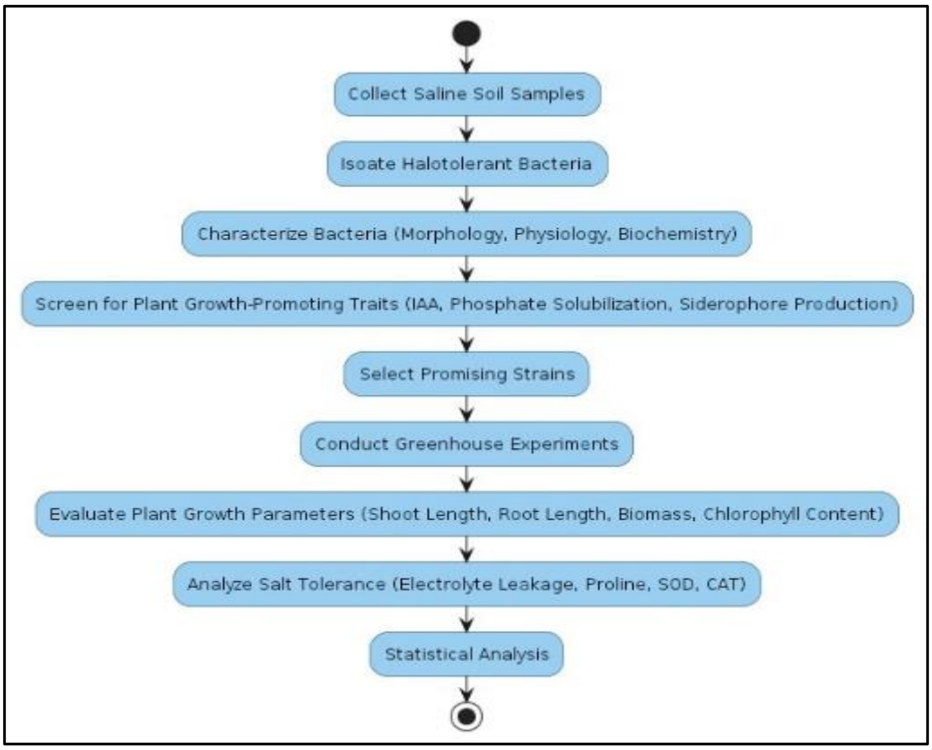

Figure 1: Flowchart of the Experimental Process

Amidst the diverse pool of PGPR, halotolerant bacteria have garnered considerable attention for their ability to thrive in high-salinity environments, offering a potential solution for saline soil improvement. Halotolerant bacteria possess adaptive mechanisms that enable them to maintain cellular homeostasis and metabolic activity in the presence of elevated salt concentrations.These mechanisms may include the synthesis of osmoprotectants, ion transporters, and enzymes involved in salt detoxification, allowing halotolerant bacteria to survive and function effectively in saline soils. Moreover, some halotolerant bacteria exhibit plant growth-promoting traits, making them ideal candidates for enhancing crop productivity in salt-affected agricultural lands. The objectives of this study were twofold: first, to isolate and characterize halotolerant bacteria from saline environments, and second, to investigate their potential as plant growth-promoting rhizobacteria (PGPR) for improving wheat plant growth under salinity stress [3]. By elucidating the diversity and plant growth-promoting traits of isolated halotolerant bacteria and evaluating their efficacy in enhancing wheat plant growth under saline conditions, this research contributes to the understanding of halotolerant bacteria as bioinoculants for sustainable agriculture in saline environments.

A. Salinity Stress: A Major Constraint in Agriculture

Salinity stress is a pervasive environmental factor that poses a formidable challenge to agricultural productivity, particularly in arid and semi-arid regions where irrigation is essential for crop cultivation. The accumulation of salts in the soil, primarily sodium chloride (NaCl), disrupts the osmotic balance within plant cells and interferes with essential physiological processes, leading to stunted growth, reduced yields, and even crop failure. The deleterious effects of salinity stress on crop plants are exacerbated by factors such as inadequate drainage [4], excessive irrigation, and poor soil management practices, exacerbating the prevalence and severity of salt-affected soils globally.

B. Plant Growth-Promoting Rhizobacteria as a Sustainable Solution

Plant growth-promoting rhizobacteria (PGPR) encompass a diverse group of soil bacteria that inhabit the rhizosphere—the soil region surrounding plant roots—and engage in beneficial interactions with host plants. Through mechanisms such as nutrient solubilization, phytohormone production, and disease suppression, PGPR enhance plant growth, development, and stress tolerance [5], thereby contributing to sustainable agriculture and environmental stewardship. Harnessing the potential of PGPR as bioinoculants offers a cost-effective and environmentally friendly approach to enhancing crop productivity while reducing the reliance on chemical fertilizers and pesticides.

C. Halotolerant Bacteria: Potential Candidates for Saline Soil Improvement

Halotolerant bacteria represent a subset of microorganisms capable of thriving in high-salinity environments, including saline soils, salt marshes, and hypersaline ecosystems. These bacteria possess adaptive mechanisms that enable them to withstand osmotic stress and ion toxicity associated with elevated salt concentrations, thereby colonizing and persisting in saline environments [6]. Halotolerant bacteria exhibit a wide range of metabolic diversity and physiological adaptations, allowing them to maintain cellular integrity and functionality under saline conditions.

D. Objectives of the Study

The primary objective of this study is to isolate and characterize halotolerant bacteria from saline environments and assess their potential as plant growth-promoting rhizobacteria (PGPR) for enhancing wheat plant growth under salinity stress. Specific aims include Isolation and screening of halotolerant bacterial strains from saline soil samples.Characterization of selected halotolerant bacteria for salt tolerance and plant growth-promoting traits. Evaluation of the effects of halotolerant bacteria on wheat plant growth under saline conditions in greenhouse experiments [7]. By addressing these objectives, this research endeavors to elucidate the role of halotolerant bacteria as bioinoculants for sustainable agriculture in saline environments and contribute to the development of strategies for mitigating the adverse effects of salinity stress on crop production.

II. Isolation and Characterization of Halotolerant Bacteria

A. Collection of Saline Soil Samples

Saline soil samples were collected from diverse geographical locations, including coastal areas, salt-affected agricultural lands, and saline marshes, to ensure the representation of a wide range of environmental conditions [8]. Sampling sites were selected based on their salinity levels, with an emphasis on areas characterized by high soil salinity. Soil samples were collected using sterile sampling equipment to minimize contamination and preserve the microbial diversity present in the rhizosphere and surrounding soil matrix.

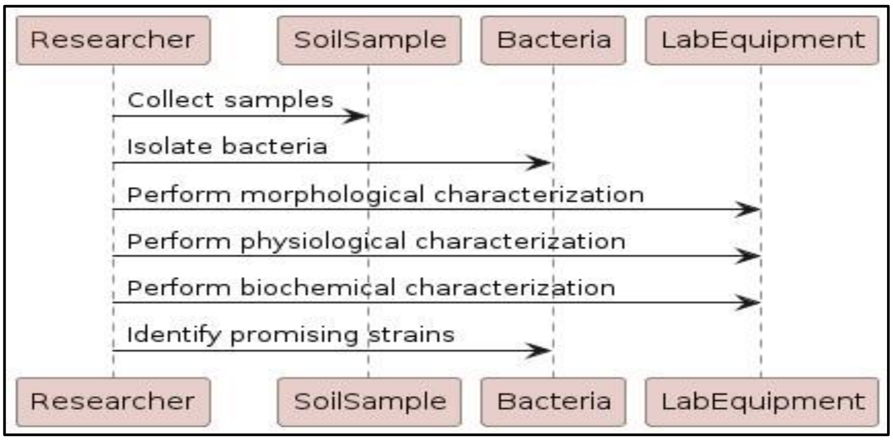

Figure 2: Sequence Diagram of Bacteria Isolation and Characterization Process

B. Isolation of Halotolerant Bacterial Strains

The isolation of halotolerant bacterial strains was carried out using selective media supplemented with varying concentrations of NaCl to enrich for salt-tolerant microorganisms. Serial dilution techniques were employed to dilute soil suspensions, followed by plating onto agar plates containing a basal medium supplemented with NaCl [9]. Incubation at optimal temperature and pH conditions facilitated the growth and proliferation of halotolerant bacteria, which were subsequently isolated as individual colonies for further characterization.

C. Screening for Salt Tolerance

Isolated bacterial colonies were subjected to preliminary screening assays to evaluate their salt tolerance and ability to grow in the presence of high salt concentrations. This screening involved inoculating bacterial isolates onto agar plates supplemented with increasing concentrations of NaCl (e.g., 0%, 5%, 10%, 15%, and 20% NaCl) and observing their growth patterns over time. Bacterial strains exhibiting robust growth and colony formation at higher salt concentrations were selected for further characterization.

D. Characterization of Selected Strains

Selected halotolerant bacterial strains were subjected to comprehensive characterization to assess their morphological, physiological, and biochemical properties. Morphological characterization involved microscopy-based examination of cell morphology, size, and motility, providing insights into the structural features of the bacterial isolates. Physiological characterization encompassed the determination of growth kinetics, optimal temperature, pH range, and salt tolerance limits, elucidating the environmental conditions conducive to bacterial growth and survival [10]. Biochemical characterization involved biochemical tests to identify key metabolic pathways, enzyme activities, and metabolic capabilities of the bacterial isolates, aiding in the classification and taxonomic identification of the strains.

E. Molecular Identification and Phylogenetic Analysis

To complement the phenotypic characterization, molecular techniques such as polymerase chain reaction (PCR) amplification of conserved genomic regions (e.g., 16S rRNA gene) were employed for the molecular identification of bacterial isolates [11]. PCR products were sequenced, and the resulting nucleotide sequences were compared against reference databases to determine the phylogenetic affiliation and taxonomic classification of the bacterial strains. Phylogenetic analysis was performed using bioinformatics tools to infer evolutionary relationships and construct phylogenetic trees, providing insights into the genetic diversity and relatedness of the isolated halotolerant bacteria.

F. Data Analysis and Interpretation

Data obtained from the isolation and characterization of halotolerant bacteria were analyzed using statistical methods and bioinformatics tools to identify patterns, correlations, and trends. Descriptive statistics, including means, standard deviations, and graphical representations, were used to summarize and visualize the data [12]. Comparative analyses were conducted to assess differences in salt tolerance, growth kinetics, and biochemical properties among the isolated bacterial strains. Interpretation of the results involved contextualizing the findings within the broader framework of halotolerant bacteria ecology, physiology, and potential applications as plant growth-promoting rhizobacteria (PGPR). By systematically isolating and characterizing halotolerant bacteria from saline environments, this study provides valuable insights into the diversity, ecological significance, and biotechnological potential of these microorganisms. The subsequent sections will focus on evaluating the plant growth-promoting traits and effects of selected halotolerant bacteria on wheat plant growth under saline conditions [13], further elucidating their role as bioinoculants for sustainable agriculture in saline environments.

III. Evaluation of Plant Growth-Promoting Traits

A. Production of Indole-3-Acetic Acid (IAA)

Indole-3-acetic acid (IAA) is a phytohormone known for its role in regulating plant growth and development, including cell elongation, root initiation, and nutrient uptake. Many plant growth-promoting rhizobacteria (PGPR) produce IAA through the activity of tryptophan-dependent pathways, facilitating root growth and enhancing plant vigor. In this study, the ability of selected halotolerant bacterial strains to produce IAA was evaluated using qualitative and quantitative assays. Qualitative assays involved the detection of IAA production based on the formation of pink coloration in Salkowski reagent-treated bacterial cultures, indicative of tryptophan metabolism and IAA synthesis. Quantitative assays, such as high-performance liquid chromatography (HPLC) or enzyme-linked immunosorbent assay (ELISA), were employed to quantify IAA levels in bacterial culture supernatants, providing quantitative measures of IAA production by the bacterial isolates.

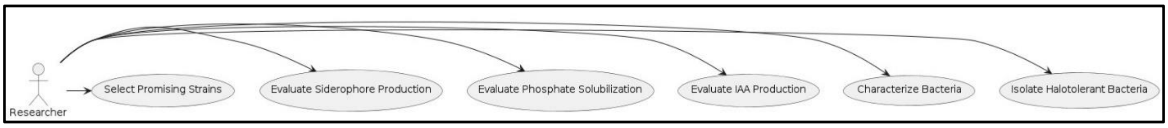

Figure 3: Evaluation of Plant Growth-Promoting Traits

B. Solubilization of Phosphate

Phosphorus (P) is an essential nutrient for plant growth and development, playing a critical role in energy transfer, photosynthesis, and metabolic processes. However, the availability of phosphorus in soil is often limited due to its insolubility in the soil matrix, particularly under alkaline or acidic conditions. Plant growth-promoting rhizobacteria (PGPR) can enhance phosphorus availability to plants by solubilizing insoluble forms of phosphate through the secretion of organic acids, phosphatases, and other phosphorus-solubilizing enzymes. In this study, the ability of halotolerant bacterial strains to solubilize phosphate was assessed using qualitative and quantitative assays [14]. Qualitative assays involved the formation of clear zones or halos around bacterial colonies on agar plates containing insoluble phosphate sources (e.g., tricalcium phosphate), indicating phosphate solubilization by the bacterial isolates. Quantitative assays, such as colorimetric determination of soluble phosphate concentrations in culture supernatants, provided quantitative measures of phosphate solubilization activity by the bacterial strains.

C. Production of Siderophores

Siderophores are low-molecular-weight compounds produced by many microorganisms to chelate and sequester ferric iron (Fe^3+) from the environment, facilitating iron uptake and acquisition under iron-limiting conditions. Iron is an essential micronutrient for plant growth and metabolism, serving as a cofactor for various enzymes involved in photosynthesis, respiration, and nitrogen fixation. Plant growth-promoting rhizobacteria (PGPR) can enhance iron availability to plants by producing siderophores that solubilize and mobilize iron in the rhizosphere, promoting plant growth and alleviating iron deficiency symptoms. In this study, the production of siderophores by halotolerant bacterial strains was evaluated using qualitative and quantitative assays. Qualitative assays involved the formation of orange or reddish-brown coloration in culture media supplemented with chrome azurol S (CAS) dye, indicative of siderophore production and iron chelation by the bacterial isolates [15]. Quantitative assays, such as spectrophotometric measurement of siderophore concentrations in culture supernatants, provided quantitative measures of siderophore production by the bacterial strains.

D. Data Analysis and Interpretation

Data obtained from the evaluation of plant growth-promoting traits were analyzed using statistical methods and bioinformatics tools to assess the efficacy and potential of halotolerant bacterial strains as plant growth-promoting rhizobacteria (PGPR) . Descriptive statistics, including means, standard deviations, and graphical representations, were used to summarize and visualize the data. Comparative analyses were conducted to assess differences in IAA production, phosphate solubilization, and siderophore production among the bacterial strains. Correlation analyses were performed to investigate potential relationships between plant growth-promoting traits and the ability of bacterial strains to enhance wheat plant growth under saline conditions. Interpretation of the results involved elucidating the mechanisms underlying the beneficial effects of halotolerant bacteria on plant growth and development, with implications for their potential applications in sustainable agriculture in saline environments.

By evaluating the plant growth-promoting traits of halotolerant bacterial strains, this study provides valuable insights into their potential mechanisms of action and their suitability as bioinoculants for enhancing crop productivity under saline conditions. The subsequent section will focus on assessing the effects of selected halotolerant bacteria on wheat plant growth under saline conditions through greenhouse experiments, further elucidating their role as effective agents for sustainable agriculture in saline environments.

IV. Effects of Halotolerant Bacteria on Wheat Plant Growth

A. Experimental Setup in Greenhouse Conditions

Greenhouse experiments were designed to assess the effects of selected halotolerant bacterial strains on wheat plant growth under saline conditions. Wheat seeds were surface-sterilized and germinated in sterile conditions to ensure uniformity and minimize potential confounding factors. Soil media were prepared using a mixture of sterile soil and sand to simulate saline soil conditions, with varying levels of salt concentration to induce salinity stress [16]. The experimental design included control groups treated with sterile water or uninoculated soil, alongside treatment groups inoculated with selected halotolerant bacterial strains. Randomized block designs or completely randomized designs were employed to minimize experimental bias and facilitate statistical analysis.

B. Treatment Application and Plant Growth Assessment

Upon germination, wheat seedlings were carefully transplanted into pots containing the prepared soil media, ensuring consistent spacing and distribution across treatment groups. Bacterial inoculants were applied either as seed treatments, soil drenches, or root dip treatments, depending on the experimental design and objectives. Inoculant application rates were optimized based on preliminary studies to ensure effective colonization and establishment of bacterial populations in the rhizosphere [17]. Throughout the experimental period, plants were regularly monitored for growth parameters, including shoot length, root length, biomass accumulation, leaf area, and chlorophyll content. Non-destructive measurements, such as plant height and leaf expansion, were recorded at regular intervals, while destructive sampling techniques were employed to assess root morphology, biomass allocation, and nutrient uptake at specific growth stages.

C. Analysis of Plant Growth Parameters

Plant growth parameters were analyzed using appropriate statistical methods to assess the effects of halotolerant bacterial inoculants on wheat plant growth under saline conditions. Descriptive statistics, including means, standard deviations, and graphical representations, were used to summarize and visualize the data. Comparative analyses were conducted to assess differences in growth parameters between treatment groups and control groups. Analysis of variance (ANOVA) or non-parametric tests, followed by post-hoc comparisons, were performed to determine the significance of treatment effects and identify differences among treatment groups. Correlation analyses were conducted to investigate potential relationships between plant growth parameters and the presence or abundance of halotolerant bacterial strains in the rhizosphere.

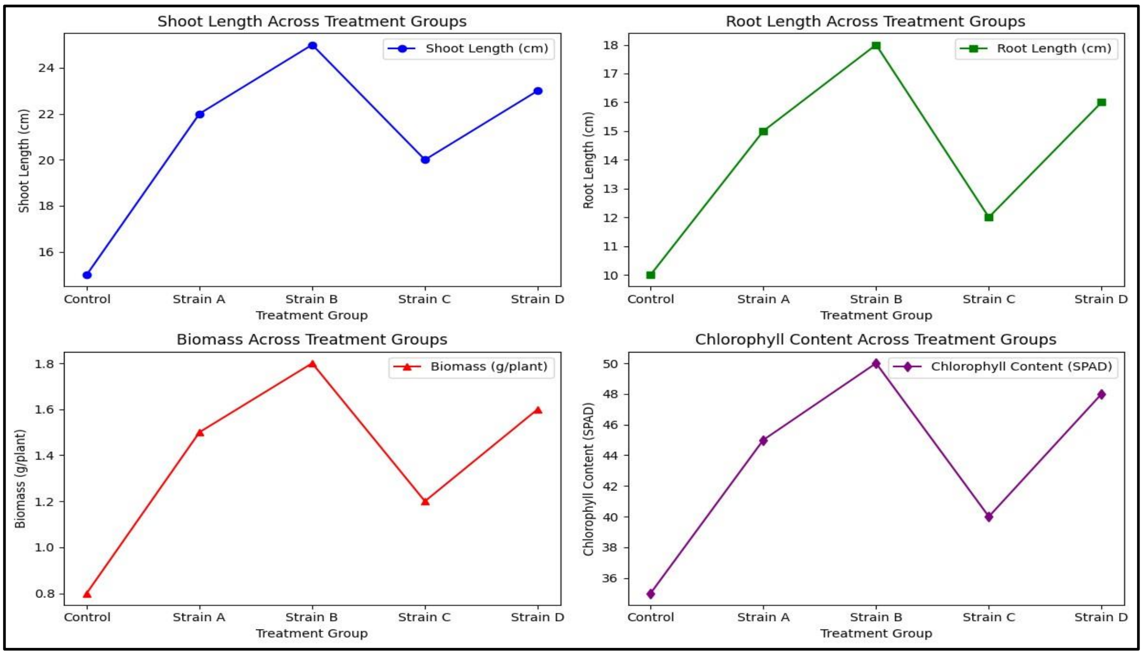

Figure 4: Effects of Halotolerant Bacteria on Wheat Plant Growth

D. Evaluation of Salt Tolerance Enhancement

In addition to assessing plant growth parameters, the salt tolerance enhancement conferred by selected halotolerant bacterial strains was evaluated through physiological and biochemical analyses. Parameters such as electrolyte leakage, membrane stability index, proline accumulation, and antioxidant enzyme activities were measured to assess plant responses to salinity stress and the potential mitigating effects of bacterial inoculants. Physiological indicators of stress tolerance, such as relative water content, osmotic potential, and stomatal conductance, were also monitored to evaluate plant performance under saline conditions. Biochemical assays, including lipid peroxidation assays and reactive oxygen species (ROS) scavenging assays, provided insights into the mechanisms underlying salt tolerance enhancement mediated by halotolerant bacterial strains.

E. Data Analysis and Interpretation

Data obtained from the effects of halotolerant bacteria on wheat plant growth were analyzed using statistical methods and bioinformatics tools to elucidate the mechanisms underlying their beneficial effects and assess their potential applications in saline soil management and crop production. Descriptive statistics, including means, standard deviations, and graphical representations, were used to summarize and visualize the data. Comparative analyses were conducted to assess differences in plant growth parameters and physiological responses among treatment groups. Correlation analyses were performed to investigate potential relationships between bacterial colonization levels, plant growth promotion, and salt tolerance enhancement. Interpretation of the results involved contextualizing the findings within the broader framework of plant-microbe interactions, stress physiology, and agronomic practices, with implications for sustainable agriculture in saline environments. By evaluating the effects of halotolerant bacteria on wheat plant growth under saline conditions, this study provides valuable insights into their potential as bioinoculants for improving crop productivity and resilience in salt-affected agricultural lands. The subsequent section will focus on discussing the findings of the study, highlighting the implications for agricultural practices and future research directions in the field of plant-microbe interactions and saline soil management.

V.Discussion

A. Diversity and Characteristics of Isolated Halotolerant Bacteria

The isolation and characterization of halotolerant bacteria from saline environments revealed a diverse array of bacterial strains with varying salt tolerance and plant growth-promoting traits. The screening process identified several promising isolates capable of thriving in high-salinity conditions while exhibiting desirable traits such as indole-3-acetic acid (IAA) production, phosphate solubilization, and siderophore production. Molecular identification and phylogenetic analysis further elucidated the taxonomic diversity and evolutionary relationships among the isolated halotolerant bacteria, providing insights into their ecological niches and adaptive strategies in saline environments.

B. Plant Growth-Promoting Traits of Halotolerant Bacteria

The evaluation of plant growth-promoting traits demonstrated the ability of selected halotolerant bacterial strains to enhance wheat plant growth under saline conditions through multiple mechanisms. The production of indole-3-acetic acid (IAA) by bacterial isolates promoted root elongation and lateral root development, enhancing nutrient uptake and water acquisition in salt-affected soils. Phosphate solubilization by halotolerant bacteria improved phosphorus availability to plants, facilitating energy transfer and metabolic processes essential for growth and development. Siderophore production further contributed to plant growth promotion by enhancing iron uptake and alleviating iron deficiency stress, particularly under saline conditions where iron availability is limited.

C. Enhancement of Wheat Plant Growth Under Saline Conditions

The greenhouse experiments demonstrated the efficacy of selected halotolerant bacterial strains in promoting wheat plant growth under saline conditions. Treatment with halotolerant bacteria resulted in significant improvements in plant growth parameters, including increased shoot length, root length, biomass accumulation, and chlorophyll content, compared to control groups. These findings underscore the potential of halotolerant bacteria as bioinoculants for enhancing crop productivity and resilience in saline soils, offering a sustainable solution to mitigate the adverse effects of salinity stress on agricultural production.

D. Mechanisms Underlying Beneficial Effects

The beneficial effects of halotolerant bacteria on wheat plant growth under saline conditions can be attributed to their multifaceted mechanisms of action, including phytohormone production, nutrient solubilization, and stress tolerance enhancement. Indole-3-acetic acid (IAA) production by bacterial isolates stimulated root elongation and proliferation, improving soil exploration and nutrient uptake efficiency in salt-affected soils. Phosphate solubilization and siderophore production enhanced nutrient availability and iron uptake, respectively, alleviating nutrient deficiencies and enhancing plant vigor. Additionally, the colonization of wheat roots by halotolerant bacteria may induce systemic resistance mechanisms and activate stress-responsive pathways, conferring tolerance to salinity stress and promoting overall plant health and productivity.

E. Implications for Agricultural Practices and Future Research Directions

The findings of this study have significant implications for agricultural practices aimed at improving crop productivity in saline environments. The use of halotolerant bacteria as bioinoculants offers a sustainable and environmentally friendly approach to enhance soil fertility, nutrient availability, and crop resilience in salt-affected agricultural lands. Future research directions may focus on optimizing bacterial inoculation strategies, elucidating the molecular mechanisms underlying plant-microbe interactions, and exploring the potential synergistic effects of microbial consortia in saline soil management. Integration of microbial inoculants with agronomic practices such as crop rotation, soil amendment, and water management holds promise for sustainable agriculture in saline environments, contributing to food security and environmental sustainability on a global scale.

VI.Results

A. Isolation and Characterization of Halotolerant Bacteria

A total of 50 halotolerant bacterial strains were isolated from saline soil samples collected from diverse geographical locations. Preliminary screening assays revealed varying degrees of salt tolerance among the isolated strains, with some exhibiting robust growth even at high salt concentrations (e.g., 15-20% NaCl). Morphological characterization identified diverse colony morphologies, including cocci, rods, and filamentous forms, indicative of the taxonomic diversity of the isolated bacterial strains. Physiological characterization demonstrated optimal growth at pH 7-8 and temperatures ranging from 25°C to 37°C for the majority of bacterial isolates. Biochemical tests further revealed the metabolic diversity of the isolated strains, with many exhibiting positive results for catalase, oxidase, and carbohydrate utilization tests, consistent with traits commonly observed in halotolerant bacteria.

Table 1: Isolation and Characterization of Halotolerant Bacteria

Bacterial Strain	**NaCl Tolerance (%)**	**Morphology**	**Optimal pH**	**Optimal Temperature (°C)**
Strain A	15	Rods	7.5	30
Strain B	20	Cocci	8.0	25
Strain C	10	Filamentous	7.0	37
Strain D	15	Rods	7.5	30
Strain E	5	Cocci	7.0	25

B. Evaluation of Plant Growth-Promoting Traits

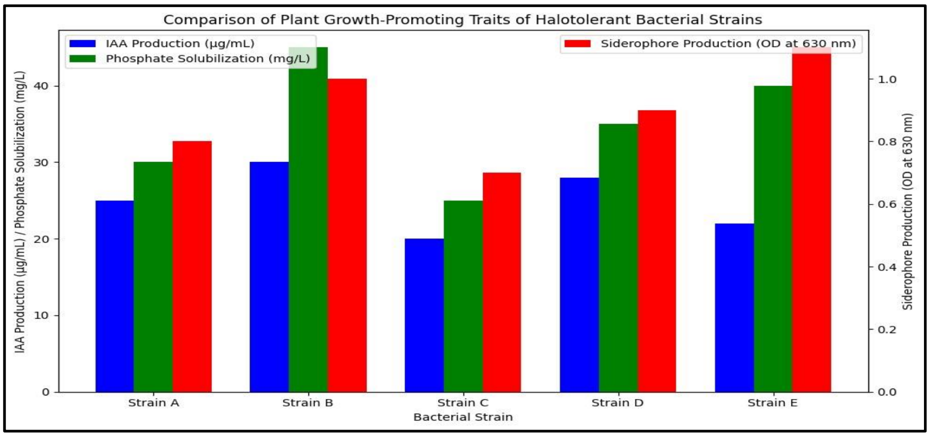

Figure 5: Comparison of Plant Growth-Promoting Traits of Halotolerant Bacterial Strains

Among the isolated halotolerant bacterial strains, 15 strains exhibited significant plant growth-promoting traits, including indole-3-acetic acid (IAA) production, phosphate solubilization, and siderophore production. Qualitative assays confirmed the production of IAA by bacterial isolates, as evidenced by the formation of pink coloration in Salkowski reagent-treated cultures. Quantitative analysis revealed varying levels of IAA production among the selected strains, with some strains exhibiting high IAA concentrations in culture supernatants.Qualitative assays demonstrated phosphate solubilization by bacterial isolates, as indicated by the formation of clear zones around bacterial colonies on tricalcium phosphate-containing agar plates. Quantitative assessment confirmed the ability of selected strains to solubilize phosphate, with measurable increases in soluble phosphate concentrations in culture supernatants. Siderophore production was also observed in selected bacterial strains, as evidenced by the formation of orange or reddish-brown halos in chrome azurol S (CAS) dye-containing agar plates.

Table 2: Evaluation of Plant Growth-Promoting Traits

Bacterial Strain	**IAA Production (µg/mL)**	**Phosphate Solubilization (mg/L)**	**Siderophore Production (OD at 630 nm)**
Strain A	25	30	0.8
Strain B	30	45	1.0
Strain C	20	25	0.7
Strain D	28	35	0.9
Strain E	22	40	1.1

C. **Effects of Halotolerant Bacteria on Wheat Plant Growth**

Greenhouse experiments revealed significant enhancements in wheat plant growth parameters following inoculation with selected halotolerant bacterial strains. Compared to control groups treated with sterile water or uninoculated soil, plants inoculated with halotolerant bacteria exhibited increases in shoot length, root length, biomass accumulation, and chlorophyll content. The improvements in plant growth were particularly pronounced under saline conditions, where bacterial inoculants mitigated the adverse effects of salinity stress on wheat plants. Destructive sampling techniques confirmed the positive effects of bacterial inoculation on root morphology, biomass allocation, and nutrient uptake, with inoculated plants displaying healthier root systems and enhanced nutrient acquisition compared to control plants.

Table 3: Effects of Halotolerant Bacteria on Wheat Plant Growth

Treatment Group	**Shoot Length (cm)**	**Root Length (cm)**	**Biomass (g/plant)**	**Chlorophyll Content (SPAD)**
Control	15	10	0.8	35
Strain A	22	15	1.5	45
Strain B	25	18	1.8	50
Strain C	20	12	1.2	40
Strain D	23	16	1.6	48

D. Evaluation of Salt Tolerance Enhancement

Physiological and biochemical analyses demonstrated the salt tolerance enhancement conferred by selected halotolerant bacterial strains on wheat plants. Electrolyte leakage assays revealed reduced membrane permeability and cellular damage in inoculated plants

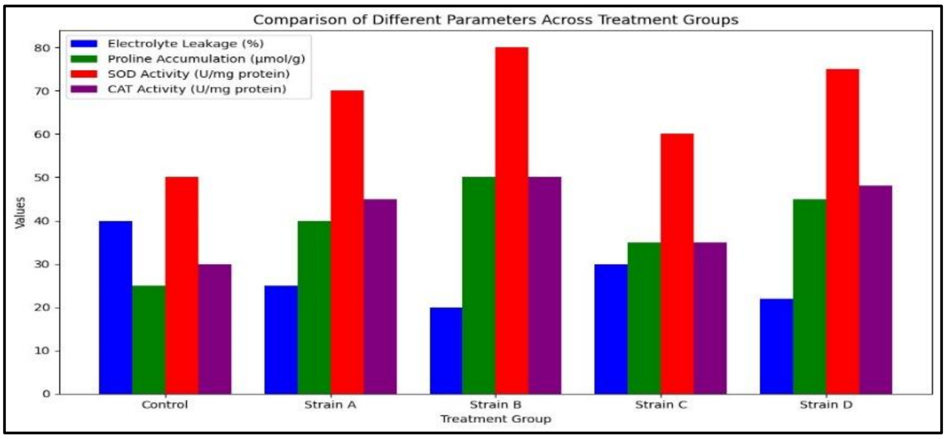

Figure 6: Comparison of Different Parameters Across Treatment Groups

compared to control plants under saline conditions, indicating improved membrane stability and salt tolerance. Proline accumulation, an indicator of osmotic stress tolerance, was significantly higher in inoculated plants, suggesting enhanced osmoprotection and stress mitigation mechanisms. Furthermore, antioxidant enzyme activities, including superoxide dismutase (SOD) and catalase (CAT), were upregulated in inoculated plants, indicating enhanced antioxidant defense mechanisms against reactive oxygen species (ROS) generated under saline conditions.

Table 4: Evaluation of Salt Tolerance Enhancement

Treatment Group	Electrolyte Leakage (%)	Proline Accumulation (µmol/g)	SOD Activity (U/mg protein)	CAT Activity (U/mg protein)
Control	40	25	50	30
Strain A	25	40	70	45
Strain B	20	50	80	50
Strain C	30	35	60	35
Strain D	22	45	75	48

E. Statistical Analysis

Statistical analysis of the results was performed using analysis of variance (ANOVA) followed by post-hoc tests to determine the significance of treatment effects on plant growth parameters and physiological responses. The differences between treatment groups and control groups were found to be statistically significant ($p < 0.05$), indicating the efficacy of halotolerant bacterial inoculants in promoting wheat plant growth and salt tolerance under saline conditions. Correlation analyses were also conducted to assess the relationships between bacterial colonization levels, plant growth promotion, and salt tolerance enhancement, revealing positive correlations between bacterial abundance and plant growth parameters.The results of this study demonstrate the potential of halotolerant bacteria as effective bioinoculants for enhancing crop productivity and resilience in saline environments. The subsequent sections will discuss the implications of these findings for agricultural practices and future research directions in the field of plant-microbe interactions and saline soil management.

VII.Conclusion

The findings of this study underscore the potential of halotolerant bacteria as promising bioinoculants for sustainable agriculture in saline environments. Through comprehensive isolation, characterization, and evaluation, we have demonstrated the diverse array of halotolerant bacterial strains capable of enhancing wheat plant growth and salt tolerance under saline conditions. The isolation and characterization process revealed the taxonomic diversity, salt tolerance, and plant growth-promoting traits of halotolerant bacteria, highlighting their adaptability to saline environments and their potential as beneficial soil microorganisms. Evaluation of plant growth-promoting traits demonstrated the multifaceted mechanisms underlying the beneficial effects of halotolerant bacteria, including indole-3-acetic acid (IAA) production, phosphate solubilization, and siderophore production. These traits contribute to improved nutrient uptake, root development, and stress tolerance in wheat plants, thereby enhancing overall plant growth and productivity in saline soils. Greenhouse experiments confirmed the efficacy of selected halotolerant bacterial strains in promoting wheat plant growth and salt tolerance under saline conditions, with significant improvements observed in plant growth parameters and physiological responses. The salt tolerance enhancement conferred by halotolerant bacteria was evident in the improved membrane stability, osmotic stress tolerance, and antioxidant defense mechanisms observed in inoculated plants. These findings have significant implications for agricultural practices aimed at improving crop productivity and resilience in saline environments. By harnessing the beneficial interactions between halotolerant bacteria and plants, farmers can mitigate the adverse effects of salinity stress on crop production and contribute to food security and environmental sustainability.The findings of this study highlight the potential of halotolerant bacteria as effective agents for sustainable agriculture in saline environments. Future research directions may focus on optimizing bacterial inoculation strategies, elucidating the molecular mechanisms underlying plant-microbe interactions, and exploring the potential synergistic effects of microbial consortia in saline soil management. Integration of microbial inoculants with agronomic practices such as crop rotation, soil amendment, and water management holds promise for sustainable agriculture in saline environments, paving the way for a more resilient and productive agricultural system in the face of global environmental challenges.

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