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Short Communication

Genomic Insights into Salt-Tolerant Plant Growth-Promoting Rhizobacteria from Mangrove Flora: Unlocking Agricultural Potential under Salinity Stress

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Abstract

Soil salinity is a major constraint on crop productivity, particularly in coastal areas of Bangladesh. To unlock microbial solutions, halotolerant plant growth-promoting rhizobacteria (PGPR) were isolated from the rhizosphere of wild plants in the Sundarbans mangrove forest. Among 23 soil samples, isolate 7A1 exhibited the strongest plant growth promoting traits, including phosphate solubilization, indole-3-acetic acid (IAA) production, hydrogen cyanide (HCN) and ammonia release, and nitrogen fixation. Inoculation of rice variety BR-28 with isolates 7A1–7A4 significantly improved seed germination and restored shoot length under salinity stress to levels comparable with non-saline conditions. Biochemical assay and 16s rRNA sequencing identified isolate 7A1 as *Enterobacter hormaechei* ML02, and whole-genome sequencing revealed genes linked to nutrient acquisition, phytohormone biosynthesis, stress mitigation, and heavy metal detoxification. These results demonstrate the potential of *E. hormaechei* ML02 as a salt-tolerant PGPR for enhancing rice growth in saline environments and provide genomic insights into its functional mechanisms and the agricultural potential of salt-tolerant PGPR under salinity stress.

Keywords: Salt-tolerant bacteria, soil salinity, whole-genome sequencing, rhizosphere bacteria, plant growth promotion

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Soil salinity is one of the most destructive forms of soil degradation that markedly diminishes the quality and productivity of agricultural fields [1]. Approximately 20% (45 million hectares) of irrigated land, which generates one-third of the global food supply, is affected by salinity [2]. The geographical position of Bangladesh makes it particularly vulnerable to soil and water salinization. Salinity is now one of the most limiting variables for agricultural productivity in Bangladesh's coastal region, as it varies across a significant portion of the coast, estimated at around 10560 km^2 (out of 16890 km^2) [3]. Mitigating soil salinity or developing salt-tolerant crop varieties are the only options to preserve the food supply; the latter is considered easier. Some wild

plants that usually grow in low-saline soils elsewhere in Bangladesh are also found under salt-stress conditions in the saline regions of the Sundarbans. There might be some special features in the soil, *e.g.*, soil bacteria, that help them to survive in the saline areas. To determine whether any bacteria were involved in promoting plant development in the high-saline region, we examined the rhizosphere soils of the wild plant varieties found in the Sundarbans. Here, we sampled the rhizosphere soil of different wild plants growing in the extreme saline areas of Sundarbans, and measured soil physicochemical properties.

Subsequently, rhizosphere bacteria were grown on TSA media supplemented with various NaCl concentrations, adjusting the EC value from around 5 dS/m to 15 dS/m to screen halotolerant rhizobacteria. We evaluated the PGP activities of the bacterial isolates according to Phosphate solubilization, IAA production, HCN production, NH_3 production, and N_2 fixation [4, 5]. The complete genome studies of the prospective bacteria were also carried out to discern the genomic description for confirming the plant-growth characteristics of the isolated bacteria.

A total of 23 rhizosphere soil samples with electrical conductivity (EC) ranging from 9.1±0.006 dS/m to 16.8±0.03 dS/m were collected in an aseptic jar and cultured in Tryptic Soy Broth (TSB, Himedia, LQ009A) supplemented with 1-15% NaCl. The EC, pH, temperature, and moisture content of the soil were recorded during sample collection. We also noticed a rich growth of the bacteria on TSA media enriched with NaCl, showing that the bacteria were halotolerant. Bacteria growth decreased as the salt content in the TSA media was higher. Most interesting is the occurrence of extremely salt-tolerant rhizosphere bacteria in the rhizosphere soil of Sample 7 that grew on TSA-salt medium containing 15% NaCl (**Figure 1**).

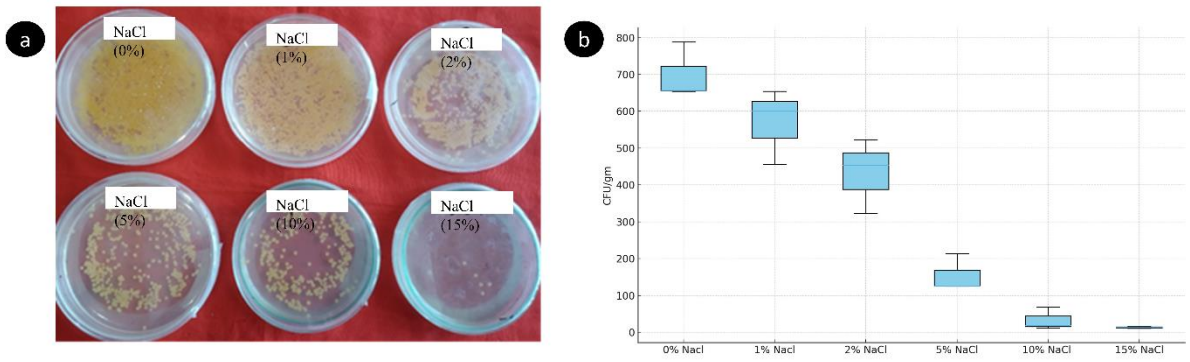

Figure 1. Microbial load analysis at different salt concentrations. (a) Culture of Rhizosphere bacteria at different concentrations. (b) Abundance of Rhizosphere bacteria at different levels of salinity.

We used the bacterial isolates to conduct plant growth-promoting activities, including phosphate solubilization, production of indole-3-acetic acid (IAA), hydrogen cyanide (HCN), and ammonia (NH_3), and biological nitrogen fixation. Among the rhizosphere bacteria isolated from sample 7, several distinct phosphate-solubilizing colonies were observed. Notably, sample 7A yielded the highest number, producing four colonies on Pikovskaya's medium. Of these, colony 7A1 demonstrated the greatest phosphate-solubilizing index, along with the highest production of NH_3, HCN, and IAA. In addition, 7A1 exhibited strong biological nitrogen fixation ability (**Table 1**).

The salt-absorbing capacity of rhizosphere bacteria can help mitigate salt stress in plants. To assess this, we measured the TDS and EC values of the culture media supplemented with NaCl before and after incubation. A reduction in TDS and EC values after incubation indicates that the bacteria absorbed salts from the solution [6]. The bacteria isolated from sample 7A1 showed a noticeable salinity reduction. The strain promoted seed germination under salt stress and enhanced shoot length. Inoculation of rice variety BR-28 with rhizosphere bacterial isolates (7A1, 7A2, 7A3, and 7A4) restored shoot length to levels comparable with those observed under low-salinity conditions.

The strain Isolate 7A1 was identified as *Enterobacter hormaechei* through 16S rRNA sequencing and biochemical tests, and it was subsequently named *E. hormaechei* ML02 according to the lab records. Whole-genome sequencing was performed using an Illumina NextSeq 550 instrument for a genome-wide association study. The genome length of the strain was 4,904,073 base pairs, comprising 225 contigs, with an average coverage of 98.38x and a GC content of 55.45%. The Pokka annotation program predicted 4,524 coding sequences (CDS), while the PATRIC tool identified 4,756 CDS across the 225 contigs. A summary of these characteristics is presented in **Table 2**. Additionally, the PATRIC tool provides insights into

the involvement of these proteins in various biological processes, as shown in **Figure 2**. The draft genome sequence of *Enterobacter hormaechei* ML02 has previously been reported in Microbiology Resource Announcements as a genome announcement [7]. The present study builds upon that genomic resource by integrating phenotypic characterization with genome-wide functional analysis to elucidate the plant growth-promoting and salinity tolerance mechanisms of the strain.

Table 1. Assay for Plant Growth-Promoting Activities of Isolated Rhizosphere Bacteria

Sample ID	Phosphate Solubilizing Index	HCN production	N2 fixation	IAA Production (µg/ml)			NH3 Production (µg/ml)		
				0 minute	30 Minutes	60 Minutes	0 minute	30 Minutes	60 Minutes
7A1	0.028	+	+	1.34	1.72	3.1	3.71	3.86	3.9
7A2	0.012	-	+	0.091	0.112	2.7	3.6	3.61	3.72
7A3	0.015	-	+	1.12	1.23	1.9	3.51	3.54	3.61
7A4	0.011	+	+	1.2	1.29	2.1	3.11	3.23	3.34

Table 2. Genomic Features of *E. hormaechei* ML02

Description of source		
Location	Sundarbans, Bangladesh	
Time	2023	
Type	Rhizosphere Soil	
Sequencing Summary		
Coverage	98.38	
Genome Length	4,904,173 bp	
Number of Contigs	34	
GC Content (%)	55.46	
Annotation Report		
Tool used	**Prokka**	**PATRIC**
Contigs	225	225
Bases	4,904,173	4,904,173
CDs	4525	4,756
mRNA	4588	-
rRNA	4	7
tRNA	58	68
tmRNA	1	-
Proteins Features (PATRIC)		
Hypothetical proteins	583	
Proteins with functional assignments	4173	
Proteins with EC number assignments	1269	
Proteins with GO assignments	1029	
Proteins with Pathway assignments	897	
Proteins with PATRIC genus-specific family (PLfam) assignments	4500	
Proteins with PATRIC cross-genus family (PGfam) assignments	4585	
Special Gene (PATRIC)		
Antibiotic Resistance	PATRIC	59
	NDARO	2
	CARD	56
Virulence Factor	VFDB	31
	Victors	153
	PATRIC_VF	128
Transporter	TCDB	582
Drug Target	DrugBank	310
	TTD	55

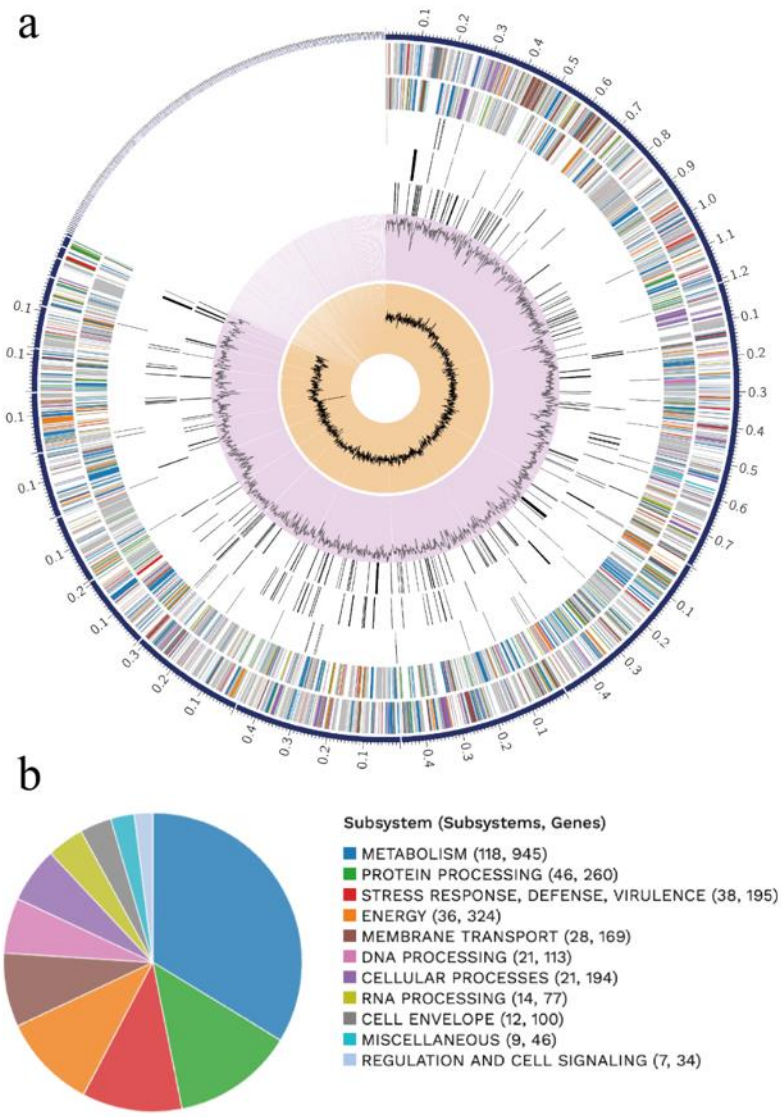

Figure 2. PATRIC annotation and subsystem analysis. (a) A circular graphical display of the distribution of the genome annotations. This includes, from outer to inner rings, the contigs, CDS on the forward strand, CDS on the reverse strand, RNA genes, CDS with homology to known antimicrobial resistance genes, CDS with homology to known virulence factors, GC content and GC skew. The colors of the CDS on the forward and reverse strands indicate the subsystem that these genes belong to (see Subsystems below). (b) Subsystem of the PATRIC annotation.

We performed an extensive analysis of the genome to identify genes associated with various plant growth-promoting activities, such as phosphate solubilization, IAA production, NH3 and HCN production, and biological nitrogen fixation. The presence of plant growth-promoting genes in *E. hormaechei* ML02 was predicted using the PGPg_finder tool [8]. Several genes related to nitrogen fixation, phytohormone production, phosphate solubilization, nitrogen acquisition, and salinity stress neutralization were identified, which confirmed the *in vitro* results obtained earlier. In addition to the genetic characterization of plant growth-promoting (PGP) activities such as phosphate solubilization, salinity stress mitigation, and the production of IAA, NH_3, and nitrogen fixation, whole-genome sequencing also revealed genetic features associated with heavy metal detoxification, biofertilization, siderophore production, seed germination stimulation, and surface attachment (**Table 3**).

In this study, *E. hormaechei* ML02 was identified from 23 soil samples based on in vitro assays of salt tolerance and plant growth-promoting activity. This strain was found to enhance seed germination and promote shoot length growth under salt stress conditions. Another notable characteristic of *E. hormaechei* ML02 is its ability to accumulate salt. Whole-genome sequencing *E. hormaechei* ML02 revealed various genomic traits associated with plant growth promotion under

Table 3. Plant growth-promoting genes in *E. hormaechei* ML02

Feature	Gene	Function
N_2 Fixation	nifA, anfA, vnfA, nifF, fldA, isiB, nifM, nifU, iscU, nifV, nifJ, nifS.	Nitrogenase Biosynthesis
	hyfA, hyfB, hyfC, hyfG, hyfH, hyfI, hyfJ, hyfR, hyaD, hybD, hupD, hypA, hybF, hypB, hupJ, hypC, hupF, hypD.	Hydrogenase Biosynthesis
Phytohormone Production	aldB, iaaT, yedL, ysnE, betB_homologous, ipdC, ppdC, aspC, bam, iaaH, aux2, trpA, trpB, trpCF, trpD, trpE, phnA, trpDG, trpS, solA, trpR, lysN, puuE, patA1, mtr.	IAA Production
Phosphate Solubilization	aphA, ppx, ppx_gppA, ppa, phy, phyA, phyB, phyC, agpP, agp.	Phosphatase Activity
	ppk, ppk2, phoR, phoB, yciG, ymdF, gsiB, phoH, phoE, pstA, pstB, phoT, pstC, phoW, pstS, phoS, phoU, phoY, pitA, pit, yjbB.	Phosphate Metabolism
Nitrogen Acquisition	nark, nrtP, nrt, narU, nasR, nasT, nirB, nirD, narJ, narW, narG, narZ, nxrA, narX, norR, norV, norW, nasA, nasC, narB, nosX, apbE, yojL, fmnB, narH.	Nitrate Reduction
	nitA, nitB, nitR, nit1, exoR, amtB, ybaG, amt, gltP, gltT, gltI, aatJ, gltK, aatM, gltJ, aatQ, gltL, aatP, ntrA, rpoN, gltS, TC_AAT, yifK, gltB, gltD, glnA, glmE, mute, mamB, glnB, glnY, glnE, glnD, glnH, glnK, glnZ, ntrB, glnL, ntrC, glnG, gdhA, glnP, glnQ.	Ammonium Assimilation
Salinity Stress Neutralization	degQ, hhoA	Salinity Stress Signaling
	yloB, ctpA, TMEM165, gdt1, chaA.	Calcium Transport
	chaB, brnQ, ybaL, yrbG, yflA, yihO, xynP.	Cation Transport
	asd, doeB, doeX, ectB, dat, lysC.	Ectoine Metabolism
	araE, ydfJ, ydjE, galP.	H+ Symporter
	atpC, atpD, atpG, atpA, atpH, atpF, atpE, atpB, lon, rpmEB, ydiY, rseA, surA, ycaD, spoT, mtnN, pfs, yadA, prkA, yeaG, smpB, tatB, acrR1, rspB, atpI.	Halotolerance Related Enzymes
	mgtE, corA, yfjQ	Magnesium Transport
	proA, proB, argHA, argAB, argO, argB, argC, argE, proC, proY.	Proline Metabolism
	panS, yocS, ybaS, nhaR, nhaK, TC_CPA1, nhaA, nhaB, TC_SSS yerK, opuE, putP, ycgO, nqrA.	Sodium Transport
	SPP_like, scrY, scrA, sacP, sacX, ptsS.	Sucrose Metabolism
	treB, crr, treC, glvA, treR, treS, treY, glgY, treZ, glgZ, otsA, otsB, malK, mtlK, thuK, treA, treF, ISA, treX.	Trehalose Metabolism
Siderophore production	speF, alcR, iucB.	Iron Acquisition
Seed germination stimulation	folE, folE2, nudB, folB, folk, folP, folA, folC, ribA, pabA, pabB, pabAB, pabC, folX, folM	Plant Germination stimulation
Colonizing plant System	lapF, ompA, ompF, sadA, btaF, bmaC, ompW, lapB-K, lapE, bcsA, bcsC, mlrA, csgA	Colonization Surface Attachment
Bio-Fertilization	cbbR, cmpR, cooF, cynT, cah-K, hcaT	CO_2 Fixation
	hutA, feoA, feoB, feoC, efeU, efeO, efeB, afuA, afuC, tonB, exbB, exbD	Iron Acquisition
Heavy Metal Detoxification	arsB, arsC1, aqpZ, glpF	Arsenic Resistance
	chrC, chrR	Chromate Resistance
	cobQ, cbiE, cobA, corC, apaG, btuC, btuD, btuF	Cobalt Resistance
	ddpA, ddpD, ddpF, ddpB, ddpC, rcnB, hoxN, dppA, dppB	Nikel Resistance
	selA, selD, selB, selU, ynfE, ynfH, dedA	Selenium Resistance

salt stress. Soils enriched with such plant growth-promoting rhizobacteria (PGPR) can boost crop production in saline environments. Therefore, genomic studies can provide valuable insights into soil quality by analyzing the PGPR community. Metagenomic analysis of rhizosphere soil will serve as a crucial parameter for agricultural practices in the coastal regions of Bangladesh.

Data Availability: The Whole-Genome Shotgun sequence for this study is available in GenBank under accession number JBJXVC000000000.1 (https://www.ncbi.nlm.nih.gov/nuccore/JBJXVC000000000.1). The raw sequencing reads are deposited in the SRA database under accession SRR31360097 (https://www.ncbi.nlm.nih.gov/sra/SRR31360097). Both datasets are linked to BioSample number SAMN44754504 (https://www.ncbi.nlm.nih.gov/biosample/SAMN44754504) and BioProject number PRJNA1186382 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1186382). The assembled genome is readily available under the accession number ASM4624445v1 (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_046244455.1). The draft genome announcement is available at Microbiology Resource Announcements (https://doi.org/10.1128/mra.00024-26).

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Conflict of Interest: The authors declare no conflicts of interest.

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