



## RESEARCH ARTICLE

### Drought-Tolerant Landrace Rice Norungan Harbors Novel Putative Competitive Endophytes

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#### ABSTRACT

The landraces and wild species wisely choose their microbiome and explored them as a second genome for nutrient acquisition from the limited resources of the soil ecosystem and can withstand drought and diseases. Hence, the present study aimed to explore the drought-tolerant rice landrace of Tamil Nadu for the putative competitive endophytes to develop a plant-growth-promoting endophytic bacterial inoculant. Twenty two putative competitive endophytes were authenticated from plant tissues and seeds of Norungan. The 16S rRNA gene sequencing revealed that Norungan rice was colonized predominantly by Firmicutes, including six species of *Bacillus*, four species of *Priestia*, and one species each of *Micrococcus*, *Geobacillus*, and *Lysinibacillus*, along with two Proteobacteria, *Enterobacter cloacae* and *Kosakonia oryzae*. These endophytes were screened for nutrient transformation, growth hormone production, drought mitigation, and antagonism against plant pathogens. *Bacillus cereus* NE07, *B. paralicheniformis* NE18, *B. subtilis* NE20, and *Priestia flexa* NE09 showed potential nutrient-transforming capability, while *Bacillus cereus* NE07 and *Priestia flexa* NE09 showed high plant growth hormones (indole-3-acetic acid and gibberellic acid production). Similarly, *Bacillus cereus* NE07 and *Priestia endophytica* (NE14) showed high ACC deaminase, proline accumulation, biofilm production,

and potential candidates for drought mitigation. *Bacillus hayneshii* NE04 and *Priestia aryabhatai* NE10, and *Bacillus cereus* NE07 showed antagonism against fungal pathogen *Rhizoctonia solani* and bacterial leaf blight causing *Xanthomonas oryzae*. The results confirmed that no single strain with all plant-growth-promoting and stress-mitigating traits found in Norungan, and these strains were performed division of labor basis. Hence, developing a putative competitive endophytic bacterial consortium with compatible strains representing cumulative beneficial traits as inoculants would be a novel approach for improving rice productivity.

**Keywords:** *Drought mitigation; Norungan; PGPR; Putative competitive endophytes; Rice landrace*

DRAFT

## INTRODUCTION

Rice is a vital food crop of India, being cultivated by nearly 45 million hectares of land with a total grain production of almost 178 million tonnes in 2021. Rice is one of the major fertilizer-consuming crops, accounted nearly 25% of annual fertilizer consumption (32.54 million tonnes in 2021). On average, one hectare of rice consumes 175 kg of fertilizers (N, P, and K) with 30% efficiency (Bora, 2022). The green revolution achieved rice productivity through high-yielding varieties and intensive fertilizer use. Consequently, the continuous overdose of imbalanced fertilizers affects the soil ecosystems, environment, and human health (Wang and Zhao, 2019). Recent IRRI reports alarm that the rice yield may drop 10% in South Asian countries during the next season of 2022 due to increased fertilizer costs. The farmers reduce their fertilizer use by 10-25%, affecting productivity. Next to fertilizer-based nutrient constraints, drought threatens the Indian rice production system. The mean yield loss of rice due to drought is 25.4% in India, and the yield loss varies depending on the crop stage (Zhang *et al.*, 2018). The third threat to rice production in India is yield losses due to soil-borne diseases. Several minor diseases have recently become major diseases (Shivappa *et al.*, 2021). Increasing or sustaining rice productivity is challenging in the future, and several efforts in crop improvement, management, and protection strategies are being intensified to improve productivity. The plant microbiome is considered the second genome of the plant, which the host plant is recruiting from the surrounding environment to improve its growth and resiliency (de Souza *et al.*, 2020). Hence, exploring the plant microbiome for nutrient acquisition, drought mitigation, and disease management has a potential future for agricultural sustainability.

Endophytes are among the microbiome communities associated with the plant. They colonize the internal tissues of the root, and shoot (El-Tarabily *et al.*, 2021). The crop's wild types, landraces, and ecotypes smartly choose their microbial partners through co-evolution processes (Pozo *et al.*, 2021). In contrast, modern cultivars and hybrids lose their partner due to breeding, agronomical, or management strategies. Consequently, the high-yielding cultivars depend heavily on external resources/inputs (chemical fertilizers, pesticides, etc.), while the landraces manage their needs through their microbial partners (Ray *et al.*, 2020). Hence, exploring the microbiome of native landraces, wild types, and ecotypes and applying them as inoculant for high-yielding cultivars would be a novel and sustainable approach to improving crop growth and fitness.

Norungan is the potential landrace of Tamil Nadu known for its drought resistance and profuse growth in nutritionally limited soil conditions (Chandra Babu *et al.*, 2001; Suji *et al.*, 2012). It is the principal donor of drought-resistant genes for most established modern rice varieties (Divya 2020). Norungan has high zinc and iron accumulation (Anuradha *et al.*, 2012). It is well known for its deep penetrating roots associated with plant production traits (Rajurkar *et al.*, 2021). Hence, exploring Norungan's endophyte as an inoculant for rice crop nutrition, drought mitigation, and disease management would be a viable approach. We hypothesize that the landrace Norungan harbors efficient endophytes through the co-evolution process for multi-faceted beneficial traits. In the current work, we assessed the diversity and plant-growth promoting potentials of cultivable, putative competitive endophytes of the Norungan landrace of rice.

## MATERIAL AND METHODS

### Isolation and characterization of Norungan endophytes

The drought-resistant landrace rice, Norungan, was used to isolate the bacterial endophytes. The crop was grown in Wetland farm soil (latitude – N 11°0' 7.315", longitude E 76°55'34.717", altitude 436 m) at Tamil Nadu Agricultural University under natural conditions. Endophytic bacteria were isolated from 25-day old plants using standard serial dilution and plating method (Gyaneshwar *et al.*,2001). The rice plant was uprooted from the soil without damage and washed several times with sterile water until free from the soil. Surface-sterilization was performed with sodium hypochlorite (3% available chlorine), 70% ethanol, and three rinsing with sterile water. The efficiency of contamination was tested by inoculating 1 mL of the last rinsed water on 10-mLmL of nutrient broth and observing the presence or absence of bacterial growth. The shoot and root portions of samples were separated and macerated with phosphate buffer and plated on a nutrient agar medium at 10<sup>-3</sup> dilution. The plates incubated at 30 °C were observed for bacterial colonies. The unique colonies on nutrient agar plates were purified by the streak plate method. For isolation of seed endophytes, the Norungan rice seeds were dehusked and adopted the same procedure.

All the isolates were authenticated for putative competitive endophytes using re-isolation and DNA fingerprinting assay (Nunna and Balachandar, 2022). This step eliminated the opportunistic and passenger endophytes from the putative competitive endophytes. The authenticated isolates were stored at -20 °C until further use.

### **Phylogeny of Norungan endophytes**

All the putative competitive endophytes were identified using 16S rRNA gene sequencing (Weisburg *et al.*,1991). The genomic DNA was extracted from the strains using the CTAB method as described earlier by (Kirby, 1997). The 16S rRNA gene was amplified by PCR using FD1 and RP2 primers (Weisburg *et al.*,1991). The full-length 16S rRNA gene was sequenced by Sanger's sequencing method in an automated sequencer (Applied Biosystems). The 16S rRNA gene sequences were further searched for the closest similarity in the GenBank database through NCBI-BLAST [<http://www.ncbi.nih.gov/BLAST>]. All the sequences were deposited in GenBank with accession numbers: plant endophytes (OM900148-57; ON413730-37), seed endophytes (OM413738-41).

### **Plant-growth promoting traits**

All the putative competitive endophytic strains were tested for plant growth-promoting traits, including nitrogen fixation as ammonia excretion in an N-free medium (Solorzano 1969), in vitro mineral-solubilizing efficiency for phosphorus (PS) (Nautiyal 1999), potassium (KS) (Aleksandrov *et al.*,1967), and zinc (Bunt and Rovira 1955), growth hormones, viz., indole-3-acetic acid (Gordon and Weber 1951), and gibberellin (Holbrook *et al.*,1961), drought-mitigating traits such as proline accumulation (Bates *et al.*,1973), 1-aminocyclopropane-1-carboxylic acid deaminase activity (Devi *et al.*,2018) and biofilm formation (Pierce *et al.*,2008; O'Toole 2011), and antagonistic activity against *Xanthomonas oryzae* and *Rhizoctonia solania* (Dennis and Webster 1971). Standard strains were used as a positive control for each trait.

### **Statistical analysis**

R software (Version 4.1.1) (R Core Team, Vienna, Austria) was used for all the statistical analyses of data. Analysis of variance (ANOVA) followed by the Tukey test (p<0.05) was performed to determine the significant difference among strains. Principal component analysis (PCA) was performed for all the assessed variables of strains using the princomp

function of the factoextra-package of R. The PCA biplot, contribution plot, and eigenvalues corresponding to the variation explained by each principal component were visualized using the viz function of factoextra. The correlation between the soil attributes of each experimental site was assessed based on Pearson's correlation and visualized through the Corrplot-package of R software.

## RESULTS AND DISCUSSION

### *Phylogeny of putative competitive endophytes of Norungan*

Twenty-seven plant endophytes and four seed endophytes were isolated and characterized from Norungan rice plant and seed. The re-isolation and DNA fingerprinting method (Nunna and Balachandar 2022) could authenticate eighteen plant-associated and four seed-associated isolates as putative competitive endophytes. The remaining were declared unworthy opportunistic and passenger endophytes (Data not shown). Except for two, all putative competitive endophytes of Norungan are Firmicutes (Gram Positive rods). *Enterobacter cloacae* (NE23) from rice tissues and *Kosakonia oryzae* (NSE2) from seed are the only Proteobacteria (Gram Negative rods) reported in the Norungan landrace. Twelve strains belong to the genus *Bacillus*, representing *B. subtilis*, *B. pumilus*, *B. haynesii*, *B. cereus*, *B. amyloliquefaciens*, and *B. paralicheniformis*, four species of *Priestia* such as *P. flexa*, *P. aryabhatai*, *P. endophytica*, and *P. megaterium*. *Micrococcus luteus*, *Geobacillus thermodenitrificans* and *Lysinibacillus fusiformis* represent one strain each.

In the present work, the landrace Norungan harbored relatively higher proportions of Gram Positives rather than Gram Negatives. Borah *et al.* (2021) compared the endophytes of wild and cultivated rice and reported that wild rice (*Oryza rufipogon*) had less diversified bacterial species than cultivated rice (*Oryza sativa*). In wild rice, *Bacillus*, *Microbacterium*, *Brevibacillus*, *Lysinibacillus*, and *Staphylococcus* were predominant, while the cultivated rice is colonized by *Bacillus*, *Stenotrophomonas*, *Microbacterium*, *Brucella*, and *Proteus*. Similarly, Liu *et al.* (2022) reported the Proteobacteria, Firmicutes, and Actinobacteria as endophytes of wild rice. Kumar *et al.* (2020) reported both Gram Positive and Gram Negative bacteria in cultivated rice.

In contrast, Chaudhry *et al.* (2017) obtained 21 Gram Positives (*Bacillus* and *Streptococcus*) from Pusa Basmathi rice. Hence, the choice of endophyte is dictated mainly by the crop's genotype, followed by the soil microbiome and environmental factors. Besides these, several reports confirmed that Bacilli are the rapid, efficient, and persistent endophytic colonizers of the host plants (Gond *et al.*, 2015; Tamošiūnė *et al.*, 2018; Zhao *et al.*, 2014). The predominance of Gram Positive endophytes in the Norungan landrace might be that Gram positives are more tolerant to environmental stresses like drought, salinity, and nutrient scarceness than Gram Negatives. Hence, Norungan might choose its microbial partner for mitigating biotic and abiotic stresses.

### *Plant growth-promoting traits*

The putative competitive endophytes are known for their plant-growth-promoting traits, including macro and micronutrient supply, growth hormone production, drought mitigation, and disease suppression (Vurukonda *et al.*, 2016). The quantitative assessment of plant-growth-promoting traits revealed that Norungan endophytes are functionally diversified. Some novel strains can be used as inoculants for rice growth and fitness.

### *Plant nutrients*

The biological nitrogen fixation and mineral solubilization are two key principle strategies the plant-growth-promoting bacteria adopted to supply macronutrients such as nitrogen, phosphorus, potassium, and micronutrient zinc (Sammauria *et al.*,2009). Out of 24 putative competitive endophytes, five strains (NE07, NE09, NE18, NE20, and NE23) reported higher ammonia excretion in the N-free medium than the standard strain (*Azospirillum* Sp7). In contrast, the others accounted for insignificant quantities of ammonia in the medium (Fig. 1A). The phosphorus solubilization of Norungan endophytes is significantly lower than the standard strain (*Bacillus megaterium* Pb1), and the plant-endophytes, NE16, NE07, NE14, NE02, and NE24 were showing relatively higher solubilization index than other strains. None of the seed endophytes showed significant P solubilization (Fig. 1B). Six plant endophytes (NE08, NE09, NE18, NE20, NE23, NE24) and two seed endophytes (NSE1, NSE3) solubilized potassium silicate present in the medium and released the potassium efficiently but lower than the standard strain (*Paenibacillus mucilaginosus*, KRB9). Among the endophytes, NE20, followed by NE24 and NE23, recorded higher K release than the rest of the strains (Fig. 1C), (NE04, NE14, NE23, NSE2, NSE3). Most Norungan endophytes except NE04, NE14, NE23, NSE2, and NSE3 showed positive Zn solubilization but lower than standard strain (*Pseudomonas chlororaphis* ZSB15). The strain NE07 followed NE21, and NE18 had a higher Zn solubilization index than others (Fig. 1D). Endophytic bacteria are well-known for their nutrient transforming ability to make them available to the colonizing host plants. The present results confirmed that the endophytes have traits to effectively provide nitrogen, phosphorus, potassium, and zinc to the host plant through biological nitrogen fixation and mineral solubilization. Several reports on rice endophytes are in support of the present findings. (Ji *et al.*,2014) reported *Paenibacillus*, *Microbacterium*, and *Bacillus* as potential nitrogen-fixing and mineral solubilizing rice endophytes. Borah *et al.* (2021) screened 70 rice endophytes and found *Bacillus tequilensis*, *Microbacterium tesaceum*, and *M. enclense* as potential nitrogen-fixing and mineral solubilizing (P and K) endophytes. Abd El-Mageed *et al.* (2022) found that out of 49 rice endophytes, 16 strains were potential nitrogen-fixers and 36 strains mineral solubilizers. Only a few shared the multiple nutrient-providing traits and authenticated *B. megaterium* and *B. subtilis* as potential nutrient-transforming (N, P and K) rice endophytes. In the present work, we identified *Bacillus cereus* (NE07), *B. paralicheniformis* (NE18), *B. subtilis* (NE20), and *Priestia flexa* (NE09) as the potential nutrient-transforming endophytes present in Norungan landrace.

#### Growth hormones

Eleven strains produced significantly higher IAA in the tryptophan medium than the standard strain (*Pseudomonas chlororaphis* ZSB15). The best strain, NE12, and NE23, had 18.6 and 18.2 µg/mL of IAA, respectively, which are five-fold higher than the standard strain. The other potential IAA-producing strains are NE14, NE04, NE16, and NE21 (Fig. 2A). Most plant-growth-promoting bacteria utilize the plant-derived tryptophan as the precursor and transform into common auxin, indole-3-acetic acid (Spaepen *et al.*,2007). The exogenous and endogenous IAA accumulation controls several plant physiological functions, including lateral root elongation, and increased root hair formation (Rehman *et al.*,2018). *Lysinibacillus fusiformis* NE12 and *Enterobacter cloacae* NE24 were identified as potential tryptophan-dependent IAA producers in the present work. The similar IAA-producing rice endophytes reported earlier were *Serratia marcescens* (Ji *et al.*,2014), *Ensifer garamanticus* (Cardinale *et al.*,2015), *Microbacterium laevaniformis* (Borah *et al.*,2021), *Bacillus megaterium* (Abd El-Mageed *et al.*,2022), *Flavobacterium* sp. (Walitang *et al.*,2017), *Herbaspirillum seropedicae*, *Azorhizobium caulinodans*, *Rhizobium daejeonense* (Liu *et al.*,2022). One of the best

strains, *Enterobacter cloacae* (NE24), reported in the present work, is well-known for IAA synthesis (Coulson and Patten 2015).

Six endophytic strains such as NE09, NE07, NE04, NE12, NE01, and NSE01 are the high GA producers, which reported significantly higher than standard strain (ZSB15) (Fig. 2B). NE09 reported 7.9 µg/mL of GA, six-folds higher than standard strain. NE07 produced 7.3 µg/mL of GA, while the third best strain, NE04, accumulated 6.6 µg/mL of GA. GA is an essential phytohormone needed in the early stage of the crop for seed germination and nutrient mobilization of young plants and stem elongation (Hou et al.,2013). The rice endophytes reported as potential GA producers so far include *Pantoea agglomerans* (Feng et al.,2006), *Bacillus amyloliquefaciens* (Shahzad et al.,2016), and *Microbacterium laevaniformis* (Borah et al.,2021). In the present work, we identified *Bacillus cereus* (NE07) and *Priestia flexa* (NE09) as potential GA-producing endophytes from rice.

### *Drought-mitigation*

All the twenty-two putative competitive endophytes were assessed for their drought-mitigating traits such as ACC deaminase, proline accumulation, and biofilm formation. The rhizosphere and root endophytic microorganisms produce ACCD to decompose the ACC, the precursor for acetylene, into ammonia and α-ketobutyrate to avoid ethylene-based injuries during drought conditions (Glick et al.,2007; Honma and Shimomura 1978). Hence, the putative competitive endophyte with ACC deaminase could be a desirable microbial resource for drought mitigation. NE07 showed significantly higher ACC deaminase activity (37%) than the standard strain (*Bacillus altitudinis* FD48). NE14, NE21, and NE09 are high ACCD strains (Fig. 3A). The potential ACCD positive endophytes reported in rice so far include *Methylobacterium oryzae* (Chinnadurai et al.,2009), *Bacillus altitudinis* (Kumar et al.,2017), *Pseudomonas fluorescens* (Etesami et al.,2014); *Bradyrhizobium* sp. (Sarapat et al.,2020), *Brevibacterium linens* (Choi et al.,2022), *Bacillus amyloliquefaciens* (Liu et al.,2022). In the present work, we reported *Bacillus cereus* (NE07) and *Priestia endophytica* (NE14) as potential ACC deaminase-producing endophytes of Norungan. Most of the drought-mitigating plant-growth promoting rhizobacteria accumulate proline as compatible solutes to cope with drought and salinity stress. Upon inoculation, these PGPR could improve the host plant's proline, glycine betaine, and trehalose, thereby conferring a stress tolerance (García et al.,2017). In the present work, five endophytes showed significantly high proline (300-400 µg/mL) accumulation, and one strain, NE16 (*Bacillus subtilis*), had substantially higher than the standard strain (Fig. 3B). *Priestia endophytica* (NE14) was another potential proline-accumulating strain reported.

Biofilm formation is another significantly important trait of PGPR to augment the drought mitigation of the host plant. The biofilm protects the cells against stress conditions, and the exopolysaccharide produced during biofilm is a potential reservoir for nutrients and water. Thus, the PGPR-biofilm will also help plant drought stress management (Ansari and Ahmad 2018; Ansari et al.,2021). In the present work, NE09 (*Priestia flexa*), NE18 (*Bacillus paralicheniformis*), NE01 (*Bacillus subtilis*), and NE07 (*Bacillus cereus*) accounted high biofilm under *in vitro* conditions (Fig. 3C) and these strains could be potential candidates for drought and salinity tolerance.

### *Biocontrol activity*

In the present study, most of the Norungan endophytes being gram Positive had a high impact in exhibiting antagonistic activity against fungal pathogen *Rhizoctonia solani* and



bacterial leaf blight causing *Xanthomonas oryzae* pv. *oryzae* (Xoo). However, all these strains showed lower antagonistic activity than the positive biocontrol agent (*Bacillus subtilis* TNAU1). *Bacillus hayneshii* NE04 and *Priestia aryabhatai* NE10 showed 62% inhibition, while the standard strain had nearly 100% (Fig. 4A). Except few (NE01, NE05, NE24, NSE2, NSE4), all strains were antagonists against *Rhizoctonia* but relatively lower than standard strain (Fig. 4B). In the recent past, several rice endophytes were reported as antagonists against Xoo, which include *Pseudomonas putida*, and *Metarrhizium anisophile* (Saikia and Bora 2021), *Azospirillum* (Yasuda et al.,2009), *Paenibacillus polymyxa* (Choi et al.,2022). The antagonists against *Rhizoctonia* reported were *Bacillus subtilis*, *B. nakamurai* (Wang et al.,2022), and *Lysinibacillus sphaericus* (Shabanamol et al.,2021). Our findings are following the earlier reports that endophytic Firmicutes are potential antagonists against rice bacterial and fungal pathogens.

### Relativeness of PGP traits

The principal component analysis (PCA) identified the relation between Norungan endophytes (NE01-NSE4) and their plant growth traits. The PCA biplot with two principal components (Dim1 and Dim2) depicted the orthogonal positions of the Norungan endophytes and their plant growth-promoting traits (Fig.5A). Dim1 and Dim2 contributed to 35.3% and 18.3% variability, respectively (Fig. 5B). Among the tested traits, the ACCD and proline had a high loading value (18.3%) and significantly contributed to the Dim1. The mineral solubilization efficiency in excretion of N, PS, KS, and ZNS traits contributed to 26.8% majorly to the Dim1 (Fig. 5C). All the seed endophytes (NSE1 to NSE4) were positioned in the negative quadrant of the PCA plot, while the potential plant endophytes (NE20, NE18, NE09, NE21, NE07, NE14) were in the positive quadrant. The PCA identified the potential putative competitive endophytes of Norungan, such as *Priestia endophytica* (NE21), *Bacillus cereus* (NE07), and NE14 *Priestia endophytica* (NE14), *Priestia flexa* (NE09), *Bacillus paralicheniformis* (NE18), and *Bacillus subtilis* (NE20).

Pearson correlation coefficient analyses determined pairwise relativeness of the plant growth-promoting traits of Norungan endophytes (Fig. 6). The correlogram was created with a scale of -1 to 1 (red to blue). Among the plant growth hormones, gibberellic acid and IAA were positively correlated. The *Rhizoctonia* antagonism had the highest positive impact on other growth traits, viz., phosphate, potassium, and zinc solubilization, followed by ACC deaminase activity, proline production, and antagonistic activity of *Xanthomonas*. ACCD is positively correlated with mineral solubilization, ammonia excretion, and biofilm formation. None of the traits had a negative impact on any other plant growth-promoting trait.

### CONCLUSION

One of the potential drought-tolerant rice landraces, Norungan harbors novel diversified Firmicutes as its putative competitive endophytes. These include species of *Bacillus*, *Priestia*, *Micrococcus*, *Geobacillus*, and *Lysinibacillus*. All these endophytes were preferentially selected by Norungan rice for their well-adopted growth and fitness in marginal soil ecosystems and stress environments. Additionally, these endophytes offer high nutrient availability, plant growth hormones, drought mitigation, and disease management to the host plant on a division of labor basis. They could be a potential inoculant with specific functioning or a consortium of compatible strains to augment rice's overall fitness and productivity.



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## Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

## Originality and plagiarism

Authors should ensure that they have written and submit only entirely original works, and if they have used the work and/or words of others, that this has been appropriately cited. Plagiarism in all its forms constitutes unethical publishing behavior and is unacceptable.

## Consent for publication

All the authors agreed to publish the content.

## Competing interests

There were no conflicts of interest in the publication of this content

## Data availability

All the data were included in the manuscript. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through the corresponding official mail; dbalu@tnau.ac.in

## Author contributions

The experiments were conceived and designed by NSA and DB. The samples were processed, and the experiments were performed by NSA. Critical analyses of the data were done by NSA and DB. The manuscript was prepared by NSA and DB.

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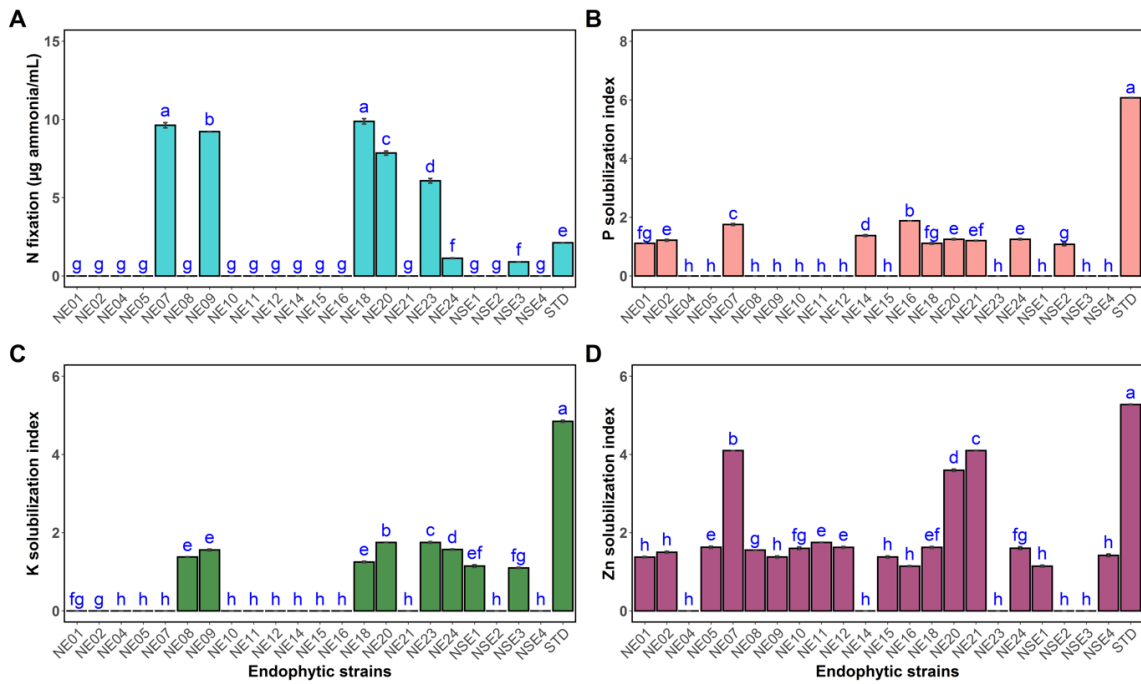
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**Table 1. Taxonomic affiliation of putative competitive endophytes of Norungan landrace**

Strain	Species affiliation	16S rRNA gene length (bp)	Closest strain	Sequence homology (%)	NCBI accession number
NE01	<i>Bacillus subtilis</i>	1418	MN227490	99.93	OM900148
NE02	<i>Bacillus pumilus</i>	1434	EE107-P5	100	ON413730
NE04	<i>Bacillus haynesii</i>	1419	19025311L2	100	OM900149
NE05	<i>Bacillus vallismortis</i>	1167	ER13	100	ON413731
NE07	<i>Bacillus cereus</i>	1413	GL2	100	OM900150
NE08	<i>Micrococcus luteus</i>	1325	BPY	100	ON413732
NE09	<i>Priestia flexa</i>	1454	L3C1L	100	OM900151
NE10	<i>Priestia aryabhatai</i>	1290	MG9	100	ON413733
NE11	<i>Geobacillus thermodenitrificans</i>	1288	MH1	100	ON413734
NE12	<i>Lysinibacillus fusiformis</i>	1450	L5C3L	99.86	OM900152
NE14	<i>Priestia endophytica</i>	1407	IHBB10265	99.93	OM900153
NE15	<i>Bacillus amyloliquefaciens</i>	1407	205	100	ON413735
NE16	<i>Bacillus subtilis</i>	1411	DE-7	99.93	OM900154
NE18	<i>Bacillus paralicheniformis</i>	1416	PL-9	99.86	OM900155
NE20	<i>Bacillus subtilis</i>	1417	B19	100	OM900156
NE21	<i>Priestia endophytica</i>	1406	2DT	99.86	OM900157
NE23	<i>Enterobacter cloacae</i>	1394	2783	99.93	ON413736
NE24	<i>Bacillus subtilis</i>	1260	SBMP4	100	ON413737
NSE1	<i>Priestia megaterium</i>	1394	FDU301	100	ON413738
NSE2	<i>Kosakonia oryzae</i>	1401	PB4	100	ON413739
NSE3	<i>Bacillus pumilus</i>	1434	Ola 51	100	ON413740
NSE4	<i>Bacillus cereus</i>	1237	FPAARGOS- 798	100	ON413741

NE01 to NE20 are Norungan plant endophytes; NSE1 to 4 are Norungan seed endophytes





**Fig. 1. *In vitro* nutrient-transforming ability of putative competitive endophytes of Norungan landrace.** A) Ammonia excretion in N-free medium; B) Phosphorus solubilization; C) Potassium solubilization; D) Zinc solubilization. NE01 to NE20 are Norungan plant endophytes; NSE1 to NSE4 are Norungan seed endophytes. Each panel represents a mean of three replicates, and the error bar indicates standard error. Endophytic strains with the same letter are not significantly different as determined by the Tukey test ( $p \leq 0.01$ ).

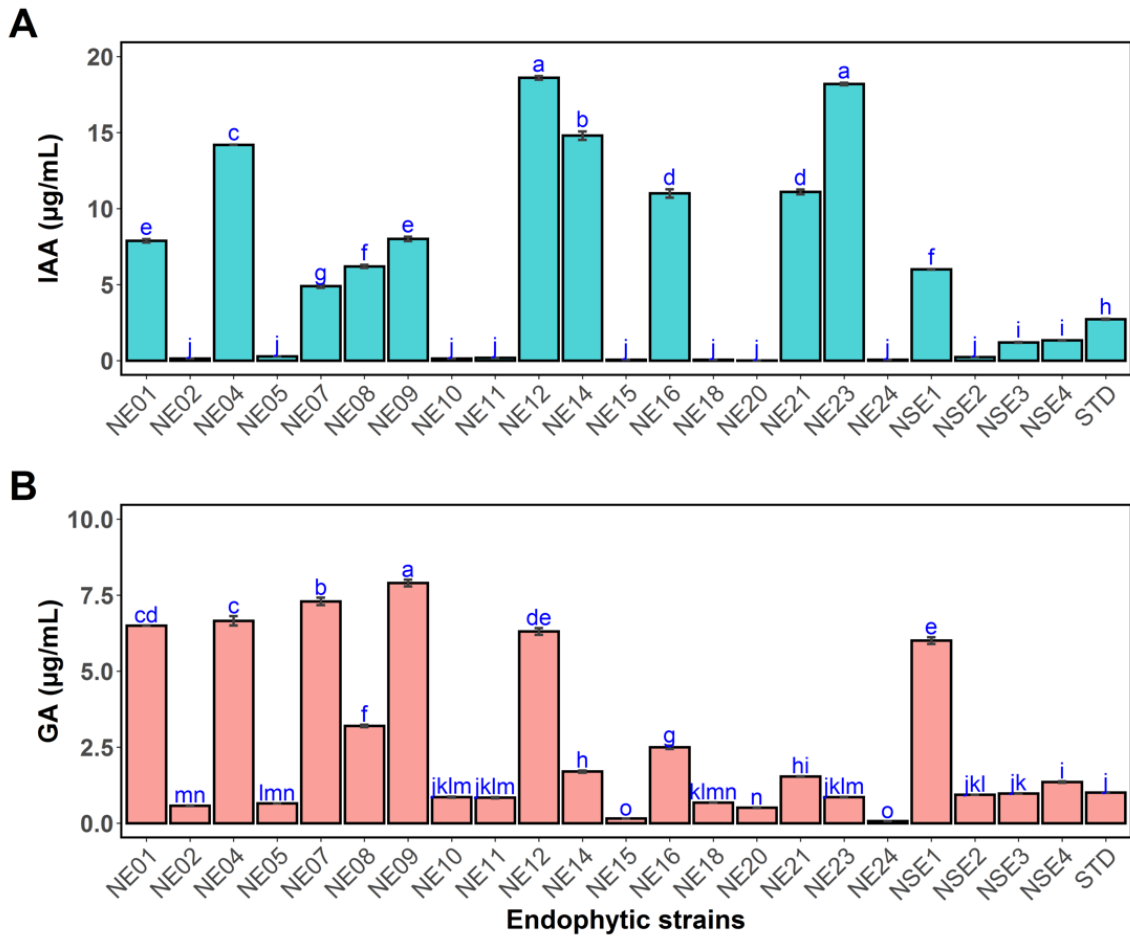
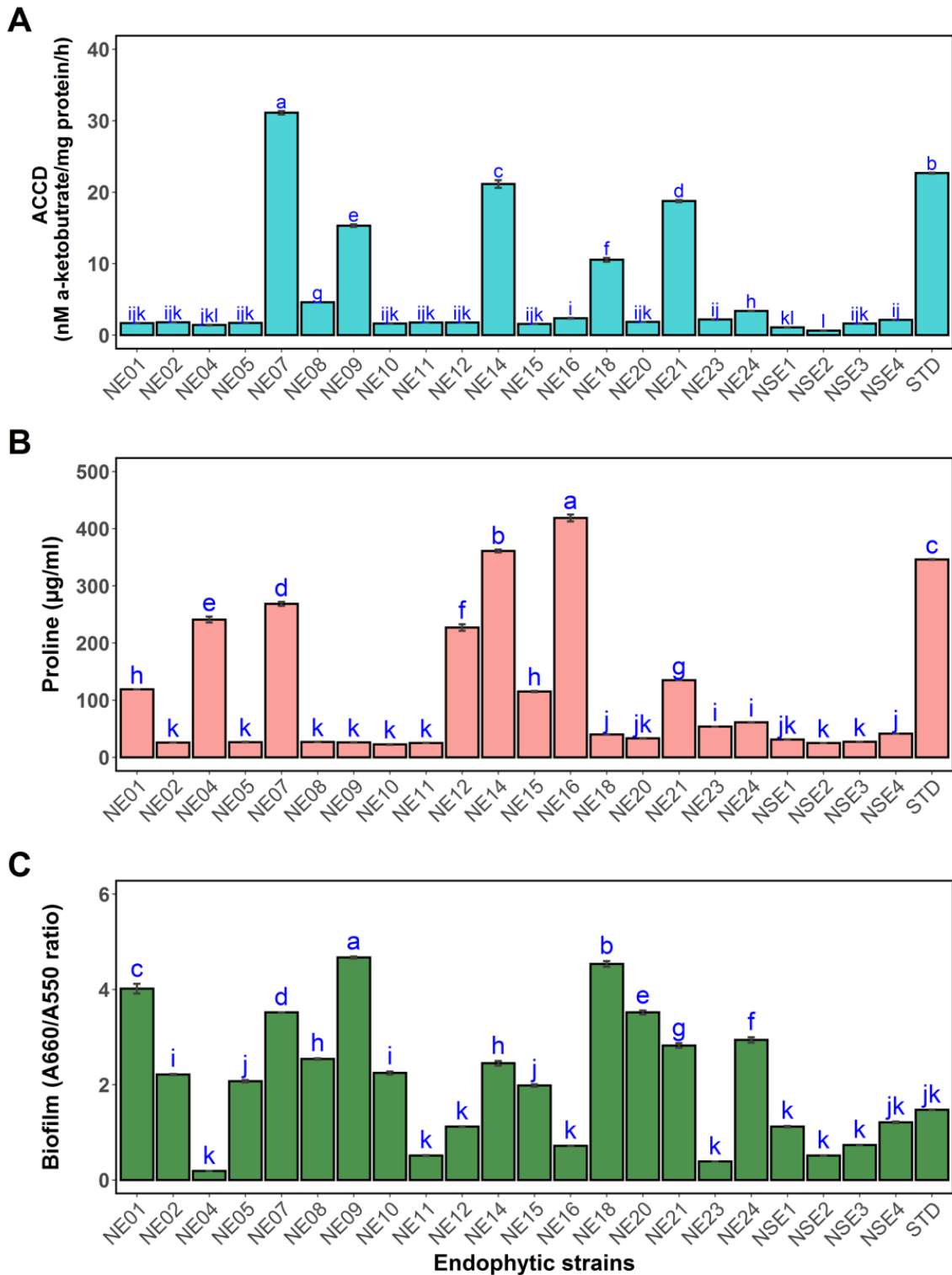
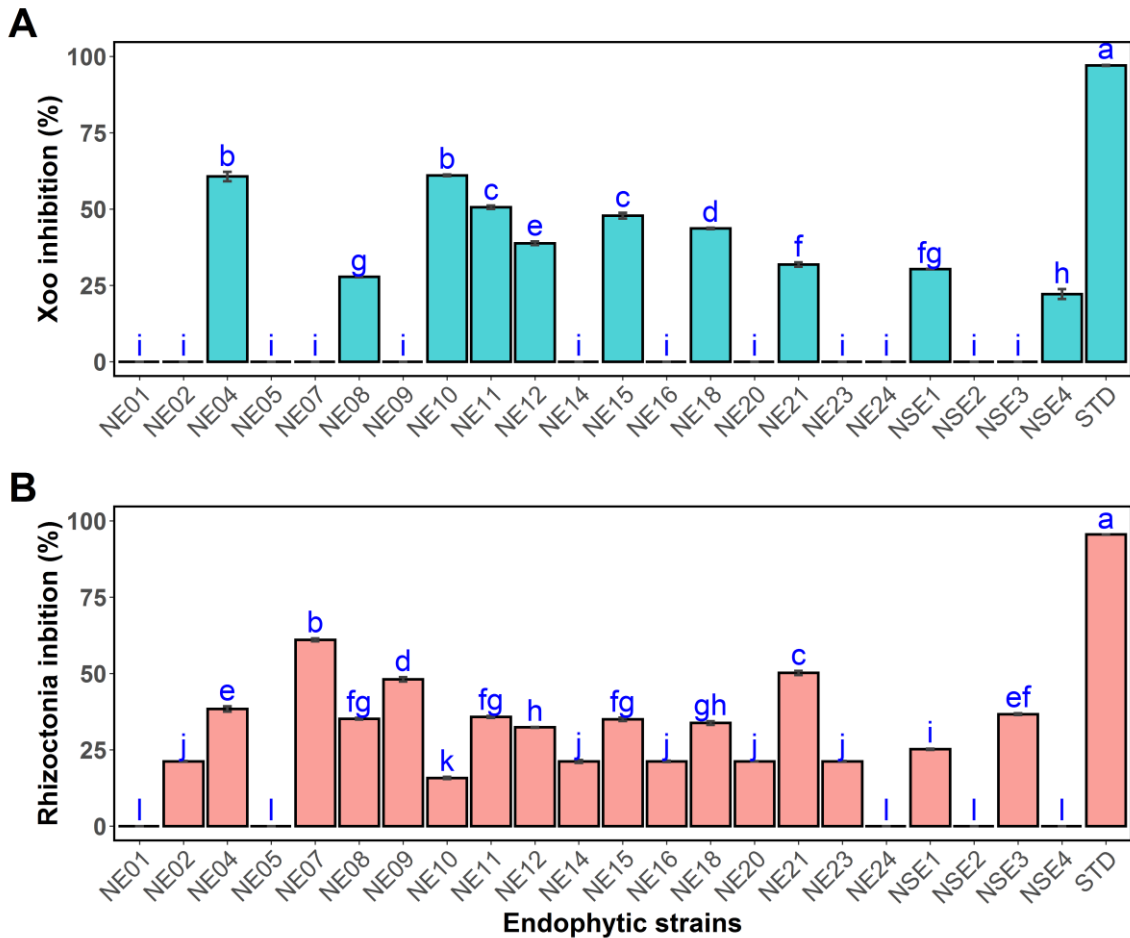


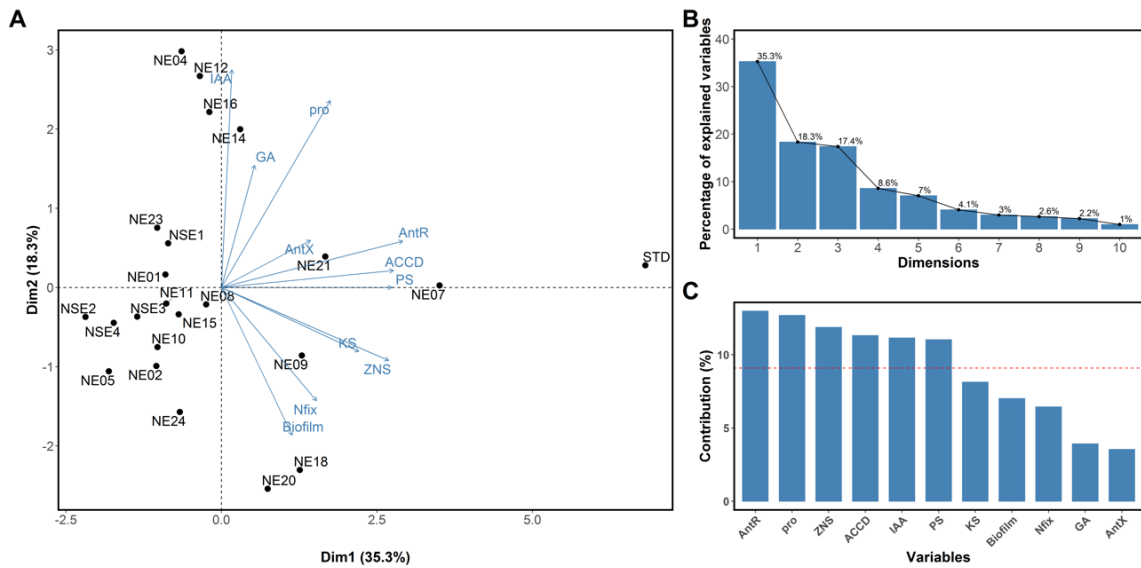
Figure 2. IAA (A) and GA (B) production by the putative competitive endophytes of Norungan landrace. NE01 to NE20 are Norungan plant endophytes; NSE1 to NSE4 are Norungan seed endophytes. Each panel represents a mean of three replicates, and the error bar indicates standard error. Endophytic strains with the same letter are not significantly different as determined by the Tukey test ( $p \leq 0.01$ ).



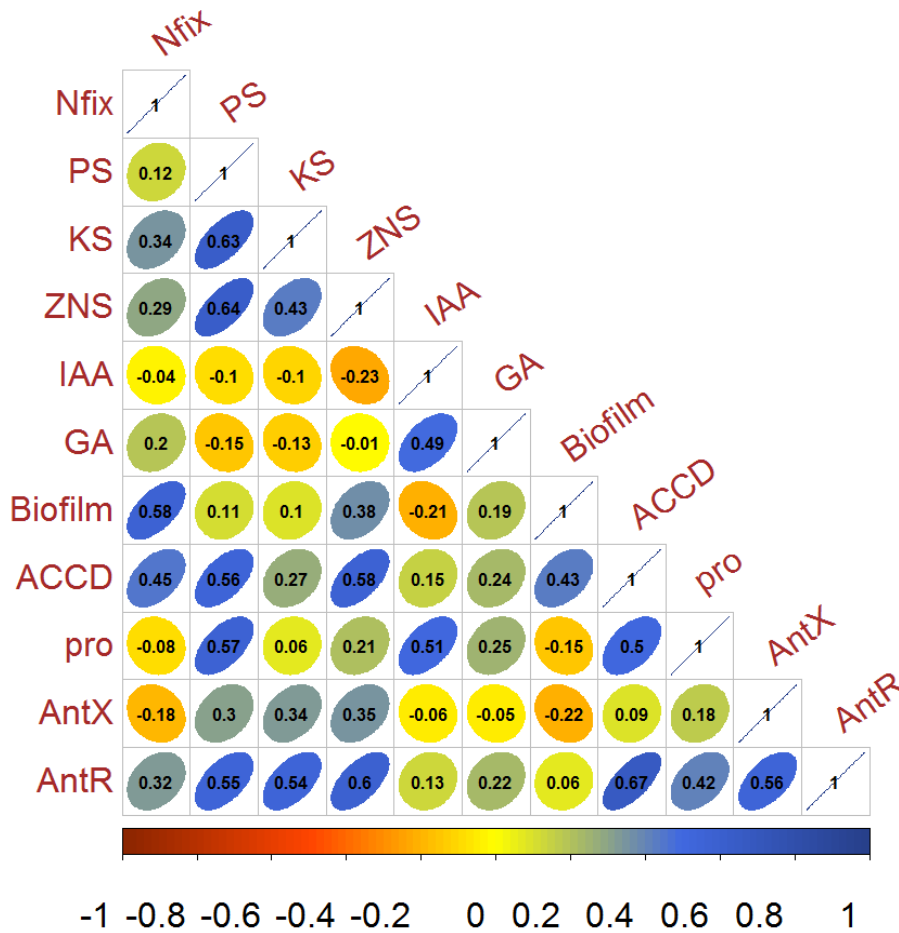
**Figure 3. Drought-mitigating traits of putative competitive endophytes of Norungan landrace.** A – ACC deaminase; B – Proline accumulation; C – Biofilm formation. NE01 to NE20 are Norungan plant endophytes; NSE1 to NSE4 are Norungan seed endophytes. Each panel represents a mean of three replicates, and the error bar indicates standard error. Endophytic strains with the same letter are not significantly different as determined by the Tukey test ( $p \leq 0.01$ ).



**Figure 4.** The antagonistic potential of putative competitive endophytes of Norungan landrace against *Xanthomonas* (A) and *Rhizoctonia* (B). NE01 to NE20 are Norungan plant endophytes; NSE1 to NSE4 are Norungan seed endophytes. Each panel represents a mean of three replicates, and the error bar indicates standard error. Endophytic strains with the same letter are not significantly different as determined by the Tukey test ( $p \leq 0.01$ ).



**Figure 5. Principal component analysis of PGP traits of putative competitive endophytes of Norungan.** A) PCA biplot showing the relation between the assessed PGP traits and the endophytic strains. NE01- to NE20 are Norungan plant endophytes; NSE1 to NSE4 are Norungan seed endophytes. B) Percent variability explained by each dimension of PCA. The individual variability percent explained by each dimension (Dim1 to Dim8) was presented on each panel. C) Percent contribution of each variable to the first two dimensions of PCA. The dashed line indicates >80% contribution to the significant component.



**Figure 6. Pearson's correlation among the PGP traits of Norungan putative competitive endophytes.** The values in the plot represent Pearson's correlation coefficients (r). The size of the ellipse and the color density of the circle reflects the scale of correlation, and blue and red indicate positive and negative correlation, respectively.