



Whole genomic sequence of *Enterobacter sichuanensis* AJI 2411 – A plant growth promoting rhizobacteria

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ABSTRACT

Enterobacter sichuanensis AJI 2411 is a rhizobacteria displaying plant growth promoting potentials, which was isolated from the rhizosphere of soybeans in Ede, Osun State, Nigeria. The full genome of *Enterobacter sichuanensis* AJI 2411 was sequenced and reported in this study to shed light on the molecular mechanisms that aids the bacteria's plant growth-promoting abilities.

Over the next few decades, humanity needs to roughly triple its food output to attain global food security. Conventional agriculture is the most common method for reaching this goal, but it has resulted in significant environmental and socioeconomic damage. According to the Sustainable Development Goals, we now need agriculture that can “multi-functionally” enhance the production of food without harm to the environment (Waldron et al., 2017). Hence, the search for environmentally friendly sources of improving plant growth is the aim of many types of research. Bacteria located in the rhizosphere that possess plant growth-promoting capacity which use a variety of processes, both direct and indirect, to enhance the growth of plant are known as Plant Growth Promoting Rhizobacteria (PGPR) (Olanrewaju et al., 2019).

Plant growth-promoting rhizobacteria (PGPR) are free-living bacteria that invade and stimulate plant development in plant roots. PGPR

may promote plant growth by directly affecting plant metabolism (increasing water and mineral uptake), enhancing root development, increasing plant enzymatic activity, “helping” other beneficial microorganisms to enhance their action on the plant, or suppressing plant pathogens (de Andrade et al., 2023; Vocciante et al., 2022).

They indirectly defend plants by competing for limited resources with diseases, biocontrolling pathogens by creating aseptic-activity chemicals, manufacturing fungal cell wall lysing enzymes, and eliciting systemic responses in host plants. By enhancing plant fitness, stress tolerance, and pollution remediation, PGPR may help plants flourish under abiotic stress. More information and a better understanding of the bacterial characteristics that drive plant growth promotion may inspire and stimulate the creation of innovative solutions employing PGPR in highly variable climatic and climatological conditions (Ole'nska et al.,

Abbreviations: PGPR, Plant Growth Promoting Rhizobacteria; IAA, Indole acetic acid; ECC, *Enterobacter cloacae* complex; WGS, Whole Genome Sequencing; DNA, Deoxyribonucleic acid; MiSeq, A sequencing platform developed by Illumina; V3, A sequencing kit for MiSeq; AJI, A strain of *Enterobacter sichuanensis*; OAT, Orthologous Average Nucleotide Identity Tool; OrthoANI, Orthologous Average Nucleotide Identity; SKESA, A genome assembly tool; shovill, A genome optimization tool.

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Table 1
Genomic characteristics of *Enterobacter sichuanensis* AJI2411.

Name	<i>Enterobacter sichuanensis</i> AJI2411
Locus	NZ_JACWFD01000000 5032127 bp DNA linear BCT 29-SEP-2020
BioProject	PRJNA224116
BioSample	SAMN16081429
Assembly	GCF_014779575.1
Assembly Method	SKESA v. 2.3.0
Type of Genome Representation	Full
Coverage of Genome	111x
Technology of Sequencing	Illumina MiSeq
Provider of Annotation	NCBI RefSeq
Pipeline of Annotation	NCBI Prokaryotic GenomeAnnotation Pipeline (PGAP)
Method of Annotation	Best-placed reference protein set; GeneMarkS-2+
Software of Annotation revision	4.13
Total Genes	4,943
Total-CDSs	4,852
Genes (coding)	4,766
CDSs (with protein)	4,766
Genes (RNA)	91
rRNAs	5, 1, 3 (5S, 16S, 23S)
complete rRNAs	5 (5S)
partial rRNAs	1, 3 (16S, 23S)
ncRNAs	77
Total Pseudo-Genes	86
Ambiguous residues Pseudo Genes	0 of 86
Pseudo-Genes (frameshifted)	34 of 86
Pseudo-Genes (incomplete)	53 of 86
Pseudo-Genes (internal stop)	17 of 86
Pseudo Genes (multiple problems)	16 of 86
Contig Num	60
WGS_SCAFLD	NZ_JACWFD010000001- NZ_JACWFD010000060

2022).

The *Enterobacter* genus comprises species that have been reported to promote plant growth due to their multiple growth-promoting activities.

Some strains that have been reported are: *E. asburiae* PDA 134 isolated from date palms (Abraham and Silambarasan, 2015; Yaish, 2016), *E. cloacae* isolated from citrus and corn plants (Araújo et al., 2002) and *E. asburiae* from sweet potato (Asis and Adachi, 2003). *Enterobacter cloacae* complex (ECC) consisting of as *E. hormaechei*, *E. sichuanensis*, *E. asburiae*, *E. kobei*, and *E. roggenkampii*, are commonly found in the environment, and are widely known to be opportunistic pathogens. In the past decades, ECC have become a global health concern (Chavda et al., 2016; Wu et al., 2018). The strain *Enterobacter* sp., P23 has also been reported as a growth promoter under conditions of abiotic stress such as salinity, due to its high ACC deaminase activity. It promotes plant growth at high temperature, Alkaline pH and in the presence of a wide variety of pesticides applied in corn, peanut and rice farming (Anzuay et al., 2017).

Bacteria specie belonging to the genus *Pantoea* (family Enterobacteriaceae) can be isolated from a wide range of habitats and hosts, such as soil, water, and plants. Unique *Pantoea* strains have been identified as plant growth-promoting rhizobacteria (PGPR), which can be exploited to create new biofertilizers or biological pesticides for sustainable agriculture. *Pantoea agglomerans* strain C410P1's assembled contigs have been uploaded to GenBank with the accession number CP016889 (Luziatelli et al., 2019).

The Plant Growth Promoting *Rhizobacterium* (PGPR) *Hartmannibacter diazotrophicus* gen. nov. sp. nov. was isolated from the rhizosphere of *Plantago winteri* from a natural salt meadow in a nature protection area. Strain E19T promote the growth of barley under salt stress conditions, the genome sequences of the *H. diazotrophicus* E19T chromosome and plasmid HDIAp1 have been deposited to the European Nucleotide Archive under accession numbers LT960614 and LT960615, respectively (Suarez et al., 2019).

Enterobacter has displayed several growth-promoting properties for plants ranging from siderophore production to the synthesis of Indole acetic acid, a plant auxin (Taghavi et al., 2010).

The Enterobacteriaceae are a Gram-negative, facultatively anaerobic, non-spore-forming rod family. This family is motile, catalase positive, and oxidase negative, and it reduces nitrate to nitrite and produces acid from glucose fermentation. With 180 full genomes representing 47 species and 21 genera, Enterobacteriaceae has been thoroughly

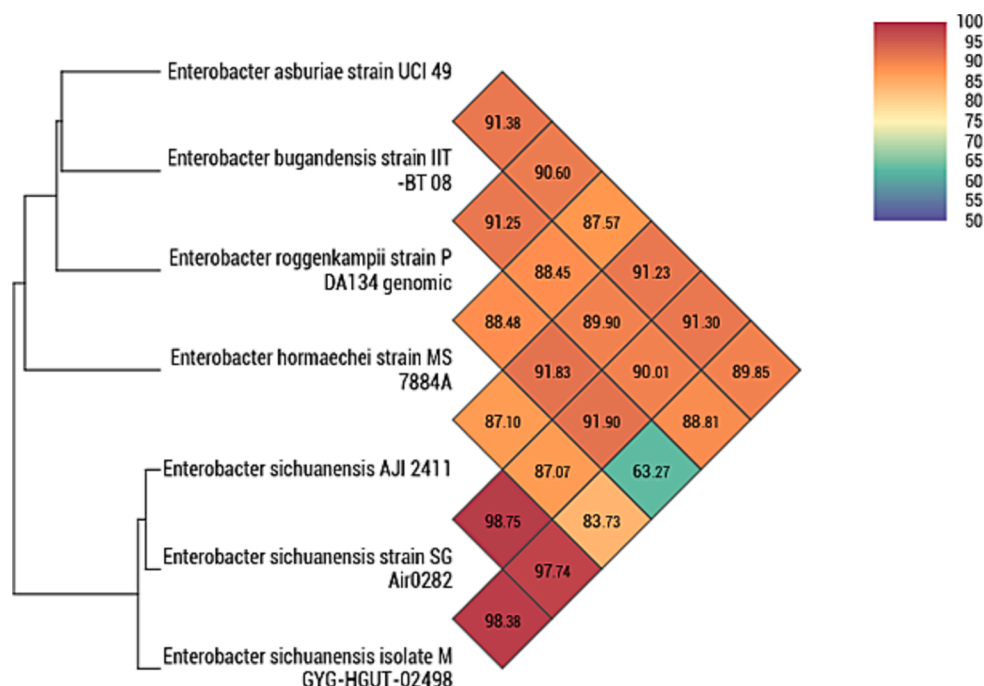
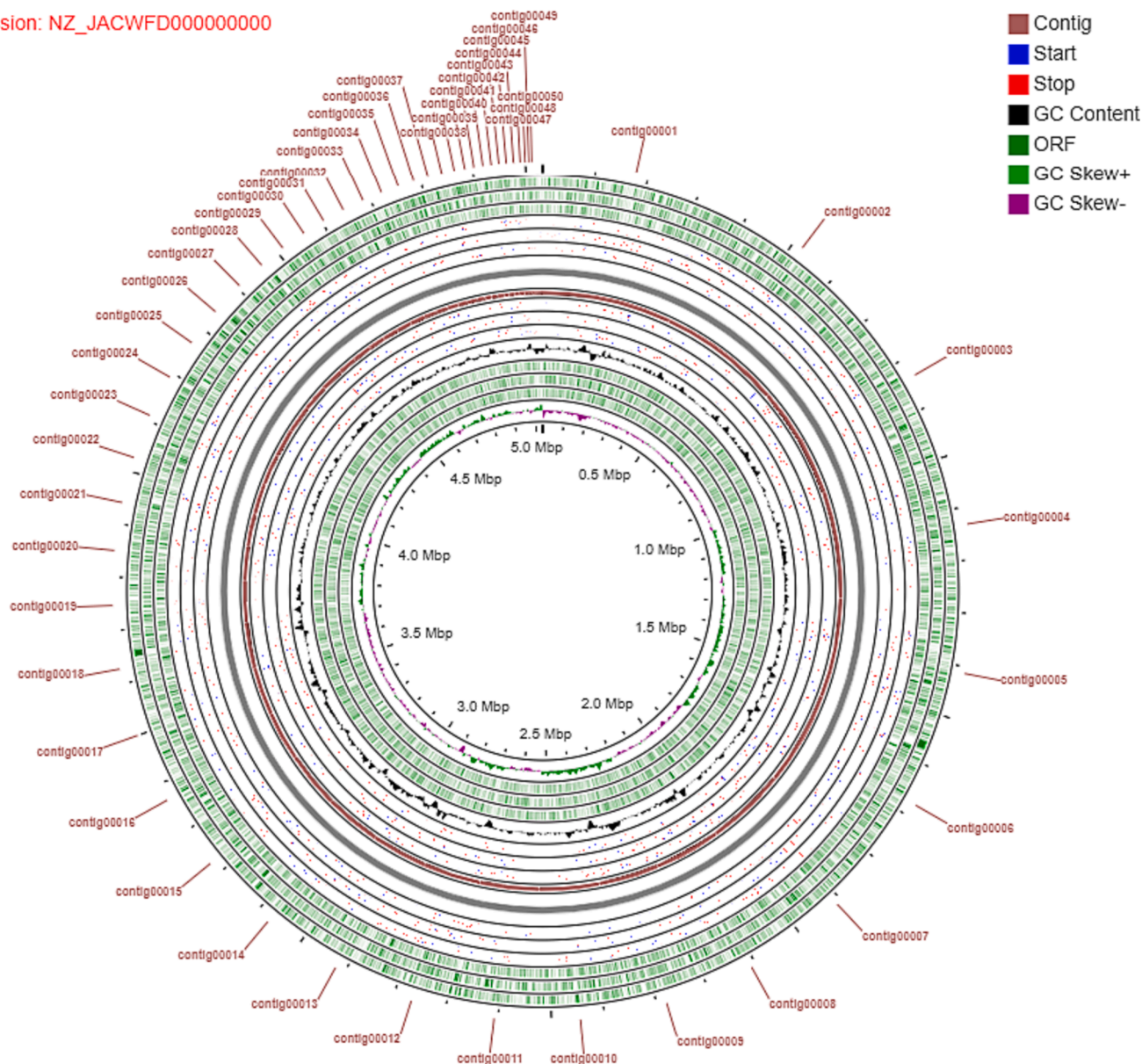


Fig. 1. Heatmap OrthoANI values of *Enterobacter sichuanensis* AJI 2411 containing *Enterobacter cloacae* complex (ECC) species.

Accession: NZ_JACWFD000000000



Enterobacter sichuanensis AJI2411

Fig. 2. The circular view of *Enterobacter sichuanensis* AJI 2411.

sequenced from throughout the family's variety. The genome size varies from 422,434 bp, which codes for just 362 ORFs, to 6,450,897 bp, which codes for 5,909 ORFs. The Enterobacteriaceae family is found all over the world. Many species may survive as free-living organisms in a variety of ecological niches, both terrestrial and aquatic, while some are only connected with animals, plants, or insects. Many are important human, animal, and/or plant pathogens that cause a variety of illnesses. There are several uses for Enterobacteriaceae members, including biocontrol in agriculture, the creation of numerous recombinant proteins and nonprotein products, disease control, anticancer medicines, biowaste recycling, and bioremediation (Octavia and Lan, 2014). The studied organism was identified as *Enterobacter sichuanensis* AJI 2411 with a total base of 588540108.

The environment is frequently home to species of the *Enterobacter cloacae* complex (ECC), which are well-known opportunistic infections. Due to widespread antibiotic resistance and the recent emergence of

multidrug resistance, ECC such as *E. hormaechei*, *E. sichuanensis*, *E. asburiae*, *E. kobei*, and *E. roggkampii* have become a global health problem. They are inherently resistant to the most widely used antibiotics, beta-lactams (Uchida et al., 2020).

In the present study, *Enterobacter sichuanensis* AJI 2411, which was isolated from the rhizosphere of Soya beans (*Glycine max*) in Ede, Nigeria was positive to some selected plant growth-promoting screening test (Omotayo et al., 2022). This is the first report of an indigenously isolated plant growth promoting rhizobacteria with sequences deposited in data banks.

In a bid to fully understand the genomic composition, and the genes that impact the plant growth promoting abilities of *E. sichuanensis* AJI2411 which can have biotechnological benefits, a complete genome sequencing of the *E. sichuanensis* AJI2411 was performed preceded by genomic DNA extraction using a DNA extraction kit (Promega, USA).

Library preparation was performed with the use of DNA Flex

Table 2Location of Genes and Some Predicted Plant growth Promoting Genes in *Enterobacter sichuanensis* AJI2411.

contig_id	feature_id	type	location	start	stop	strand	Function	pgfam
contig00005	fig 91347.113.peg.1406	CDS	contig00005_156719 + 2247	156,719	158,965	+	Phosphocarrier protein kinase/phosphorylase, nitrogen -regulation- associated	PGF_05135990
contig00008	fig 91347.113.peg.2101	CDS	contig00008_176930 + 324	176,930	177,253	+	Periplasmic divalent cation tolerance protein <i>cutA</i>	PGF_03205642
contig00005	fig 91347.113.peg.1406	CDS	contig00005_156719 + 2247	156,719	158,965	+	Phosphocarrier- protein kinase/phosphorylase, nitrogen- regulation associated	PGF_05135990
contig00011	fig 91347.113.peg.2519	CDS	contig00011_81946 + 273	81,946	82,218	+	Phosphocarrier protein, nitrogen regulation associated	PGF_08059713
contig00020	fig 91347.113.peg.3708	CDS	contig00020_13751-1050	13,751	12,702	-	Nitrogen- regulation- protein <i>ntrB</i> (EC 2.7.13.3)	PGF_00450848
contig00015	fig 91347.113.peg.3140	CDS	contig00015_82817-1071	82,817	81,747	-	Glycerol-3-phosphate- ABC transporter, ATP-binding -protein- <i>ugpC</i> (TC 3.A.1.1.3)	PGF_09994213
contig00015	fig 91347.113.peg.3141	CDS	contig00015_83664-846	83,664	82,819	-	Glycerol-3-phosphate -ABC transporter, permease -protein- <i>ugpE</i> (TC 3.A.1.1.3)	PGF_08154601
contig00015	fig 91347.113.peg.3142	CDS	contig00015_84548-888	84,548	83,661	-	Glycerol-3-phosphate ABC transporter, permease protein <i>ugpA</i> (TC 3.A.1.1.3)	PGF_02903501
contig00015	fig 91347.113.peg.3143	CDS	contig00015_86021-1317	86,021	84,705	-	Glycerol-3-phosphate ABC transporter, substrate-binding protein <i>ugpB</i>	PGF_07282910
contig00001	fig 91347.113.peg.328	CDS	contig00001_338781-645	338,781	338,137	-	Chemotaxis response - phosphatase <i>cheZ</i>	PGF_07267579
contig00014	fig 91347.113.peg.2964	CDS	contig00014_38023 + 1080	38,023	39,102	+	Chemotaxis- response- regulator protein-glutamate methylesterase <i>cheB</i> (EC 3.1.1.61)	PGF_00417580
contig00014	fig 91347.113.peg.2966	CDS	contig00014_39484 + 639	39,484	40,122	+	Chemotaxis response - phosphatase <i>cheZ</i>	PGF_07267579
contig00001	fig 91347.113.peg.328	CDS	contig00001_338781-645	338,781	338,137	-	Chemotaxis response - phosphatase <i>cheZ</i>	PGF_07267579
contig00014	fig 91347.113.peg.2964	CDS	contig00014_38023 + 1080	38,023	39,102	+	Chemotaxis response regulator protein-glutamate methylesterase <i>cheB</i> (EC 3.1.1.61)	PGF_00417580
contig00001	fig 91347.113.peg.328	CDS	contig00001_338781-645	338,781	338,137	-	Chemotaxis response - phosphatase <i>cheZ</i>	PGF_07267579
contig00001	fig 91347.113.peg.328	CDS	contig00001_338781-645	338,781	338,137	-	Chemotaxis response - phosphatase <i>cheZ</i>	PGF_07267579
contig00005	fig 91347.113.peg.1322	CDS	contig00005_78671 + 660	78,671	79,330	+	Ribose-5-phosphate isomerase A (EC 5.3.1.6)	PGF_05657253
contig00023	fig 91347.113.peg.4031	CDS	contig00023_66614 + 1485	66,614	68,098	+	Guanosine-5'-triphosphate,3'-diphosphate pyrophosphatase (EC 3.6.1.40) @ Exopolyphosphatase (EC 3.6.1.11)	PGF_05837876
contig00024	fig 91347.113.peg.4119	CDS	contig00024_64640 + 2115	64,640	66,754	+	Guanosine-3',5'-bis(diphosphate) 3'-pyrophosphohydrolase (EC 3.1.7.2) / GTP pyrophosphokinase (EC 2.7.6.5), (p)ppGpp synthetase II	PGF_00010349
contig00001	fig 91347.113.peg.137	CDS	contig00001_153581 + 948	153,581	154,528	+	Ribose-phosphate pyrophosphokinase (EC 2.7.6.1)	PGF_00048782
contig00005	fig 91347.113.peg.1444	CDS	contig00005_204124 + 2232	204,124	206,355	+	Inactive (p)ppGpp 3'-pyrophosphohydrolase domain / GTP pyrophosphokinase (EC 2.7.6.5), (p)ppGpp synthetase I	PGF_00013857
contig00007	fig 91347.113.peg.1798	CDS	contig00007_63859 + 480	63,859	64,338	+	2-amino-4-hydroxy-6-hydroxymethylidihydropteridine pyrophosphokinase (EC 2.7.6.3)	PGF_10123167
contig00005	fig 91347.113.peg.1296	CDS	contig00005_52956-594	52,956	52,363	-	Nucleoside- 5-triphosphatase- RdgB- (dHATP, dITP, XTP-specific) (EC 3.6.1.66)	PGF_00026362

(Nextera). Library preparation kit (Illumina, San Diego, CA, USA) and then sequenced using MiSeq sequencer with operational parameter 2 × 300 cycle V3 kit, following the manufacturer's recommended protocol. The pipeline jekesa (<https://github.com/stanikae/jekesa>) was used to analyze the bacterial Whole Genome Sequencing (WGS). The filtration of quality pair reads was achieved using Trim-galore v (Lindgreen, 2012). The methodology of Souvorov et al. (2018) was utilized to perform the de novo assembly through SKESA v2.3.0 and the optimization was achieved via shovill (<https://github.com/tseemann/shovill>). long-read data, which would produce a genome assembly of higher quality would give a better result, however, the Illumina sequencing is limited in this regard.

Table 1 lists the genome features of *Enterobacter sichuanensis* AJI 2411, and Fig. 1 shows the OrthoANI values of *Enterobacter sichuanensis* AJI 2411.

Utilizing the Orthologous Average Nucleotide Identity Tool (OAT) software (<https://www.ezbiocloud.net/tools/orthoani>),

The heatmap OrthoANI values of *Enterobacter sichuanensis* AJI 2411 vis -a vis *Enterobacter cloacae* complex (ECC) species was determined. OrthoANI values for the six closely related *Enterobacter* species that make up the *Enterobacter cloacae* complex (ECC) (Lee et al., 2016). The taxonomic assignment was confirmed using JSpecies (Richter et al., 2016), where the *Enterobacter sichuanensis* WCHECL1597 a type strain had an ANIb [98.27%], Aligned[86.61%], Aligned[bp]4358324, and Total [bp]5032230 with *Enterobacter sichuanensis* AJI 2411, the micro-organism under study.

Fig. 2 shows a circular view of the *Enterobacter sichuanensis* AJI 2411 genome with its characteristics. Table 2 lists the predicted plant growth promoting genes.

The NCBI database has validated this whole-genome shotgun project and assigned it the accession number NZ_JACWFD010000001-NZ_JACWFD010000060.

Literature has shown that these genes are implicated in providing Nitrogen for plants which ultimately promote plant growth. The presence of genes that are known to promote plant growth was identified. PTS IIA-like nitrogen-regulatory protein PtsN (Huergo et al., 2013), Nitrogen regulatory protein P-II, GlnK, Nitrogen regulation protein NtrB and Nitrogen regulation protein NR(I), GlnG (=NtrC) promotes plant growth. (Zimmer et al., 2000).

The Inactive (p)ppGpp 3'-pyrophosphohydrolase domain/GTP pyrophosphokinase (EC 2.7.6.5) is an enzyme involved in the metabolism of guanosine tetraphosphate (ppGpp), a signaling nucleotide implicated in bacteria's stringent response (Sinha and Winther, 2021; Gratani et al., 2018). The stringent response is a stress response mechanism that regulates gene expression and physiological activities in bacteria to adapt to changing environmental circumstances. While the precise role of this enzyme in plant growth promotion is unknown, there are studies that show the impact of ppGpp on plant growth and development. (Gratani et al., 2018) According to research, ppGpp may impact photosynthesis, growth, and development in plants. ppGpp regulates plastid gene expression and controls photosynthesis, plant growth, and development in the model flowering plant *Arabidopsis*. The gene *ppGpp* buildup has been demonstrated to limit photosynthetic ability and promote a quiescent-like condition with reduced proliferation and growth in the diatom *Phaeodactylum tricorutum* (Avilan et al., 2021). ppGpp has also been linked to chloroplast biogenesis during early leaf development in rice, influencing chloroplast differentiation and gene expression (Ito et al., 2022).

The gene *cheZ* have been implicated in aiding phosphate solubilization (Bagewadi et al., 2022).

The study acknowledges that the identification of only 1 copy of the 16S rRNA gene in *Enterobacter sichuanensis* genome, which typically carries 8 copies of 16S rRNA genes (Uchida et al., 2020) may be due to assembly artifacts.

CRediT authorship contribution statement

We extend our appreciation to Oluwatosin Ajibade and Olubukola Oyawoye for their conceptual contributions. The formal analysis was expertly conducted by Stanford Kwenda, with Zamantungwa Khumalo leading the methodology. Resources were generously provided by Arsad Ismail. Olubukola Oyawoye oversaw the supervision of this research, while project administration was competently managed by Julius Oloke. The initial draft of this paper was crafted by Oluwatosin Akinola Ajibade and Elijah Kolawole Oladipo; subsequent reviews and edits were carried out by Elijah Kolawole Oladipo and Helen Onyeaka. We also gratefully acknowledge the funding support from Helen Onyeaka.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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