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Genomic Assessment of *Enterobacter mori* AYS9: A Potential Plant Growth-Promoting Drought-Resistant Rhizobacteria

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Drought stress poses a serious danger to agricultural production. Recent studies have revealed that most of the chemical methods used in the mitigation of its effects on plant production pose a serious threat to humans and the environment. Therefore, the demand for ecologically friendly solutions to ensure the security of the world's food supply has increased as a result. Plant growth-promoting rhizobacteria (PGPR) treatment may be advantageous in this situation. *Enterobacter mori* is a promising rhizobacteria in this regard. However, information on the genome analysis of *E. mori* linked to the rhizosphere soil of the sorghum plant has not been extensively studied. In this study, we present a genomic lens into functional attributes of *E. mori* AYS9 isolated from sorghum plants, as well as assess its drought tolerance and plant growth-promoting potentials. Our results showed the drought tolerance and plant growth-promoting potentials of the AYS9. Whole genome sequencing (WGS) results revealed that the genome yielded 4,852,175 bp sequence reads, an average read length of 151 bp, 1,845,357 bp genome size, 67 tRNAs, 3 rRNAs, and a G + C content of 55.5%. The functional genes identified in the genome were linked to processes including phosphate solubilization, iron transport, hormone regulation, nitrogen fixation, and resistance to oxidative and osmotic stress. Also, secondary metabolites supporting bacterial biocontrol properties against phytopathogens, and abiotic stress such as aerobactin-type non-ribosomal peptide siderophore, Stewartan-type ladderane, and Colicin type NRPS were discovered in the AYS9 genome. Our findings however establish that the intricate metabolic pathways mediated by the projected new genes in the bacterial genome may offer a genetic foundation for future understanding of rhizosphere biology and the diverse roles that these genes play in plant development and health.

Keywords: sustainable agriculture, abiotic stress, plant health improvement, rhizobacteria, crop productivity

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INTRODUCTION

A major abiotic stress that poses a danger to global agricultural productivity is drought (Fadiji et al., 2022b; Fadiji et al., 2022c). Lack of access to fresh water to satisfy the needs of humans and the environment causes and sustains drought, which is a natural phenomenon (Balint et al., 2013; Ojuederie et al., 2019). Since drought is frequently not localised to a certain time or place, monitoring

it may be challenging. Lack of water causes serious socioeconomic problems, and crop loss, and is fatal to plants (Salehi-Lisar and Bakhshayeshan-Agdam, 2016; Ahluwalia et al., 2021). Ramakrishna et al. (2019) and Fadiji et al. (2022b) reported that roughly half of the arable land will likely be severely drought-restricted in terms of plant growth by 2052. According to (Mach et al., 2019), drought stress has lowered grain yields by up to 10% over the past 40 years and is expected to have an impact on the output above 50% of most fertile land by the year 2050 (Vinocur and Altman, 2005; Jochum et al., 2019). Research has concentrated on enhancing germplasm and creating techniques for crop management to boost water usage efficiency in order to meet this worldwide challenge in agriculture (Ngumbi and Kloepper, 2016). Due to technological advancements in microbiomics and next-generation sequencing, however, the focus has recently shifted to the use of beneficial microbes that mediate tolerance to drought and increase plant water usage efficiency (Dimkpa et al., 2009; Ngumbi and Kloepper, 2016; Vurukonda et al., 2016).

A sustainable biological strategy to address agricultural production's water shortage is the use of plant growth-promoting rhizobacteria (PGPR). Intimate and free-living relationships with host plants are quickly established by PGPR in the root rhizosphere. Through a number of processes, these interactions frequently result in an improvement in agricultural yield and the reduction of abiotic and biotic stressors (Vurukonda et al., 2016; Barnawal et al., 2017; Forni et al., 2017). Having been reported as plant disease suppressors, biofertilizers, abiotic stress relievers, and soil toxin removers, PGPR may be extremely important (Naveed et al., 2014; Timmusk et al., 2014). Changes in the structure of the osmoregulation, host root system, management of oxidative stress through the biosynthesis and metabolism of phytohormones or the production of antioxidants for scavenging reactive oxygen species (ROS), the production of large chain exopolysaccharide (EPS), which may act as a humectant, and transcriptional control of the genes responsible for host stress response are some of the mechanisms connected to PGPR-derived drought tolerance (Ngumbi and Kloepper, 2016; Forni et al., 2017; Fadiji et al., 2022a; Fadiji et al., 2022c).

The genus *Enterobacter* has a total of 23 known species (http://www.bacterio.cict.fr/e/enterobacter.html). Both as pathogenic and beneficial species, *Enterobacter* species have been implicated in plant diseases and human opportunistic pathogens (Nishijima et al., 2007), as well as essential genetic engineering and plant growth-promoting bacteria (Nie et al., 2002). Others also play vital roles in biocontrol (Zhu et al., 2011). However, only a limited number of *Enterobacter* species have been identified in plants and soils, although they play numerous functions in plant metabolism and physiology (Zhu et al., 2011; Jochum et al., 2019). For instance, due to their capacity to produce osmoprotective genes and substances, *E. mori* strains have been widely isolated from the rhizosphere soil of many plants and have been actively involved in the reduction of drought stress in plants. Hence, it may be concluded that *E. mori* has a potential future in agricultural sustainability.

The identification of key genes in the genome of *Enterobacter* species might greatly improve these bacteria's roles in the development of root-bacterial, soil-bacterial, and soil-root interactions for better plant stress response mechanisms (Vurukonda et al., 2018). The lifestyle and operations of the bacteria in the host plants can be impacted by these genes. For instance, the discovery of genes known in the development of biofilm chemotaxis can support plant defense systems and colonization of the root. Based on these functional presumptions, *E. mori* can be a viable bioagent in the formulation of biostimulants and bioinoculants against drought and for better agricultural yield. In this study, we present a genomic lens into the functional attributes of *E. mori* AYS9, as well as assess its drought tolerance and plant growth-promoting potentials. As far as we know, this is one of the foremost reports on the WGS of *E. mori* AYS9 isolated from the rhizosphere of sorghum plants in Southern Africa. Furthermore, this study assessed the plant growth-promoting activities, presence of functional genes and secondary metabolites of the strain.

MATERIALS AND METHODS

Sampling and Isolation of Rhizobacteria *Enterobacter mori* AYS9

The rhizobacterium was isolated from 10 g of rhizosphere soil from healthy sorghum plants cultivated in the research farm of the North-West University, South Africa (250 47′25.24056″ S 250 37′30 8.17464″ E). The isolation and characterization of the rhizobacterial isolate were carried out using the method of Sha'arani et al. (2021). A pure rhizobacterium was isolated from 10 g soil tightly adhering to the root of the sorghum through serial dilution and subsequent culturing on Luria–Bertani (LB) agar plates using the method of Majeed et al. (2015). After a series of subculturing, the identified single colonies were characterized using morphological and biochemical tests. The distinct colony was further selected and purified, which was then maintained on a sterile nutrient agar slant for further analyses.

Morphological, Microscopic Appearance, and Biochemical Characterization of *Enterobacter mori* AYS9

The pure culture of *E. mori* AYS9 was plated on Nutrient agar and incubated at 28°C for 24 h after which the morphological characteristics of the isolate were examined. The colonies' morphology, which includes shape, size, pigmentation, and color was properly documented after incubation. Furthermore, the biochemical characterization such as Gram staining, including oxidase, nitrate reduction, Voges Proskauer, catalase, indole, citrate, and starch hydrolysis test by using the standardized method as reported by Clarke and Cowan (1952) and Ahmad et al. (2008). While carbohydrate utilization potentials of the AYS9 were carried out using carbon sources such as galactose, maltose, xylose, lactose, fructose, glucose and sucrose (Sati and Bisht, 2006).

Screening of the *Enterobacter mori* AYS9 for Drought Tolerance Under Increasing Concentrations of Polyethylene Glycol (PEG6000)

Assessment of the osmotic tolerance of *E. mori* AYS9 was carried out by documenting their growth within the nutrient-broth medium adjusted with varying PEG6000 concentrations (0%–30%). The cultures were then incubated for 48 h at 28°C under shaking at 200 rpm at the same time (Sandhya et al., 2009). Estimation of the bacterial growth was done by properly recording the cultures' optical density of the medium at various concentrations of PEG 6000 using Merck's UV spectrophotometer at 600 nm.

Screening of *Enterobacter mori* AYS9 for PGP Traits

The standardized methods, as described by Cappuccino and Sherman (1992), as well as Ahmad et al. (2008), were utilized in the testing of *E. mori* AYS9 for NH_3 production in peptone water. The test for phosphate solubilization was done employing a modified procedure as described by Shakeela et al. (2017). The procedures of Ahmad et al. (2008), in addition to those of Masciarelli et al. (2014), were employed to test isolated bacteria for siderophore production. The nutrient broth culture of the isolate was used to inoculate tri-calcium phosphate-supplemented Pikovskaya's agar. The emergence of yellow coloration around the bacterial inoculation spot, thus, implied a positive result. The nitrogen fixation characteristics of the endophytic bacteria were determined according to Ahmad et al. (2008) in Jensen's medium. Bacterial growth in the medium indicated the nitrogen-fixing potential.

Furthermore, modified procedures of Loper and Schroth (1986) and Ahmad et al. (2008) were used to determine indole-3-acetic acid (IAA) qualitatively at varying concentrations of tryptophan of 0, 100, and 200 mg within the nutrient broth. The IAA production was signaled by the emergence of pink coloration. Screening of isolate for hydrogen cyanide production (HCN) was also carried out using the procedure described by Ahmad et al. (2008) with slight modifications. The HCN production was indicated by the emergence of orange-to-red coloration. Also, the potential of the isolate to produce 1-aminocyclopropane-1-carboxylate deaminase (ACCd) enzyme was examined on account of the capability to utilize ACC as the only source of nitrogen within the minimal medium (Duan et al., 2009). Bacterial growth on the supplemented ammonium sulphate medium indicated ACCd activity.

Whole-Genome Sequencing (WGS) and Analysis of *Enterobacter mori* Strain AYS9

The extraction of the DNA of *E. mori* strain AYS9 was carried out using the Quick-DNA™ Miniprep Kit specific for bacteria and fungi (Zymo Research, United States), using the method stipulated by the manufacturer. DNA quality checks were carried out using a NanoDrop spectrophotometry (Thermo Fischer Scientific, United States). The molecular identification and analysis of the stain AYS9 based on 16S rDNA sequence data was carried out using the method of Majeed et al. (2015). The genome sequencing of the strain AYS9 was performed at the Novogene Company Limited, Singapore.

The WGS of the rhizobacteria strain AYS9 was carried out using the employing of the standard Illumina platform. Furthermore, fragmentation of the genomic DNA of the bacteria was done employing the NEB Ultra II FS kit enzymatic technique. AMPure XP beads were used to assess the resulting fragments of the DNA based on size range (200–650 bp). Also, end-repairing of the DNA was achieved through fragmentation, and the fragment was ligated to Illumina-specifc adapter sequences. Consequently, the sample indexing and selection depending on the size in the second step were carried out. The 4 nM quantity dilution of the standard dilution concentration of the samples was achieved using the fluorometric method. Consequently, sequencing was performed using the pair-end Illumina library and was loaded into the NovaSeq 6000 (2 × 150 bp) instrument for cluster generation and sequencing (300 cycles).

The WGS analysis of the sequence in FASTQ file format was uploaded to the Department of Energy Systems Biology Knowledgebase (KBase) (Arkin et al., 2018). The assessment of the quality of each sequence reads was performed with FastQC tool (version 0.11.5) (Andrews, 2010), while the removal of low-quality bases and sequence adaptor was performed using trimmomatics (version 0.36) (Bolger et al., 2014). Also, sequence reads were assembled by SPAdes (version 3.13.0) (Nurk et al., 2013). Taxonomic annotation was carried out using the GTDB-Tk - v1.7.0 (Chaumeil et al., 2020), while functions annotation was carried out using Rapid Annotations using Subsystems Technology toolkit (RASTtk) (version 1.073) (Aziz et al., 2008) and SEED online server (Overbeek et al., 2014) to group the functions and distribution of the predicted genes. The prediction of functional protein-coding genes was performed using the genomic protein data after processing the genome on the National Center for Biotechnology Information (NCBI) database (Li et al., 2021). All analyses in this study were done using default parameters except otherwise stated. Determination of secondary metabolites in the genome was carried out using antiSMASH (version 6.0.0) (Weber et al., 2015). The circular visualization of the genome with the important genomic feature was plotted using Circular Genome Viewer (CGview) (Arkin et al., 2018), while the phylogeny analysis was performed using MrBayes (Huelsenbeck and Ronquist, 2001). The antiSMASH version 6.0 (Blin et al., 2021) was used for the detection of important metabolites and their biosynthetic gene clusters.

RESULTS

Biochemical, Cultural Features and Drought Tolerance Assay

The cultural, and biochemical characterization alongside plant growth promoting (PGP) features of *Enterobacter mori* AYS9 from the rhizosphere of the sorghum plant was presented in **Table 1**. The results showed that AYS9 is Gram-negative, rod-

TABLE 1 | Cultural, biochemical and plant growth-promoting test of *Enterobacter mori* AYS9.

Cultural and biochemical assay		Plant growth-promoting qualitative tests	
Characteristics	Result	PGP traits	Results
Shape	Rods	Ammonia production	++
Gram reaction	Gram-negative	Phosphate solubilization	++
Color	Cream	Siderophore	+
Surface texture	Smooth shiny	Nitrogen fixation	+
Citrate	+	IAA	+
Catalase	+	HCN	–
Nitrate	+	ACCd	++
Maltose	+		
Oxidase	+		
Casein hydrolysis	+		
Fructose	+		
Glucose	+		
Sucrose	+		
Trehalose	+		
Turanose	+		
Galactose	+		
Xylose	+		
Mannitol	+		
Arabinose	+		
Raffinose	+		
Maltose	+		

TABLE 2 | Response of *Enterobacter mori* AYS9 to drought stress amended with various concentrations of PEG-6000.

Treatment	O.D at 600 nm
0%	1.38 ± 0.06
5%	1.04 ± 0.01
10%	0.88 ± 0.03
15%	0.74 ± 0.02
20%	0.76 ± 0.01
25%	0.89 ± 0.02
30%	0.96 ± 0.03

Values are represented as the mean of 3 replicates (n = 3) ±SE.

TABLE 3 | General characteristics of the genome and annotation details of *Enterobacter mori* AYS9.

Treatment	***Enterobacter mori* stain AYS9**
Domain	Bacteria
Taxonomy	Proteobacteria, Gammaproteobacteria; Enterobacterales, Enterobacteriaceae, *Enterobacter*
Size(bp)	4,852,175
G + C content (%)	55.5
*N*50	632,440
*L*50	2
Number of contigs (with PEGs)	34
Number of subsystems	372
Number of coding sequences	4,446
Number of RNAs	76

shaped, creamy in colour and catalase positive. Furthermore, strain AYS9 utilizes all the sugars tests carried out and grows optimally between the pH range of 4–10 and temperatures of 20°C–30°C. The plant growth-promoting assay carried out on the strain showed that the strain AYS9 tested positive for all the PGP traits, such as the production of ammonia, phosphate, siderophore, indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylate deaminase (ACCd) as well as nitrogen fixation. But tested negative for hydrogen cyanide production (**Table 1**). A drought tolerance assay carried out on the strain showed that the strain exhibited increased tolerance to Polyethylene glycol (PEG6000) even to a 30% concentration (**Table 2**).

WGS Information of the Rhizobacterium *Enterobacter mori* AYS9

The WGS assessment of *E. mori* AYS9 showed 7,301,524 bp sequence read count, genome size is 4,852,175 bp, and a G + C content of 55.5% as seen in **Table 3**. The read length average was 151 bp, while L_{50} and N_{50} were 2 and 632,440 bp, respectively while the number of contigs is 34. Furthermore, the genome analysis showcased 4,546 coding sequences, 67 tRNA and 3 rRNA. The circular genome visualization of *E. mori* strain AYS9 is shown in **Figure 1**. The statistics of the subsystem revealed 27 subsystem features of the coding protein into functional groups based on the annotated genome grouping by the SEED subsystem in RAST online server. The functional groups of 4,546 protein-coding genes (PCG) were assessed using the RAST and KEGG databases. The 1,732 genes annotated using SEED were classified into cellular components, molecular functions, and biological processes. The six functional groups were found to be prominent these groups are stress response (95 genes), Protein Metabolism (220 genes), carbohydrates (329 genes), Amino Acids and Derivatives (350 genes), respiration (99 genes), cofactor vitamins, prosthetic groups, and pigments (157 genes) (**Figure 2**).

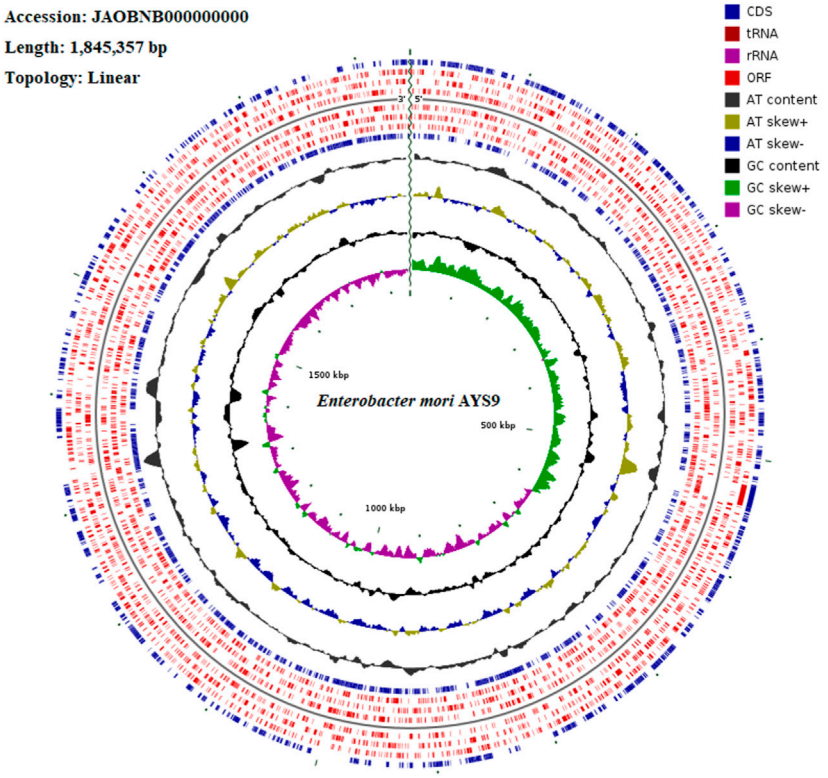

FIGURE 1 | Circular genome visualization of whole-genome *Enterobacter mori* AYS9.

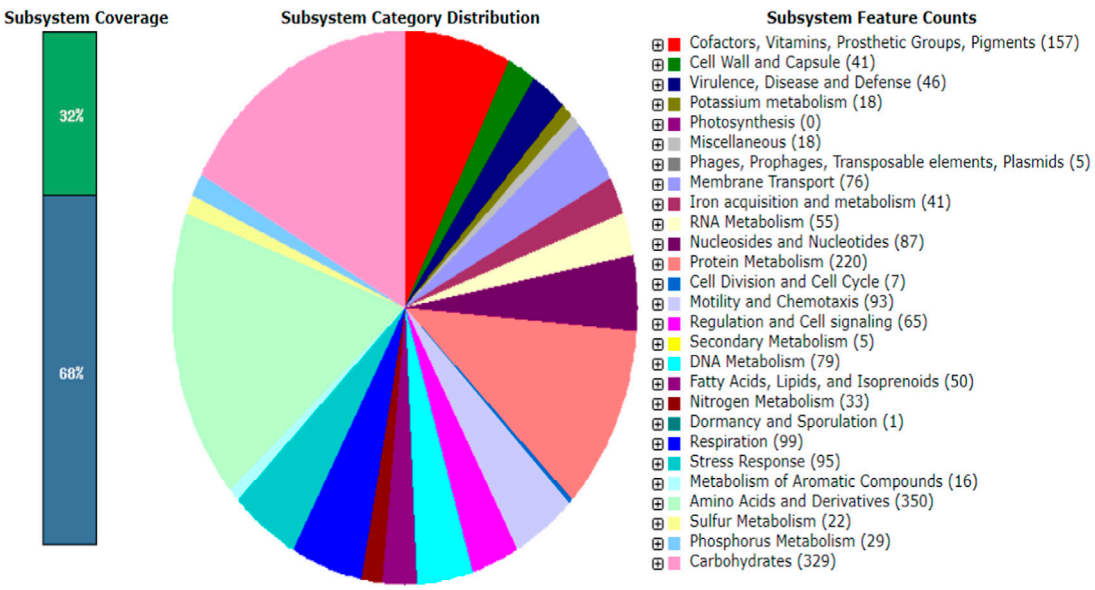

FIGURE 2 | Major PCG of *Enterobacter mori* strain AYS9 annotated in the RAST and SEED Viewer annotation online server. The green/blue bar on the left shows the coverage of the subsystemin percentage. The blue bar shows the proteins percentage present.

Functional Genes Annotation Information of *Enterobacter mori* AYS9

Notable metabolic genes were identified in the AYS9 genome (**Tables 4**–**6**). Notable genes involved in phosphate solubilization and pathways, such as phosphate assimilation and phosphate transport, are presented in **Table 4**. The genes, such as *fbpC* and *feoA*, coding for iron transport in the Iron (III) transport and Iron (II) transport were detected in strain AYS9 (**Table 5**). Genes, such as *trpA*, *trpB miaA*, *miaB speA*, *speB*, *speD*, *speE speG*, *nirB*, *nirD*, *amtB*, *ureC*, *ureG*, *ureF*, *ureE* coding for iron transport, modulation and nitrogen metabolism in the L-tryptophan production, CK biosynthesis, putrescine biosynthesis, spermidine biosynthesis, N1- and N8-acetylspermidine formation, dissimilatory nitrate reduction, ammonia transport, urea degradation and transport respectively are similarly presented in **Table 5**. Other important genes involved in the mitigation of osmotic and oxidative pathways, such as glycine betaine biosynthesis, proline biosynthesis, glutamate biosynthesis, glycine betaine/proline transport system, glutamate transport, degradation of hydrogen peroxide and degradation of hydrogen peroxide and organic hydroperoxides (**Table 6**).

Predicted Secondary Metabolite Cluster Genes Through antiSMASH

The secondary metabolite gene clusters identified for strain AYS9 using antiSMASH analysis are reported in **Table 7**. The identified biosynthetic gene clusters are aerobactin type non-ribosomal peptide siderophore, Stewartan type ladderane, and Colicin type NRPS. The major secondary metabolite gene clusters detected are additional biosynthetic genes, transport-related genes, core biosynthetic genes, regulatory genes, resistance, and other genes (**Figures 3A, B**). The genes cluster exhibiting 66% and 88% similarity for gene type siderophore and arylpolyene with the most similar known cluster Aerobactin and arylpolygenes node regions 1.1 and 7.1 were also presented (**Table 7**).

DISCUSSION

The goal of the study's design was to identify and describe rhizosphere bacterial strains from the sorghum plant that are capable of stimulating plant development and reducing the effects

TABLE 4 | Notable genes identified in phosphate transport and solubilization.

Genes	Locus Tag	Product	Pathways
ppa	N4Q52_12890	Inorganic pyrophosphatase	Phosphate assimilation
pgl	N4Q52_18515	6- phosphogluconolactonase	
ppx	N4Q52_08230	Exopolyphosphatase	
*pst*C	N4Q52_08250	P (ABC)T permease subunit *Pst*C	
*pho*U	N4Q52_18765	Phosphate signaling complex protein *Pho*U	
*pho*A	N4Q52_15190	Alkaline phosphatase	
*pst*A	N4Q52_18755	Phosphate ABC transporter (P (ABC)T) permease PstA	Phosphate transport
*pst*B	N4Q52_18760	P (ABC)T ATP-binding protein *Pst*B	
*pst*S	N4Q52_18745	P (ABC)T substrate-binding protein *Pst*S	

TABLE 5 | Notable genes identified in iron transport and hormonal modulation.

Genes	Locus Tag	Product	Pathways
Iron transport			
FbpC	N4Q52_20740	Iron (III) transport system ATP-binding protein	Iron (III) transport
FeoA	N4Q52_20745	ferrous iron transport protein A	Iron (II) transport
Hormonal modulation			
TrpA	N4Q52_04370	tryptophan synthase subunit alpha	L-tryptophan production
TrpB	N4Q52_04365	tryptophan synthase subunit beta	
MiaA	N4Q52_12590	tRNA dimethylallyltransferase	CK biosynthesis
MiaB	N4Q52_16645	tRNA-2-methylthio-N6-dimethylallyladenosine synthase	
SpeA	N4Q52_10515	arginine decarboxylase	Putrescine biosynthesis
SpeB	N4Q52_10520	Agmatinase	
SpeD	N4Q52_14365	S-adenosylmethionine decarboxylase	Spermidine biosynthesis
SpeE	N4Q52_14370	Spermidine synthase	
SpeG	N4Q52_02295	Spermidine/Spermine N (1)-acetyltransferase	N1- and N8-acetylspermidine formation
Nitrogen metabolism			
NirB	N4Q52_04595	nitrite reductase (NADH) large subunit	Dissimilatory nitrate reduction
NirD	N4Q52_20880	nitrite reductase (NADH) small subunit	
AmtB	N4Q52_15550	ammonium transporter, Amt family	Ammonia transport
UreC	N4Q52_09930	urease subunit alpha	Urea degradation and transport
UreG	N4Q52_09910	Urease accessory protein UreG	
UreF	N4Q52_09915	Urease accessory protein UreF	
UreE	N4Q52_09925	Urease accessory protein UreE	

TABLE 6 | Notable genes identified for osmotic and oxidative stress.

Genes	Locus Tag	Product	Pathways
BetB	N4Q52_16210	Betaine-aldehyde dehydrogenase	Glycine betaine biosynthesis
ProA	N4Q52_16210	Glutamate-5-semialdehyde dehydrogenase	
ProB	N4Q52_15005	Glutamate 5-kinase	Proline biosynthesis
ProC	N4Q52_15215	Pyrroline-5-carboxylate reductase	
GltB	N4Q52_09025	glutamate synthase (NADPH/NADH) large chain	Glutamate biosynthesis
GltD	N4Q52_09020	glutamate synthase (NADPH/NADH) small chain	
GlnA	N4Q52_21360	Glutamine synthetase	
ProX	N4Q52_11795	Glycine betaine/proline transport system substrate-binding protein	Glycine betaine/proline transport system
ProW	N4Q52_11800	Glycine betaine/proline transport system permease protein	
ProV	N4Q52_11805	Glycine betaine/proline transport system ATP-binding protein	
GltP	N4Q52_22150	proton glutamate symport protein	Glutamate transport
KatE	N4Q52_01085	Catalase	Degradation of hydrogen peroxide
Tpx	N4Q52_01085	Thiol peroxidase	Degradation of hydrogen peroxide and organic hydroperoxides
Bcp	N4Q52_08155	Peroxiredoxin Q/BCP	

TABLE 7 | Secondary metabolite gene clusters in the genomes of *Enterobacter mori* strain AYS9.

Node region	Type	From	To	Most similar known cluster		Similarity
Rg 1.1	Siderophore	1,270,777	1,285,205	Aerobactin	Other	66%
Rg 4.1	Ladderane	2009	44,567	Stewartan	Saccharide	14%
Rg 4.2	NRPS	322,675	366,532	Colicin	RiPP	3%
Rg 5.1	Thiopeptide	130,773	157,075	O-antigen	Saccharide	14%
Rg 7.1	Arylpolyene	73,134	116,769	Aryl polygenes	Other	88%

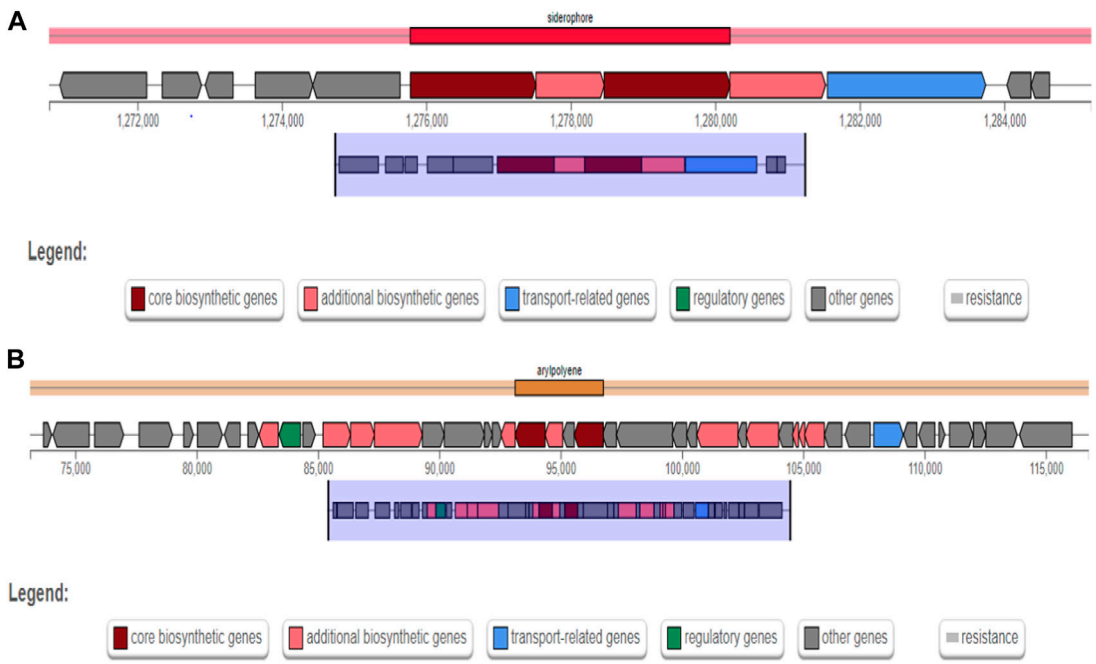

FIGURE 3 | *Enterobacter mori* strain AYS9 biosynthetic gene clusters for secondary metabolites from antiSmash. **(A)** siderophore gene clusters and **(B)** Arylpolene gene clusters.

of drought. A range of hosts, including plants, water, animals, and humans, have been observed to demonstrate an intimate interrelationship with *Enterobacter mori*, which has been isolated from many ecosystems. In general, it has been found that *E. mori*, one of the most prevalent species connected to plants, possesses a number of traits that support plant growth (Jha et al., 2011). As a result, knowledge based on their environmental and agricultural value in many crops and plants, such as white

mulberry (Zhu et al., 2011), maize (Khan et al., 2022), and peanut (Ludueña et al., 2019) has been reported. This is true to a larger extent due to their potential for plant growth promotion. Whole-genome analysis for identifying several *E. mori* genes implicated in PGP characteristics, phytohormones, and stress reduction have not yet been investigated. As a result, we presented the first report on the genomic data for *E. mori*, which was isolated from the sorghum rhizosphere in South Africa.

Enterobacter mori AYS9 was isolated on Luria-Bertani (LB) medium, evaluated on growth-promoting media, and finally sequenced. From both functional and taxonomic data, it was found that the number of genes found in strain AYS9 agrees with those tested in *in vitro* experiments. In addition to the genes that had been previously assessed, other significant genes of agricultural significance were also found. The strain AYS9 might control plant development in a variety of ways, according to the findings of studies that promoted plant growth. For example, in the solubilization of phosphate, IAA and biosynthesis of siderophore. Our results are consistent with past reports on the properties of *Enterobacter* strains and other rhizosphere bacteria associated with the root that promote plant development (Kämpfer et al., 2005; Mukherjee and Roy, 2016). Strain AYS9 showed the prospects of being able to mitigate drought stress through its ability to withstand exposure to the PEG 6000. Although its growth was observed to be decreasing initially at 5%–10% concentration of PEG, it started thriving at 20%–30% concentration of PEG. This may be a result of the ability of the rhizobacterium to produce IAA and ACC which has been widely reported to be an important drought-tolerant attribute of rhizobacteria (Fadiji et al., 2022c).

Microbes' capacity to fix atmospheric nitrogen for the use of the plant is dependent on certain enzymes and genes that they generate (Wang et al., 2004; Peng et al., 2009). Strain AYS9 was found to contain the nitrite reductase enzyme, which codes for by the genes *nirB* and *nirD* involved in the dissimilatory nitrate reduction pathway. Other genes discovered are involved in the transport of ammonia (*amtB*) and urea (*ureC*, *ureG*, *ureF*, and *ureE*), which may increase the amount of nitrogen in the soil. This outcome is comparable to one from a previous investigation in which flavodoxin and the ammonia transport gene (*amtB*) were found in MSR2 genome (Nascimento et al., 2020a).

The potential of strain AYS9 to dissolve phosphate under *in vitro* conditions on tricalcium media was verified by the discovery of many phosphate solubilization and transport-related genes. In a variety of bacterial phyla, gluconic acid (GA) is recognized as a precursor that promotes the solubilization of phosphate. Microbial activities and uses have been influenced by the production of GA by the enzyme glucose-1-dehydrogenase and its nonprotein chemical component pyrroloquinolone quinine (pqq) (Ramachandran et al., 2006). Despite the absence of *pqq* genes in AYS9 strain, investigations have revealed that the diazotroph bacterial endophyte *Herbaspirillum seropedicae* Z67 connected to commercial plants expresses heterologous *pqq* genes (*pqqABCDEF*) that exhibit phosphate solubilization capacity (Wagh et al., 2014). Similar to this, *Klebsiella* sp. D5A's genome was also found to be devoid of *pqq* genes (Guerrieri et al., 2021). Due to their high affinity and the presence of phosphate transport genes *pstABCS*, found in strain AYS9 may be better able to absorb phosphates. These results support those made earlier by Guerrieri et al. (2021) and Shariati J et al. (2017) regarding the phosphate transport genes *pstABCS* expression, on the WG analysis of *Klebsiella variicola* UC4115 and *Pantoea agglomerans* P5 respectively. Phosphorus absorption by soil and plant bioavailability may both be improved by the presence of *pst* genes. The discovery of the *phoA* gene, which codes for the alkaline phosphatase enzyme involved in numerous phosphorus metabolic processes including phosphate assimilation, was also present in strain AYS9, which was also consistent with the findings for *Burkholderia multivorans* WS-FJ9 genome (Liu et al., 2020). In addition, the discovery of the genes *ppx, pgl* and *pgl* in the AYS9 genome, which respectively encodes for 6-phosphogluconolactonase and exoployphosphatase for the synthesis of d-gluconate and inorganic pyrophosphatase degradation, may have influenced the bacterial strain's capacity to solubilize phosphate in the plant's rhizosphere. Our findings, however, are consistent with those made by (Nascimento et al., 2020b), who found that the genome of *Bacillus megaterium* STB1 contains *phoAD* genes that boost the bacterial strain's capacity to solubilize phosphate.

The expression of genes active in the synthesis of IAA and siderophore was found in the rhizosphere bacterial strain AYS9, which is consistent with earlier studies on the PGP traits of rhizobacteria (Peng et al., 2009; Tariq et al., 2014). It has been hypothesized that many pathways, including tryptamine, indole-3-acetonitrile (IAN), indole-3-acetamide, and indole-3-pyruvate (IPA), are involved in the production of IAA in plants and microbes (Spaepen and Vanderleyden, 2011). IAA, a key auxin found in plants, may influence gene expression and biosynthesis in microbes via signaling. Therefore, the production of auxin signal molecules by the microbes may be related to plant responses against naturally occurring harmful pathogens (Spaepen and Vanderleyden, 2011; Fadiji et al., 2022c). In addition, the identical functions of the *ipdC* gene in *Enterobacter cloacae* have been discovered (Koga et al., 1991) but were not observed in the genome of AYS9.

It's interesting to note that strain AYS9 has tryptophan synthase, which is irreversibly implicated in the pyridoxal phosphate pathway and reversibly catalyzes the condensation of serine and indole to create glyceraldehyde-3-phosphate from indole-3-glycerol phosphate (Ireland et al., 2008). The production of IAA by bacteria through the IAN route may be aided by the presence of amidase enzymes. Furthermore, strain AYS9's participation in two distinct IAA metabolic pathways demonstrated their capacity to produce IAA, which is essential for the creation and growth of plant roots. The AYS9 genome was then found to harbour *miaAB* genes involved in cytokinin production and transformation. The bacterial genome contains enzyme genes for tRNA dimethylallyltransferases that may improve the generation of iPR—N6-(dimethylallyl) adenosine.

In the *Pantoea phytobeneficialis* MSR2 genome, various cytokinin genes, such as *miaA* and *miaB*, are expressed. These genes change *Ipr* to 2-methylthio-N6-(dimethylallyl) adenosine and then to 2-methylthio-cis-ribozeatin (Nascimento et al., 2020a). The immediate effects of this specific gene on plant growth

promotion imply that cytokinin production may be crucial for promoting plant health and growth (Wani et al., 2016). Despite this, reports on cytokinin genes from this rhizobacterium strain AYS9 are not yet published, necessitating future comparative studies into agriculturally significant *E. mori* strain AYS9 to learn more about their unique genes. Plant development may be aided by the identification of siderophore and iron-related genes in the *E. mori* strain AYS9. These genes may increase the accessibility of plants to the soil's mineral resources. A crucial function in the mineralization of insoluble iron and its bioavailability for plant use may be played by iron transport genes identified in strain AYS9. Additionally, the bacteria's ability to produce 2,3-butanediol, induce systemic resistance and increase tolerance of the plant to drought have all been connected to their capacity for biocontrol (Madhaiyan et al., 2010; Samaras et al., 2021).

The pathogen-suppressing abilities of rhizobacteria depend on their capacity to create biocontrol agents. Rhizobacteria have used indirect processes in the creation of metabolic chemicals to control phytopathogens (Santoyo et al., 2016; Orozco-Mosqueda et al., 2021). It is possible that siderophore production, which increases the strain AYS9's antibiosis activity against plant pathogens, is the cause of the strain's capacity for biocontrol and stress reduction (Maheshwari et al., 2019). It has been determined that the siderophore catecholate present in the bacterial genome is crucial for bacterial adhesion to receptor surfaces, transport and iron chelation (Pedraza et al., 2010). In strain AYS9, iron transport-related genes like *fbpC* and *feoA* were discovered. Contrary to earlier findings, no prominent genes involved in siderophore formation and transport that are necessary for the change of chorismate into enterobactin, such as *fepABCDG* and *entABCDEFGHS* (Hubrich et al., 2021), we observed in this present study.

The quantity of intracellular polyamines is controlled by production, breakdown, excretion, and absorption from the environment (Kurihara et al., 2011). L-arginine is converted into putrescine via processes that are catalyzed by the enzyme's arginine decarboxylase and agmatinase. The *speA* and *speB* genes encode these enzymes. The two ornithine-decarboxylation enzymes (OCDs), which are either encoded by *speC* or *speF*, may also convert L-ornithine into putrescine (Kurihara et al., 2011; Schneider and Wendisch, 2011). Putrescine and decarboxylated S-adenosylmethionine (SAM) served as the building blocks for the production of spermidine, which needs the enzymes *speD* and *speE* and were identified in this AYS9 strain. Although it is unclear how these biosynthetic activities are controlled, the intracellular concentration of spermidine appears to be self-regulated (Shah and Swiatlo, 2008).

Plant sensitivity to heat or cold shock may be improved by the presence of osmotic stress-regulating genes. Similarly, to this, the protein-coding genes that control the effects of heat and cold stimuli can work through several gene families and differentiating regulations (Wani et al., 2016). The strain AYS9 included genes such as *betB*, a component of the glycine betaine biosynthesis pathway known for its osmoprotective properties. This supports past research on *Bacillus substilis* (Boch et al., 1996; Hussain Wani et al., 2013). Also, genes such as *proB* and *proC* involved in proline biosynthesis were identified in the strain AYS9. Additionally, strain AYS9 included genes related to proline biosynthesis, including *proB* and *proC,* which is one of the notable mechanisms used by beneficial microbes for the mitigation of drought stress. Pyroline-5-carboxylate synthetase (*P5CS1*), a stress-induced gene in *Arabidopsis*, limits L-proline incorporation in chloroplasts (Strizhov et al., 1997; Stein et al., 2011; Fadiji et al., 2021).

In any living creature, glutamine and glutamate act as the primary amino group donors for all nitrogen-containing substances, such as other amino acids and the building blocks for the creation of RNA and DNA. The strain AYS9 has genes for the production and transport of glutamate, including *gltB*, *gltD*, and *gltP*. Glutamate serves as a key intracellular potassium antagonist in addition to its involvement in anabolism (McLaggan et al., 1994). Additionally, certain bacteria and archaea use it as an osmoprotectant (Saum et al., 2006). Our findings are consistent with a previous study on *B. subtilis*, in which it was shown that glutamate is the precursor to proline, which is present in molar quantities in hyperosmotic circumstances and acts as a compatible solute to protect the cells (Brill et al., 2011).

In this strain AYS9 genome, it was discovered that *KatE*, which encodes catalase and is involved in the breakdown of hydrogen peroxide, was present. The transgenic indica rice cultivar is more tolerant to salt stress when the catalase genes are overexpressed in *E. coli* (Moriwaki et al., 2008). Additionally, *Escherichia coli* strain K12 catalase gene expression increases the jute plant's resistance to salt stress (Islam et al., 2013). Furthermore, the genome of strain AYS9 had the thiol peroxidase gene, which was shown to degrade organic hydroperoxides and hydrogen peroxide. This gene is well-known for its function in the biocontrol of wheat blast by *Bacillus* spp. This function is related to induced systemic resistance and the generation of antimicrobial chemicals in host plants like tomatoes (Baier and Dietz, 1996; El-Gaied et al., 2013).

CONCLUSION

In-depth Information on the prominent plant growth-promoting genes present in *E. mori* AYS9 with diverse roles and potential to mitigate drought were explored in this study. Some significant regulatory and plant growth-promoting genes were discovered by genome analysis. Notable genes that encourage plant development were found in *E. mori*. Their importance and promise in agriculture cannot be underestimated. The numerous genes discovered had biotechnological significance in the interactions between microorganisms and plants, in plant resistance to environmental stress (drought, osmotic and oxidative), and in organic compounds that could improve soil health and plant growth and the yield of the crop. The existence of these notable genes has important implications for understanding *E. mori* AYS9's genome PGP activities alongside showing promise in establishing sustainable agriculture. Additionally, according to the data on *E. mori* AYS9, future research is encouraged to take advantage of its potential as a prospective option for creating

bioinoculants to deal with abiotic stress-related agricultural issues in the future, most importantly through field trials.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

AUTHOR CONTRIBUTIONS

AF, AA, and OB conceived the ideas. AF collected the data and developed the manuscript. AA and OB provided technical input and proofread the manuscript. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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