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Effect of endophytic bacterium, *Stenotrophomonas maltophilia* JVB5 on sunflowers

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Abstract: Identifiable endophytic bacteria with plant growth-promoting traits promise to ensure sustainable agriculture. However, information on the versatility and exploration of sunflower-associated bacteria as bioinoculants is less studied. Here, we present the whole-genome sequence and annotation of *Stenotrophomonas maltophilia* JVB5 isolated from the sunflower root endosphere from the North West province, South Africa. The whole-genome analysis revealed a genome size of 4 771 305 bp, a sequence read count of 8 764 890, a 66% guanine-cytosine content, 57 tRNAs, 268 contigs, and 4 160 protein-coding genes with functions in various metabolic pathways. Pathways involved in the indole acetic acid production were found in the *S. maltophilia* JVB5 genome. The whole-genome annotation predicted notable genes involved in bacterial colonisation, antibiosis, and plant growth promotion. The predicted genes are involved in the sulfur metabolism, and the oxidative stress may enhance the plant growth promotion and boost plant the resistance to stress. Upon inoculation, *S. maltophilia* JVB5 efficiently colonised the sunflower root under greenhouse conditions with a significant improvement on the wet plant weight of 437.20 g compared to the uninoculated control with a 331.04 g wet weight. The genomic analysis revealing specific functional genes in the bacteria genome suggests their bioprospecting in agriculture. Hence, understanding the mechanisms employed by *S. maltophilia* JVB5 based on the predicted multifunctional genes will help harness their bioresource in sustainable plant health.

Keywords: bacterial genome; crop improvement; putative phytohormone genes; root adherence; sunflower; sustainable agriculture

The endosphere is an entire region within plant tissues colonised by endophytic microbes (Cavalcanti et al. 2020; Fadiji & Babalola 2020). In recent times, research into the endosphere microbial community to explore for beneficial endophytes to improve crop health is a promising feature in agriculture. Many studies have demonstrated biotechnological applications of endophytic microbes in combating future food scarcity and insecurity (Alkahtani et al. 2020). However, information on the novel agriculturally important endophytic bacterial strains associated with sunflower in Southern Africa is limited, thus necessitating this study.

Bacterial endophytes can form a mutual or antagonistic association with plants, with beneficial

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types classified as plant growth-promoting endophytes (PGPEs) (Adeleke & Babalola 2020). PGPEs enhance plant growth by supplying essential soil nutrients, producing growth hormones, fixing nitrogen, and synthesising chelating compounds (siderophores) that stimulate the plant response in the control of phytopathogens (Wang et al. 2020). Some examples of endophytic bacterial genera employed as bioinoculants include *Acinetobacter*, *Agrobacterium*, *Azospirillum*, *Bacillus*, *Brevibacillus*, *Burkholderia*, *Herbaspirillum*, *Mycoplasma*, *Pantoea*, and *Pseudomonas* (Haidar et al. 2018; Rahman et al. 2018).

Based on the ecological threats posed by the use of chemical fertilisers, the exploration and use of bacterial inoculants as alternatives stand as a promising and efficient method in developing a stable ecosystem and environmentally friendly agriculture practices (Nwachukwu et al. 2021). The bacterial genera *Stenotrophomonas* colonising the plant endosphere and the expression of certain functional traits enhance their metabolic activities in producing several metabolites and enhance their survival in different habitats (Rojas-Solís et al. 2018). Like other identified *Stenotrophomonas* species, *S. maltophilia* is a Gram-negative, rod-like, spore former, and facultative anaerobe, that is commonly found in a plant-soil environment. Recent findings on some agriculturally important *Stenotrophomonas* species are evident in literature, which suggests their future exploration as a suitable candidate in the formulation of bioinoculants to provide for a stable ecosystem, provide biosafety, and create sustainable agriculture (Alexander et al. 2019; Ulrich et al. 2021; Egamberdieva et al. 2022).

Stenotrophomonas maltophilia is one of the dominant groups of bacteria with diverse ecological functions ranging from agricultural to industrial applications. Reports on *S. maltophilia* producing secondary metabolites, extracellular enzymes, and plant growth-promoting genes are less documented, and information on the biotechnological application of *S. maltophilia* isolated from sunflowers is rare in literature. Insights into the genomic study of *S. maltophilia* will provide new information on the novel genes involved in the different biological functions. Hence, we performed genome sequencing of *S. maltophilia* JVB5 to identify its functional genes involved in plant growth and sustainable health.

MATERIAL AND METHODS

Isolation and screening of *S. maltophilia* JVB5

Sunflower roots were sourced from farmlands in Lichtenburg, South Africa (26°4'31.266"S, 25°58'44.442"E) in February 2020. An endophytic bacterium, *S. maltophilia* JVB5 was isolated from the sunflower roots on a Luria Bertani agar medium based on the modified method of Khamwan et al. (2018). Briefly, the plant roots obtained were placed in zip-lock bags in an ice-box for transportation to the laboratory within 4 h of collection for further analysis. For the bacterial isolation, the roots were cut into small sizes and surface-sterilised by immersion in 70% (v/v) ethanol for 1 min, followed by a 2% (v/v) hypochlorite solution for 3 min, and finally, immersed in 70% (v/v) ethanol for 30 seconds. The samples were thoroughly rinsed five times with sterile distilled water to remove the surface sterilisation agents. The sterility level of the samples was assessed by pour-plating onto the Luria-Bertani medium using the last water used to rinse the plant samples. The surface-sterilised root samples were macerated manually and serially diluted. From the serial dilution process, 0.1 mL from 10^6 dilutions were aseptically dispensed into sterile Petri dishes and poured onto plates with the sterilised Luria Bertani medium. The Petri dishes were then incubated at 28 °C for 24 h and checked for colony growth. The pure isolates were obtained by streaking a bacterium inoculum on fresh Luria Bertani agar plates. The pure isolates were kept on slants at 4 °C for further analysis.

The morphological and biochemical characterisation of *S. maltophilia* JVB5

The cultural attributes of *S. maltophilia* JVB5 were evaluated on the Luria Bertani (LB) media after incubation at 28 °C for 24 hours. The morphology of *S. maltophilia* JVB5 was visualised under a light microscope (ECLIPSE E200; Nikon, Japan). Biochemical tests, such as sugar fermentation tests (mannitol, glucose, sucrose, maltose, fructose, galactose, and raffinose), a catalase test, an oxidase test, a citrate utilisation test, starch and casein hydrolysis were performed using standard biochemical methods described by Bashir et al. (2020).

Plant growth-promoting screening

Siderophore production. The siderophore-producing potential of *S. maltophilia* JVB5 was investi-

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gated on chrome azurol S (CAS) Khan et al. (2020). The quantity of the siderophore produced was determined by inoculating the LB broth solution containing CAS with 0.1 mL of a 24-hour-old bacterial culture and then incubated at 28 °C at 180 rpm on a rotary shaker for seven days. Centrifugation of the cell suspension was achieved at 10 000 × *g* for 8 minutes. Zero-point five millilitres (0.5 mL) of the cell filtrate was added to 0.5 mL of the CAS reagent and properly mixed, then incubated for 2 minutes. The quantity of the siderophore released was measured at 630 nm using a spectrophotometer (Thermo Spectronic, Merck Chemicals, South Africa).

Phosphate solubilisation. The qualitative screening of the bacterial isolates for the phosphate solubilisation was performed according to the modified method of Khan et al. (2020). For the quantitative assay, the phosphate solubilisation tendencies of the bacterial isolates were performed by inoculating 10 mL of sterile Pikovskaya broth in 50 mL Falcon tubes with 0.1 mL (10^6 CFU/mL) of a freshly grown bacterial culture, incubated at 30 °C for five days at 160 rpm on a rotary shaker. A bacterial supernatant was obtained after centrifuging 10 mL of the bacterial culture at 4 °C (10 000 rpm for 5 min). Four millilitres of a colour reagent (1 : 1 : 1 : 2 ratio 3 M H_2SO_4, 10% (w/v) ascorbic acid, 2.5% (w/v) ammonium molybdate and distilled water were added to 10% (w/v) of the 5 mL trichloroacetic acid inside the test tubes. The tubes were incubated at normal room temperature for 15 min. The quantity of the phosphate was measured according to phosphomolybdate, a blue method at an absorbance of 820 nm. The medium without a bacterial inoculation served as control.

Exopolysaccharide (EPS) production. The bacterial isolates were qualitatively screened for EPS production following the method of Igiehon et al. (2019). Square-shaped sterile Whatman filter paper No. 1 was aseptically and gently placed onto the sterile molten LB agar plates. Two microlitres of the freshly grown 24-hour bacterial culture were directly inoculated onto the surface of the filter paper placed inside the sterilised media plates supplemented with sucrose (10%) with the pH adjusted to 7. Subsequently, the plates were aseptically inoculated and incubated at 30 °C for 2 minutes. The mucoid formation around the colonies on the square-shaped filter paper depicted the EPS production.

Indole acetic acid (IAA) screening. The indole acetic acid synthesis by *S. maltophilia* JVB5 was conducted according to Gutierrez et al. (2009). Ten millilitres of LB broth supplemented with tryptophan was aseptically inoculated with 100 mL of the freshly grown bacterial culture (10^6 CFU/mL) and incubated at 28 °C for seven days at 120 rpm in a rotary shaker incubator (SI-600; LAB Companion, South Korea). The bacterial culture was cold centrifuged (4 °C) at 10 000 rpm for 5 min to obtain the supernatant. From the supernatant, 1 mL was measured into a clean tube, and 2 mL of Salkowski's reagent (1 : 30 : 50 ratio of 0.5 M $FeCl_3$ solution: 95% w/w sulfuric acid : distilled water) was added. Then, a drop of 10 mM orthophosphoric acid was also added to the mixture and incubated for 10 min for the observable colour development. The appearance of pink coloration in the tubes after incubation in the dark indicated a positive result. An uninoculated tube served as the control. The samples were measured using a UV-Vis spectrophotometer (Thermo Fisher Scientific, Carlsbad, CA, USA) at 530 nm from the reacting mixture and the control tube.

Whole-genome sequencing of S. maltophilia JVB5. The bacterial identification was confirmed by 16S rRNA gene sequencing before the whole-genome sequencing (WGS). The standard Illumina sequencing method was employed for the WGS at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa. The genomic DNA was fragmented using an enzymatic approach (NEB Ultra II FS kit; New England Biolabs, Ltd, UK). The resulting DNA fragments were selected by size (200–700 bp), using AMPure XP beads (Beckman Coulter, USA). Each DNA fragment was end-repaired and ligated to Illumina-specific adapter sequences. The DNA samples were singularly indexed, and a second size selection step was achieved. A fluorometric method was used to quantify each sample at a dilution standard concentration (4 nM). The sequencing was performed on Illumina's NextSeq platform, using a NextSeq mid-out kit (300 cycles), according to the manufacturer's instructions. The resulting 400 Mb of the 2 × 150 bp paired-end reads dataset was obtained from *S. maltophilia* JVB5.

The genome analysis of the sequences was performed on Kbase (https://kbase.us/) (Arkin et al. 2018). A quality check of the sequence was performed by FastQC (version 0.11.5) (Babraham Bioinformatics 2011), and Trimmomatic (version 0.36) (Bolger et al. 2014) was used for the trimming and further assembled by SPAdes (version 3.13.0) (Nurk

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et al. 2013). The gene annotation was performed on RASTtk (version 1.073). The bioinformatics analysis was performed using the default settings. The whole-genome sequence at GenBank was annotated with the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline (PGAP) during submission as a fully automated process. The metabolic pathways of the biomolecules were obtained from KEGG on RAST (https://rast.nmpdr.org/rast.cgi/). The biosynthetic gene clusters present in the genome of the organism were detected using antiSMASH (version 6.0.0) (https://antismash.secondarymetabolites.org) (Weber et al. 2015). The circular genome visualisation was obtained from PATRIC (https://www.patricbrc.org/) (version 3.6.9), while the phylogenetic analysis of the whole genome sequence was performed using MrBayes (http://www.phylogeny.fr/one_task.cgi?task_type=mrbayes) (version 3.2.6) (Huelsenbeck & Ronquist 2001; Arkin et al. 2018).

Availability of data and material

The data available from the NCBI database output include the Bioproject number (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA706608), Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra/?term=SRR13908543), and accession and strain numbers (https://www.ncbi.nlm.nih.gov/genome/?term=JAGEKL000000000) and JVB5 search (https://www.ncbi.nlm.nih.gov/genome/browse/"\l"!/prokaryotes/880/JVB5).

Greenhouse experiments

The effect of *S. maltophilia* JVB5 on the sunflower growth was tested in the greenhouse. Briefly, the experiment was carried out using sterile and non-sterile soil sourced from agricultural farmlands at North-West University, the Mafikeng Campus (25°49'26.256"S, 25°36'31.536"E). The soils were collected at the depth of 0–20 cm. The soil debris and other plant materials were removed, oven-dried at 70 °C for 48 h, and sieved with a 2-mm stainless steel mesh, and then autoclaved at 121 °C for 15 minutes. The sterilised soil was allowed to cool for 2 days after which 15 kg of soil was aseptically transferred into plastic pots.

Sunflower seeds were surface-sterilised by washing, first in 70% ethanol for 3 min, then in 3% hypochlorite for 1 min, and finally dispensed in 70% ethanol for two minutes. The seeds were further washed with water to remove the disinfectants. The bacterial isolate was cultured on the LB medium at optimum growth conditions. The broth suspension was centrifuged at 4 °C (1 000 rpm for 5 min) using a centrifuge and then the pelletised bacterial cells were washed in 0.85% (w/v) normal saline. The inoculum size was standardised to 0.5×10^6 CFU/mL at OD_{600}. The seeds were inoculated in the bacterial suspension while seeds suspended in sterile distilled water served as the control. Twenty-four plastic pots containing the experimental soils were randomly arranged in the greenhouse at 10 cm apart. Ten seeds were planted in each pot containing sterilised moistened soil. After planting the seeds, the moisture content of the soil was maintained by adding 500 mL of sterile water. The light conditions in the greenhouse were a 14-hour photoperiod and 10 h of dark, and the temperature ranged between 25 °C and 30 °C. At maturity, i.e., 132 days, the sunflowers were harvested. The sunflower roots were thoroughly washed with water and then excised from the shoots. The root and stem weight (dry and fresh) parameters were recorded after determining the weights on a weighing balance (Wagi Elektroniczne, Poland). The experiments were performed in triplicate. The experimented soil contains 68.30% of sand, 7.92% of silt, 22.20% of clay, and 3.20% of organic matter. The soil pH was 7.4. Six weeks after the sunflower seeds were inoculated and placed in the greenhouse, re-isolation of the bacterial strain was performed from the plant roots and the same endophytic bacterium was identified, which confirm the adherence of *S. maltophilia* JVB5 to the sunflower roots.

Data analysis

The analysis of the data from this study was performed using the Statistical Package for the Social Sciences (SPSS; version 6.0) and Microsoft Excel (version 13). A one-way analysis of variance (ANOVA) was performed for the data, followed by Duncan's test at a 5% level of significance to determine the differences between the means. The obtained data were presented as mean ± standard deviation.

RESULTS

The morphological, biochemical, and plant growth-promoting features of the endophytic bacterium, *S. maltophilia* JVB5

The morphological, biochemical, and plant growth-promoting features of the endophytic

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Table 1. Morphological, biochemical, and screening of *Stenotrophomonas maltophilia* JVB5

Cultural characteristics							
GR	SHP	CT	SH	NT	OXD	CIT	CSH
(–)	Rod	(+)	(+)	(+)	(+)	(+)	(++)
Growth conditions							
Salt tolerance (% v/v)		temperature (°C)		pH			
3.5		25–40		4–10			
Isolate screening				**enzyme assay**			
	quantitative	qualitative		ZC (mm)	qualitative		
Siderophore	79.90 ± 0.15^{c}	(++)	xylanase	22.00 ± 0.01^{d}	(+)		
IAA	30.0 ± 0.01^{a}	(+)	protease	2.00 ± 0.01^{b}	(+)		
Phosphate	32.23 ± 0.21^{b}	(+)	amylase	9.00 ± 0.01^{c}	(+)		
EPS		(+++)	mannanase	0.00 ± 0.00^{a}	(+)		
			cellulase	50.00 ± 0.01^{e}	(+)		
Sugar fermentation							
Glucose	xylose	galactose	arabinose	sucrose	raffinose	mannitol	maltose
(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)

(–) = negative reaction; (+) = positive reaction; CIT = citrate; CSH = casein hydrolysis; CT = catalase; EPS = exopolysaccharide; GR = Gram-reaction; IAA = indole acetic acid; NT = nitrate; OXD = oxidase; SH = starch hydrolysis; SHP = shape; ZC = zone of clearance measurement

[a–e]Mean ± standard deviation values with different superscripts (small letters) within the same column represent significant difference

bacterium, *S. maltophilia* JVB5 are presented in Table 1. *S. maltophilia* JVB5 is Gram-negative, catalase-positive, rod-like in shape, ferments the tested sugars, and the growth conditions ranged between a temperature of 25 °C and 40 °C, and a pH of 4 and 10. The bacteria yielded approximately 80% siderophore activity, 32 µg/mL of phosphate activity, and 30 µg/mL of IAA activity. Varied enzyme activity [xylanase (22.00 ± 0.01 mm), protease (2.00 ± 0.01 mm), amylase (9.00 ± 0.01 mm), mannanase (0.00 ± 0.00 mm), and cellulase (50.00 ± 0.01 mm)] on the plate assay was exhibited by *S. maltophilia* JVB5 (Table 1).

Whole-genome information of *S. maltophilia* JVB5

The resulting output from the whole genome analysis is presented in Table 2. A total genome size of 4.77 Mb, a read count of 8 764 890 sequences, 1 323 498 390 bases, a 66% GC content, a mean read length of 151 bp, and 268 contigs were obtained from *S. maltophilia* JVB5. The L_{50} and N_{50} values were 49 and 28 858 bp, respectively. Figure 1 shows the whole genome circular view of *S. maltophilia* JVB5. Also, the phylogenetics of the whole genome sequence of *S. maltophilia* JVB5 is presented in Figure 2.

Gene prediction of *S. maltophilia* JVB5

The predicted genes responsible for the bacterial functions were predicted in the genome of *S. maltophilia* JVB5 [Tables 3–5, and supplementary Tables S1–S13 in electronic supplementary material (ESM) (for the supplementary material see the electronic version)]. Table S1 in ESM shows the genes involved in the secretion system. Similarly, the predicted genes involving carbohydrate, such as mannose, hexose, rhamnose, erythrulose,

Table 2. Whole-genome characteristics and resources of *Stenotrophomonas maltophilia* JVB5

Whole-genome information	*S. maltophilia* JVB5
Size	4 771 305 bp
G + C content	66%
Number of contigs	268
Number of genes coding RNAs	58
L_{50}	49
Number of subsystem	305
N_{50}	28 858 bp
Number of coding sequences	4 446

G + C = guanine + cytosine; L_{50} = least number of contigs that are summed up to 50% genome; N_{50} = length of a contig

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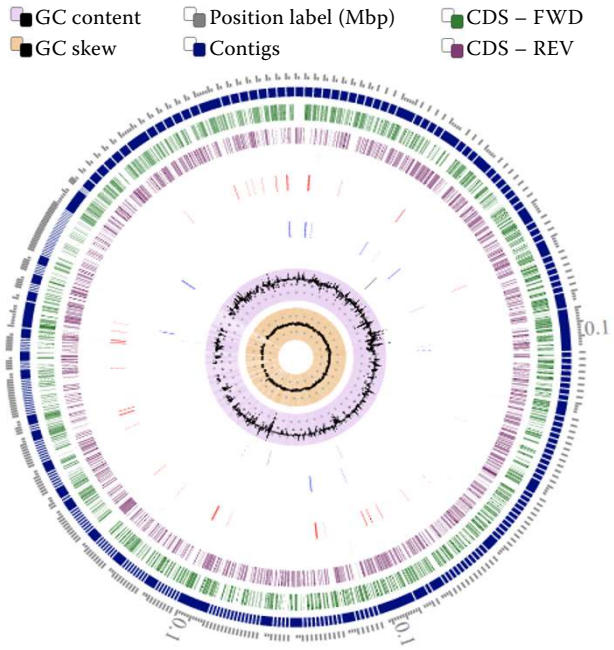

Figure 1. Circular visualisation of the whole-genome of *Stenotrophomonas maltophilia* JVB5

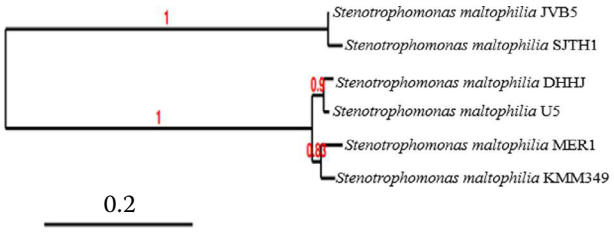

Figure 2. Phylogenetic tree of the whole genome-sequence of *Stenotrophomonas maltophilia* JVB5

fructose, and inositol, degradation and metabolism are shown in Table S2 in ESM. The functional genes responsible for the carbohydrate, transport, amino acid, and organic acid metabolism were detected in the genome of *S. maltophilia* JVB5 (Tables S3–S5 in ESM). The nitrogen fixation genes, *nif*SF, *amt*, and *azt*F coding for cysteine desulfurase, flavodoxin, ammonium transport, and allophanate hydrolase are presented in Table S6 in ESM. The other phosphate solubilisation and transport genes, such as *pho*U, *agp*, and *pst*ABCS, and the sulfate transport pathway, *cys*KQ were predicted in the genome of *S. maltophilia* JVB5 (Table 3). The sulfur metabolism genes were also profound in the bacterial genome (Table S7 in ESM).

The siderophore genes, such as *fur*, *feo*AB, *fhu*E, *tes*B, and *ent*ABCF, responsible for the enterobactin synthesis and iron transport were detected in the genome of *S. maltophilia* JVB5 (Table 4). Several genes involved in the chemotaxis (*che*ABDRYZ, *mot*AB, *mcp*, *flg*ABCDEFGHM, and *flh*ABF), flagella biosynthesis (*fli*DEGFMNPRS), pilus and fimbriae (*fim*V, *pil*BGHOVW, and *cpa*B), type IVa pilus homologue protein (*pil*C, *cpa*B, and *tad*D) were also found (Table S8 in ESM). Genes responsible for the bacterial attachment to plant surfaces are presented in Table S9 in ESM. Quorum-sensing genes were also predicted in the genome (Table S10 in ESM). Genes, such as *sod*1, *sod*2, *kat*EG, and *ahp*CF, coding for superoxide anion radicals, hydrogen peroxide, and organic hydroperoxide degradation for plant protection against oxidative and nitrosative stress were profound in the bacterial genome (Table S11 in ESM). Furthermore, *S. maltophilia* JVB5 harboured lignin-degrading genes (Table S12 in ESM). Several genes, such as *trp*ABCD, *ami*E, and *mia*A, involved in the IAA production in the indole-3-acetamide pathway (IAM) were predicted in the bacterial genome (Table 5). Notable osmotic stress tolerance genes (*bet*ABLT) involved in the glycine betaine and proline biosynthetic pathways

Table 3. Predicted genes involved in the phosphate metabolism and transport

Gene	Locus tag	Function	Pathway
*pho*U	J0661_14530	phosphate signaling complex protein *Pho*U	organic phosphate solubilization
ppa	J0661_00840	inorganic pyrophosphatase	degradation of inorganic polyphosphates
ppx	J0661_03465	exopolyphosphatase	
*phn*D	J0661_03670	phosphate/phosphite/phosphonate ABC transporter substrate-binding protein (SBP)	degradation of phosphonates
*pst*S	J0661_14510	phosphate transporter (PT) ABC	phosphate transport
	J0661_17540	SBP	
*pst*C	J0661_14515	(PT) ABC – *Pst*C – permease enzyme	
*pst*A	J0661_14520	(PT) ABC permease *Pst*A	
*pst*B	J0661_14525	(PT) ABC – *Pst*B	

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Table 4. Predicted genes involved in the iron metabolism and siderophore synthesis

Gene	Locus tag	Function/product	Pathway
fur	J0661_18340	ferric iron uptake transcriptional regulator	iron(III) transport
–	J0661_19495	iron ABC transporter permease	
*feo*A	J0661_01485	iron transporter protein A	iron(II) transport
*feo*B	J0661_01490	iron transporter protein B	
*fhu*E	J0661_10800	ferric-coprogen receptor *Fhu*E	iron complex transport
*ent*H	J0661_00050	thioesterase	enterobactin production
	J0661_01835		
	J0661_02500		
	J0661_03535		
	J0661_08840		
	J0661_09715		
*tes*B	J0661_17755	acyl-CoA thioesterase II	
*ent*B	J0661_20195	isochorismatase	
*ent*C	J0661_20205	isochorismate synthase	
*ent*F	J0661_20185	enterobactin synthase component F	
*ent*A	J0661_20180	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase	*ent*A

Table 5. Predicted genes involved in the phytohormone synthesis and modulation

Gene	Locus tag	Function/product	Pathway
*trp*A	J0661_15745	subunit alpha (tryptophan synthase)	L-tryptophan production; indole acetic acid production
*trp*B	J0661_15735	subunit beta (tryptophan synthase)	
*trp*D	J0661_17720	anthranilate phosphoribosyltransferase	
*trp*C	J0661_17725	indole-3-glycerol phosphate synthase *TrpC*	
*ami*E	J0661_12320	amidase	indole acetic acid production; indole-3-acetamide pathway
*mia*A	J0661_15225	*mia*A	cytokinins biosynthesis and transformation
*mia*B	J0661_08550	*mia*B	
*xdh*C	J0661_04265	xanthine dehydrogenase iron-sulfur-binding subunit	

were predicted in *S. maltophilia* JVB5 (Table S13 in ESM).

Identifiable secondary metabolite gene clusters, such as arylpolyene, non-ribosomal peptides, lanthipeptide class II, and RiPP-like, were predicted in the bacterial genome (Table 6). Several of notable secondary metabolite gene clusters detected include regulatory genes, additional biosynthetic genes, other genes, resistance transport-related genes, and core biosynthetic genes (Figures 3A and 3B). Gene clusters with 17% and 42% non-ribosomal peptides (NRPS) and arylpolyene, similar to the known cluster for arylpolyene and streptobactin, were detected at node regions 1.1 and 258.1 (Table 6), respectively.

Table 6. Secondary metabolite gene composition in *Stenotrophomonas maltophilia* JVB5

Region	Kind/type	Start/from	To/stop	Most similar known cluster	%Score
1.1	arypolyene	1	27 392	arypolyene (other)	42
64.1	RiPP-like	42 315	48 197	–	–
208.1	RiPP-like	1	5 829	–	–
22.1	lanthipeptide class II	1	2 889	–	–
258.1	non-ribosomal peptide	1	27 229	streptobactin (non-ribosomal peptide)	17

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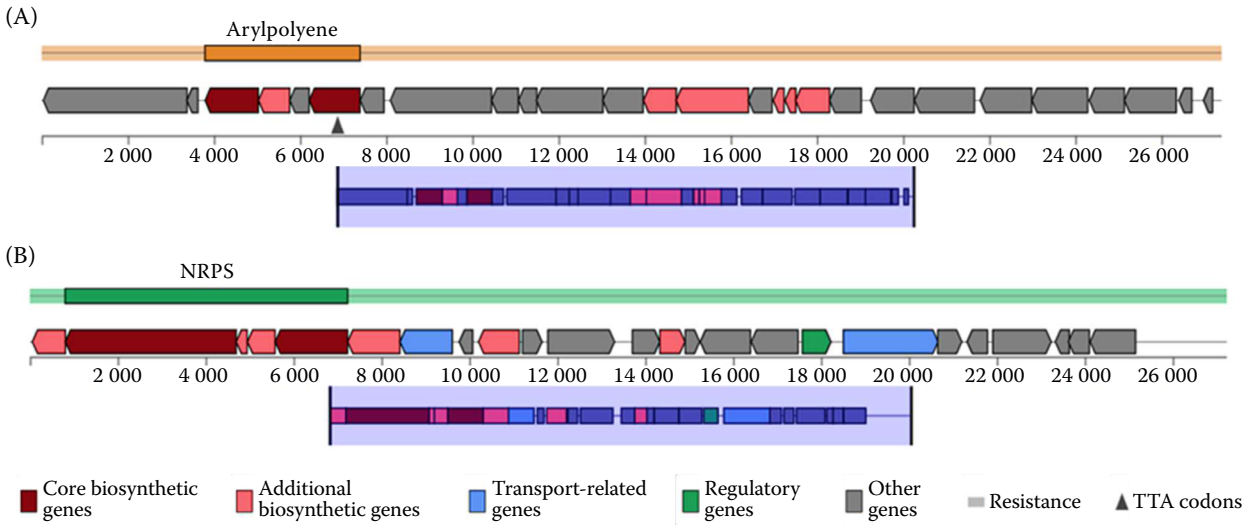

Figure 3. Arylpolyene and non-ribosomal peptide (NRPS) encoding genes

Plant yield output

The comparison between the inoculated and uninoculated sunflower yield parameters obtained from the greenhouse is presented in Table 7. Except for the taproot width and seed weight (wet and dry), the yield output above and below the ground levels of the inoculated sunflower show a significantly different results compared to the uninoculated sunflower plants. The inoculated plant yielded a 437.20 g plant wet weight compared to the uninoculated plant with a 331.00 g wet weight. Similar results were obtained on the shoot dry weight with 57.3 g (uninoculated) and 76.2 g (inoculated), uninoculated head fresh and dry weight of 153.7 g and 41.6 g, and inoculated head fresh and dry weight of 200.8 g and 55.1 g, respectively. Also, the root wet and dry weight values of the inoculated and uninoculated sunflower were recorded at 84.2 g and 19.4 g and 44.1 g and 8.4 g, respectively.

DISCUSSION

In this study, we investigated the whole genome sequence of *S. maltophilia* JVB5 and its potential for improving the sunflower yield under greenhouse experimental conditions. The preliminary *in vitro* screening reveals the plant growth-promoting traits of *S. maltophilia* JVB5. The ability of bacterial endophytes to produce certain phytostimulants and metabolic compounds, such as siderophores, IAA, ammonia, hydrogen cyanide, lytic enzymes,

Table 7. Sunflower yield output

Growth parameters (belowground)							
	TRL (mm)	TRW (g)	RL (mm)	LRN	RWW (g)	RDW (g)	NR
Inoculated	191.7 ± 10.69^{b}	6.3 ± 0.58^{a}	294.3 ± 12.50^{b}	31.7 ± 0.58^{b}	84.2 ± 13.78^{b}	19.4 ± 19.41^{b}	1163.7 ± 61.92^{b}
Uninoculated	146.7 ± 58.33^{a}	5.3 ± 0.58^{a}	217.0 ± 81.22^{a}	25.7 ± 1.53^{a}	44.1 ± 16.12^{a}	8.4 ± 1.55^{a}	935.0 ± 11.30^{a}
Growth parameters (aboveground)							
	SWW (g)	SDW (g)	HFW (g)	HDW (g)	PWW (g)	SHWW (g)	SHDW (g)
Inoculated	0.1 ± 0.01^{a}	0.03 ± 0.01^{a}	200.8 ± 32.58^{b}	55.1 ± 13.55^{b}	437.2 ± 21.08^{b}	204.6 ± 8.07^{b}	76.2 ± 10.09^{b}
Uninoculated	0.1 ± 0.01^{a}	0.03 ± 0.01^{a}	153.7 ± 13.94^{a}	41.6 ± 4.27^{a}	331.0 ± 20.16^{a}	165.8 ± 5.16^{a}	57.3 ± 7.04^{a}

HDW = head dry weight; HFW = head fresh weight; LRN = lateral root number; NR = number of roots; PWW = plant wet weight; RDW = root dry weight; RL = root length; RWW = root wet weight; SDW = seed dry weight; SHDW = shoot dry weight; SHWW = shoot wet weight; SWW = seed wet weight; TRL = taproot length; TRW = taproot width

^{a,b}Mean ± standard deviation values with different superscripts (small letters) within the same column represent a significant difference

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and antibiotics, suggests their use in sustaining plant growth and providing immunity (Alkahtani et al. 2020; Fouda et al. 2021). The results obtained in this study conformed to those of Bashir et al. (2020), who, in their findings, evaluated the plant growth-promoting attributes of endophytic bacteria isolated from sunflowers. Nevertheless, few reports have been documented on the genome analysis of agriculturally important *Stenotrophomonas* species (Pinski et al. 2020), thus findings on endophytic bacteria *S. maltophilia* JVB5 can serve as a model in future studies. Therefore, in our view, this is the first documentation on the genome analysis of endophytic bacterium *S. maltophilia* JVB5 isolated from the sunflower root endosphere, although other *Stenotrophomonas* species might have been reported in sunflowers.

The genomic assessment of *S. maltophilia* JVB5, as investigated in this study, is aimed to provide insights into the PGPE genes and other metabolic genes underlining the bacterial functions in enhancing the sunflower yield. Interestingly, notable genes involving the siderophore production, phosphate solubilisation, carbon metabolism, phytohormone synthesis, cell attachment, and colonisation were predicted in the *S. maltophilia* JVB5 genome. Bacterial DNA contains an array of genes with varied genome sizes and specific functions (Westoby et al. 2021). In this study, a strong correlation existed between the genome size (4.7 Mb) of *S. maltophilia* JVB5 compared to the genome size (4.3 Mb) and (4.7 Mb) of endophytic bacteria *S. maltophilia* strains RR-10 and B418 isolated from rice and barley, respectively (Zhu et al. 2012; Wu et al. 2015). Evidence has shown that bacteria with a bigger genome size tend to adapt to different habitats because of their additional encoding gene products involved in the metabolism of organic compounds and osmotic stress (Westoby et al. 2021). Also, a finding has argued about the ability of bacteria with small genome sizes to efficiently participate in the metabolism of organic substrates (Zeng et al. 2018).

In line with plant growth-promoting screening, genes involved in the phytohormone synthesis were predicted in the genome of *S. maltophilia* JVB5. Insights into phytohormone genes from endophytic bacteria with important functions in plant growth promotion have been studied (Nascimento et al. 2020a; Samaras et al. 2020), but such reports are less documented in the bacteria genera *Stenotrophomonas*. In this study, genes (*trp*ABCD) involved in the IAA biosynthesis were predicted in the genome of the *S. maltophilia* JVB5 genome. The detection of IAA genes in the genome of *S. maltophilia* JVB5 can mediate their actions in enhancing the root development in plants. The results obtained in this study corroborate the findings of Singh et al. (2021), who reported IAA genes, such as *trp*BE, *trp*ABCDE, and *trp*ABD, from the whole genome analysis of endophytic bacterial strains from plants. From a previous study, the *in vitro* screening of IAA-producing endophytic *Stenotrophomonas* isolated from sunflowers has been documented (Majeed et al. 2015), but their genome assessment for detecting IAA genes is still lacking, hence limiting their full exploration in agricultural biotechnology. However, the results obtained in this study validate the report of Khamwan et al. (2018) on the potential use of IAA-producing *S. maltophilia* isolated from *Helianthus tuberosus* in enhancing the plant root development and growth promotion.

The predicted genes involved in the metabolism of energy-yielding compounds in the whole genome of *S. maltophilia* JVB5 show their preference in metabolising complex organic substrates, which enhance their colonisation efficiency in the plant endosphere. A plant's adaptation and survival under stress conditions have been linked to the microbial actions in utilising accumulated trehalose in the soil environment (Pinski et al. 2020). The detection of enzymes malto-oligosyltrehalose synthase and trehalose-phosphatase coding genes (*tre*YZ) involved in the trehalose degradation and uptake of exogenous trehalose in *S. maltophilia* JVB5 can be an indication in boosting the sunflower tolerance to high osmotic or salinity stress (Pinski et al. 2020).

The secretion system genes detected in the genome of *S. maltophilia* JVB5 corroborate the findings of Liu et al. (2016), who reported secretion system and type I-VI genes were found in the endophytic *Klebsiella* sp., *Variovorax paradoxus*, and *Pseudomonas fluorescens* genome. The presence of these genes in *S. maltophilia* JVB5 may contribute to plant growth promotion and bacterial attachment/colonisation in the root endosphere. Other types of secretion system genes, type I-IV have also been reported in the genome of *Stenotrophomonas* strain 169 isolated from the field-grown poplar (Ulrich et al. 2021). Interestingly, similar genes in the genome of *Bradyrhizobium diazoefficiens* and *Mesorhizobium loti* have been identified with multiple func-

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tions in biofilm production and the establishment of plant-bacterial interactions (Li et al. 2020).

Nitrogen, phosphorus, and potassium are essential nutrients required by plants, and the role of endophytic bacteria in sustainable plant growth through nitrogen fixation and soil mineral solubilisation has been reported in several studies (Bashir et al. 2020; Khalil et al. 2021). Based on the gene functions, the potential of endophytic bacteria, such as *Bacillus*, *Pseudomonas* and *Enterobacter*, to fix nitrogen to the soil is important in improving the soil profile and plant growth (Guo et al. 2020). The effects of *S. maltophilia* on the sunflower yield upon inoculation, as reported in this study, can be due to the expression of the nitrogen fixation genes in the bacterial genome and this agrees with the report of Singh et al. (2020). The predicted multiple nitrogen fixation genes, *nif*ABDEFHJKLMNQSTUVWZYZ in the genome of *Klebsiella* have been employed as a stereotype for studying nitrogen fixation in diverse bacterial strains (Samaras et al. 2020). In this study, the detection of nitrogen fixation genes, such as *nif*SF, ammonia transport gene (*amt*), and urea degradation, and the metabolism gene in the bacterial genome may suggest their use in formulating bioinoculants as an alternative to nitrogen fertiliser in an organic farming system. Similar genes involved in nitrogen metabolism, such as *nif*HU, *aat*JMPQ, and *glt*BDPS, have been detected in the genome of *P. aeruginosa* B18 (Singh et al. 2021).

The ability of bacterial endophytes to fix phosphorus in the soil has played an important role in plant nutrition (Passari et al. 2016). Endophytic *S. maltophilia* strains, namely, SEN_1 (Etesami & Alikhani 2016), B11 (Kasim et al. 2021), SY-2 (Liaquat et al. 2020), have been identified as phosphate solubilisers with the potential of enhancing plant growth. The phosphorus-solubilising potential of *S. maltophilia* JVB5 in a tricalcium medium was confirmed in the genome analysis, as diverse genes involving phosphate transport and metabolism were predicted in the genome. The detection of phosphate transport genes (*pst*ABCS) and other regulatory genes, such as *pho*U, *phn*D, and *agp*, as revealed in this study, suggest the role of *S. maltophilia* JVB5 in the degradation and assimilation of phosphate compounds, thus making phosphorus available in the soil for plant use. Besides phosphates, studies argued phosphonates are another source of phosphorus in the soil (Oliverio et al. 2020), with a similar gene identified in this study. The results obtained in this study corroborate that of Singh et al. (2021) and Nascimento et al. (2020b) who reported *pst*ABCS and *pho*U in the genome of *P. aeruginosa* B18 and *Pantoea phytobeneficialis* MSR2, respectively.

The genome analysis revealed genes (*cys*KQ) involved in the sulfur transport in *S. maltophilia* JVB5, thus classifying these bacteria as a sulfur producer. The predicted genes in the bacterial genome can mediate the mineralisation of sulfur-containing compounds, recycling of organic sulfate, root colonisation, plant tolerance to stress (Battu & Ulaganathan 2020). The enzyme-coding genes, bisphosphate nucleotidase and cysteine synthase A, detected in *S. maltophilia* JVB5 might be a unique feature of this bacteria which was compared to the *cys*A, *cys*W, and *cys*P genes identified in the genome of *Enterobacter* and *Pseudomonas* in transporting thiosulfate or sulfate molecules in the living cell (Guo et al. 2020; Singh et al. 2021). Exploration of *S. maltophilia* JVB5 in agriculture, based on their ability to degrade phosphate and phosphonate, can improve the soil mineral levels and supply to the plants.

The siderophore is another key plant growth-promoting trait exhibited by *S. maltophilia* JVB5. Identification of siderophore genes, such as siderophore enterobactin genes, *fes*, *ent*D, and *fep*A, has been reported to reduce the iron level and capture the fixed iron siderophore in the soil (Nascimento et al. 2020b; Singh et al. 2021). Several siderophore genes mediating the plant response against osmotic stress and pathogens have been identified in *Pseudomonas* species (Kang et al. 2020). The detection of siderophore genes, *ent*ABCFH, in the genome of *S. maltophilia* JVB5 agrees with Guerrieri et al. (2021) and Hubrich et al. (2021), who reported similar genes in the genome of the endophytic bacterium *Klebsiella variicola* UC4115, with functions in deriving enterobactin from chorismate.

Various chemotaxis genes identified in this study suggest the bacterial activity to adhere, penetrate and colonise plant endosphere in overcoming the surface repulse, cell displacement, and biofilm formation due to the production of extra polymeric substances (cellulose and exopolysaccharide) and attachment organelles (flagella, fimbriae, and pili) (Zheng et al. 2015).

The adherence of endophytic bacteria on surfaces can help build up signal molecules for the gene expression and regulation, cell-to-cell com-

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munication, and bacterial adaptation under stress conditions (Adeleke et al. 2021). Also, the detection of the flagella protein in the bacterial genome may stimulate host defensive mechanisms against plant pathogens, enhance the bacterial colonisation and plant tolerance to unfavourable conditions, thus increasing the endosphere competence (Shastry et al. 2020). The predicted genes mediating chemotaxis, flagellar biosynthesis, fimbriae, and pilus production/biosynthesis in *S. maltophilia* JVB5 confirm the findings of Singh et al. (2021), who reported similar genes in *P. aeruginosa* B18. Nevertheless, the whole-genome sequencing of the agriculturally important *S. maltophilia* associated with the sunflower reveals that the motility, chemotaxis, and biofilm production genes have not been documented.

A gene (*cyc*A) mediating quorum-sensing (QS) signal molecule, *N*-acyl-L-homoserine lactones, was found in the bacterial genome. The detection of *N*-acyl-L-homoserine lactones hydrolase in *S. maltophilia* JVB5 suggests their ability in the metabolism and modulation of AHLs, which may directly influence their lifestyle and ecological functions. Bacteria respond to oxidative stressors based on their ability to produce certain enzyme regulatory proteins and metabolic compounds. The predicted enzyme-coding genes, such as catalase, catalase-peroxidase, peroxiredoxin, superoxide dismutase, and alkyl hydroperoxide reductase in *S. maltophilia* JVB5 and their function in degradation free radicals, hydrogen peroxide, organic hydroperoxides, and superoxide anion can mirror their ability in mitigating oxidative stressors in plants. The detection of *sod* genes in the genome of *P. putida* has been reported to stimulate plant resistance and defence against pathogens (Gupta et al. 2014). In addition, the detection of oxidative stress genes in *S. maltophilia* JVB5 can inform us on their potential in mitigating oxidative stress in plants. Nevertheless, the exact mechanisms employed by endophytic bacteria in mitigating oxidative stress need further study.

Different secondary metabolite gene clusters, such as arylpolyene, NRPS (streptobactin), lanthipeptide class II, and RiPP-like, were identified in *S. maltophilia* JVB5. Interestingly, six biosynthetic genes coding for secondary metabolites were predicted in *S. maltophilia* JVB5. Exploring these products and their biocontrol activities against plant pathogens can ensure sustainable plant health. The detection of arylpolyene gene clusters in *S. maltophilia* JVB5 may stimulate bacterial functions against oxidative stressors. Similar genes with similar functions have been reported in stress-tolerant *B. megaterium* STB1 and other prokaryotes (Cimermancic et al. 2014).

Sunflower inoculation with *S. maltophilia* JVB5 under greenhouse experiments showed an improvement in the sunflower yield compared to the uninoculated plants. The root development and early flowering in sunflowers may be linked to the phytohormone synthesis and detection of phytohormone genes in the bacterial genome. The effect of the co-inoculation of endophytic bacteria genera, such as *Stenotrophomonas, Bradyrhizobium, Pseudomonas, Kosakonia,* and *Azospirillum*, on the growth parameters and yield of sunflowers and other crops under greenhouse experiments have been performed with success (Egamberdieva et al. 2016; Youseif 2018; Singh et al. 2020; Adeleke et al. 2021; Kasim et al. 2021). Furthermore, the sunflower yield enhancement by *S. maltophilia* JVB5 upon inoculation in the greenhouse corroborates the results of Adeleke et al. (2021) who reported a significant difference in the yield of sunflowers inoculated with endophytic *S. indicatrix* BOVIS40.

CONCLUSION

In conclusion, *S. maltophilia* JVB5 exhibited positive reactions to the *in vivo* plant growth-promoting assay. The genomic elucidation of *S. maltophilia* JVB5 has provided novel insights into the functional genes responsible for plant-bacteria interactions with profound plant growth-promoting activities rather than being an opportunistic pathogen. A sunflower yield improvement was observed based on the bacterial colonisation and the presence of diverse functional genes involved in the phytohormone synthesis, carbohydrate metabolism, quorum-sensing, secretion system, lignin degradation, and oxidative and nitrosative stress. *S. maltophilia* JVB5 harbouring oxidative and putative secondary metabolite genes can be employed as a biocontrol agent against phytopathogens for improved crop production. Therefore, harnessing *S. maltophilia* JVB5 can serve as a model in formulating bioinoculants with bioprospecting in developing eco-friendly agriculture sustainably.

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