Genomic Assessment of *Stenotrophomonas Indicatrix* for Improved Sunflower Plant

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Abstract

Plant growth-promotion screening and genome analysis of *Stenotrophomonas indicatrix* BOVIS40 were presented in this study. The genomic information reveals various genes underlining plant growth promotion and resistance to environmental stressors. The genome of *S. indicatrix* BOVIS40 harbors genes involved in the degradation and biotransformation of organic molecules. Also, other genes involved in biofilm production, chemotaxis, and flagellation that facilitate bacterial colonization in the root endosphere and phytohormone genes that modulate root development and stress response in plants were detected in strain BOVIS40. IAA activity of the bacterial strain may be a factor responsible for root formation. Nevertheless, the results highlighted here provide insights into the genomic functions of *S. indicatrix* and which can be explored in agricultural management. Hence, a measurable approach to the *S. indicatrix* lifestyle can strategically provide several opportunities in their use as bioinoculants in developing environmentally friendly agriculture sustainably.

1.0 Introduction

Plant growth promotion depends on natural bioactive compounds, which are inherent or produced by its associated microorganisms (Palanichamy et al. 2018). Either from microorganisms or plants, their immense contributions in the establishment of ecological balancing between plants and microbes remain fundamental (Fadiji et al. 2021). The importance of plant growth-promoting (PGP) genes and secondary metabolites from microorganisms are not only applicable in agriculture or restricted to plant improvement, but with wider therapeutic importance (Manganyi et al. 2019; Adeniji et al. 2021).

To know the composition of the different genes present in the genome of bacteria, the need for their isolation, characterization and analysis using bioinformatics tools became important (Mamphogoro et al. 2020). In recent times, the advancement in biotechnological findings into endophytic studies using various bioinformatics tools has revealed various PGP genes and secondary metabolites in bacteria (Mohotti et al. 2020; Nascimento et al. 2020b). Each of these gene clusters can code for different metabolic compounds such as siderophores, arypolyene and lanthipeptide-class-II with specific functions. The siderophore genes in the genome of bacterial genera such as *Bulkhoderia, Bacillus, Pseudomonas* and *Rhizobium* have been reported in host plants with a strong arsenal that suppresses the level of pathogenicity for improved plant growth (Ludwig-Müller 2015; Bhattacharyya et al. 2017). Similarly, the detection of several other secondary metabolite genes from endophytic bacteria colonizing medicinal plants has been documented (Ludwig-Müller 2015; Dinesh et al. 2017).

The survival and adaptive mechanism of bacteria in extreme environments can be linked to their ability to produce vital PGP genes and synthesis of secondary metabolites, thus making them suitable candidates with great values for various biotechnological, agricultural, and industrial applications (Nascimento et al. 2020a; Chukwuneme et al. 2021). The genomic analysis of sequenced bacterial genome revealing conservative and functional genes have been reported (Mitter et al. 2013; Zeng et al. 2018; Akinola et al. 2021), but information regarding novel plant-growth-promoting and secondary metabolite genes of *S.*

indicatrix isolated from sunflower root endosphere has not been documented, thus necessitating this study. *Stenotrophomonas* spp is Gram-negative, spore formers and rod-like. Few of these species and their functions in plant physiological functions have been studied (Kumar and Audipudi 2015; Singh and Jha 2017). Nevertheless, the genus *Stenotrophomonas* are dominant in diverse environments with a promising outlook in agricultural biotechnology (Alexander, et al. 2019).

The detection of important genes in the genome of *Stenotrophomonas* spp can significantly enhance their functions in the establishment of root-soil, soil-bacterial, and root-bacterial interactions for improved plant adaptation mechanisms to soil stressors (Vurukonda et al. 2018). These genes can affect the lifestyle and bacterial functions in the host plants. For instance, the detection of chemotaxis and biofilm production genes can contribute to root colonization and plant defense mechanisms. To these functional premises, *S. indicatrix* can be a potential biological tool in the formulation of bioinoculants for improved crop productivity. In this study, the authors presented genomic insights into endophytic *S. indicatrix* BOVIS40 obtained from sunflower root endosphere with significant effects on sunflower yield. Whole-genome sequencing (WGS) analysis unraveled vital genes involved in various biological functions and plant growth promotion. To the best of our knowledge, this is the first report on the WGS of *S. indicatrix* BOVIS40 sourced from sunflower root. Hence, this study aims to provide insights into *S. indicatrix* BOVIS40 about (i) plant growth-promoting activities, (ii) whole-genome sequencing, (iii) functional and secondary metabolite genes, and (iv) sunflower improvement under greenhouse experiment.

2.0 Materials And Methods

2.1 Source of plant growth-promoting endophytic bacterium *Stenotrophomonas indicatrix* BOVIS40

The healthy sunflower roots used in this study were sourced from Lichtenburg, South Africa (26°4'31.266' 'S, 25°58'44.442"E). The plant surface-cleaning, isolation, and characterization of the bacterial isolates were performed according to the modified methods of Forchetti et al. (2007). Bacterial isolates were screened for various PGP traits. Phosphate solubilization (PS) assay was performed according to the methods described by Pikovskaya (1948) with little modifications, while indole acetic acid synthesis by bacterial strains was conducted following the methods of Bric et al. (1991). Similarly, methods described by Sun et al. (2006) and Schwyn and Neilands (1987) were employed for exopolysaccharide test and siderophore screening on chrome azurol S (CAS) medium, respectively.

2.2 Molecular identification and whole-genome sequencing

The genomic content of strain BOVIS40 was extracted using a commercial Quick-DNA^{TM} Miniprep Kit specific for fungi or bacteria (Zymo Research, Irvine, CA, USA; Cat. No. D6005), as stipulated in the manufacturer's guide. Determination of quantity and quantity of the extracted DNA was achieved using a NanoDrop spectrophotometer (Thermo Fischer Scientific, CA, USA). The molecular identification of bacterial isolate as *S. indicatrix* based on 16S rDNA sequence data analysis was according to the method

described by Araújo, et al. (2002). The WGS of strain BOVIS40 was performed at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa.

The WGS of endophytic bacterial strain BOVIS40 was performed according to the standard Illumina method. Succinctly, fragmentation of bacterial genomic DNA was performed using the NEB Ultra II FS kit enzymatic approach. AMPure XP beads were employed for the selection from the resulting DNA fragments based on size range (200 - 700 bp). Subsequently, DNA end-repaired were achieved by fragmentation, and each fragment was ligated to Illumina-specific adapter sequences. Furthermore, the indexing of each sample and selection based on the size in the second step was performed. The quantity of samples at dilution of standard concentration to a 4 nM was determined using a fluorometric method. After that, sequencing was performed using a NextSeq mid-out kit (300 cycles) on Illumina's NextSeq platform, following a guideline as described by the manufacturer. The resulting 400 mb of data (2x150 bp long paired-end reads) were obtained for each sample.

For WGS analysis, each sequence (FASTQ file) was submitted to the predictive biology online server and data science platform (KBase - https://kbase.us/) (Arkin et al. 2018). First, sequences were uploaded for reads processing and read quality assessment was achieved using FastQC (version 0.11.5) (Bioinformatics 2011). The removal of sequence adaptor and low-quality bases of the pair-end Illumina raw sequence reads were processed with a flexible read trimmer to obtain high-quality sequence reads (trimmomatic (version 0.36)) (Bolger et al. 2014). Furthermore, sequence reads assembly was processed by SPAdes online (version 3.13.0) (Nurk et al. 2013), then annotated by employing RASTtk (Rapid Annotations using Subsystems Technology toolkit – version 1.073) and SEED online server to categorize the distribution and functions of the predicted genes into subsystems (https://rast.nmpdr.org). The prediction of functional protein-coding genes (PCG) was obtained from the genomic protein output after processing in NCBI (https://www.ncbi.nlm.nih.gov/). Bioinformatics probe was performed using default settings. For the evaluation of metabolic compounds in the bacterial genome, antiSMASH programs – version 6.0.0 alpha 1-60 bffdb (https://antismash.secondarymetabolites.org) was used (Weber et al. 2015). The circular genome visualization with the well-informed genomic feature was generated using an online tool (CGView) (http://cgview.ca/maps/) by uploading the genome assembly fasta file (Stothard and Wishart 2005), while the phylogenies and pairwise comparison of the genome dataset were created using Type (Strain) Genome online server (https://tygs.dmsz.de./) (Meier-Kolthoff et al. 2013).

2.3 Accession number of *Stenotrophomonas indicatrix* BOVIS40

From the NCBI database output, the Bioproject number is PRJNA706595, BioSample number is SAMN18138830, while the whole genome accession number is JAGENA000000000.

2.4 Inoculum preparation and seed treatment

A seed inoculation assay was used to facilitate bacterial adherence to the disinfected sunflower seeds. The effectiveness of sunflower seed inoculation was performed following the methods of Gholami et al. (2009). The bacterial inoculum size in LB broth at 24-hour incubation was standardized to 0.5 (10^6

CFU/ml) at OD_{600}. Cleaning of the seeds was performed by washing in sterile distilled water to remove floating-unhealthy seeds and dirt, and disinfected in 70 percent ethanol for 3 minutes, followed by 3 percent hypochlorite for 1 minute, then immersed in 70 percent alcohol for 2 minutes with final washing with sterile distilled water (5 times). Bacterial inoculated in LB broth was incubated on a rotary incubator machine at 180 rpm for 24 hours. Broth culture was centrifuged to obtain the pelletized cells after washing in 0.85% normal saline and re-suspended in the same solution. The sterilized seeds were suspended in LB bacterial culture ntaining 1% (v/w) carboxymethyl cellulose (CMC) as an adhesive (binder) in a 250 ml flask for 60 minutes. The seeds suspended in sterile distilled water without bacterial inoculum serve as the control.

2.5 Greenhouse experimental study

The soil used for planting in the greenhouse was sourced from agricultural farmlands in North-West University, Mafikeng Campus (Figure 1). Soil debris and other plant materials were removed, air dry, and sieved with a 2 mm micro stainless steel mesh sieve. Equal weight of soil, approximately 5 kg, was put inside autoclavable plastic bags and sterilized at 121 ^{o}C for 900 seconds. This step was repeated three times to ascertain all spore formers, vegetative cells, and forms of life were eliminated.

The inoculated and non-inoculated pots were randomly arranged by employing a complete randomized design (CRD) approach with 8 replicates for each treatment at a 10 cm distance apart in a greenhouse under natural light. Bacterial strains inoculated in 1 Liter LB broth were incubated in a rotary shaker (SI-600, LAB Companion, Korea) at 180 rpm for 2 days for optimal growth density. The broth was centrifuged (8000 x g for 600 seconds) and pelletized bacterial cells obtained were suspended in saline solution (0.85%). The centrifugation and washing of the pellets were performed under sterile experimental conditions.

The plastic pots measurement of 34 cm in diameter and 29 cm tall were washed with sterile water and sterilized with 15% sodium hypochlorite solution before filling with 15 kg dry-sterilized loamy soil. The surface-sterilized sunflower seeds were inoculated with 150 ml standardized bacterial inoculum, agitated in a shaker incubator at 120 rpm at 30^{o}C for 2 hours. Then, the filtrate suspension was gently poured out by decanting. Seeds were placed on a foil paper under sterile laminar flow (Filta Matix Laminar Flow Cabinet) and air-dried before taken to the greenhouse. Air-drying was performed to ensure the sticking of the bacterial inoculum to the surface of the seeds. The sterile plastic pots containing 15 kg sterile autoclaved soil were moistened with 500 ml sterilized water before sowing. Ten seeds maximum were sown per pot (at 1.5 cm deep). After seed emergence, i.e., at 8 days, thinning was performed, leaving one seedling in a pot. Growing seedlings were maintained at a temperature of 30±2^{o}C, a day-night cycle of approximately 14 hours under natural light, and relative humidity of 85%. The pots were moistened with an equal amount of water and maintained daily. Pots containing seedlings without inoculation serve as the control. The plants were harvested at maturity after 132 days of planting.

2.5.1 Sunflower morphological parameters below-and-above-ground level

The data collected based on sunflower morphological parameters after harvesting include the root weight (dry), number of lateral roots, root number, shoot weight (dry), taproot length, root weight (fresh), etc. These parameters were considered following the methods described by Igiehon et al. (2019). After harvesting from a greenhouse, sunflower plants were taken to the research laboratory. The soil adhering to sunflower roots was thoroughly cleansed/washed under running sterile water. The roots and shoot weight were weighed for fresh weight determination on a weighing balance (Wagi Elektroniczne, Poland). Also, lateral roots and root numbers were determined. Furthermore, plant roots and shoots were packed/wrapped in foil paper and oven-dry at 60 °C for 24 hours and re-weighed on a weighing machine for the determination of roots and shoot dry weights.

2.5.2 Determination of sunflower yield parameters

The sunflower yield parameters such as seed weight (fresh), seed weight (dry), and total seed weight (dry) were determined. After drying, sunflower seeds were manually separated from the sunflower head containing seeds. Seeds were oven-dried at 60°C for 24 hours, and seed weight (dry) was measured on a weighing balance. The plant samples were kept in plastic bags for further analysis. The whole experiment was repeated for each treatment.

2.6 Soil variable analysis

Approximately 0.5 kg of sieved soil used in the greenhouse experiment was used for soil physical and chemical parameters determination (Ali, et al. 2009). The chemical parameters such as silt, clay, and sand, and chemical parameters such as organic matter, magnesium, iron, pH, manganese, potassium, phosphorus, total nitrogen, and organic carbon were evaluated.

3.0 Results

3.1 Biochemical and cultural features

The biochemical and cultural characterization and PGP features of *S. indicatrix* BOVIS40 from the sunflower root endosphere are shown in table 1. The results revealed that strain BOVIS40 is rod-like, catalase-positive, and Gram-negative. Also, strain BOVIS40 utilizes all the sugars tested and grows between pH ranging from 4 to 10, temperature from 25 to 40°C, and 3.5% normal saline. Plant growth-promoting screening showed a significant difference in siderophore value of 77.54%, phosphate content of 32.93 µg/ml, and IAA of 16.15 µg/ml, respectively (Table 1). Bacterial endophytes exhibited significant reactions to cellulase, xylanase, and protease compared to amylase and protease production with no significant difference from the plate assay experiment.

3.2 Whole-genome sequence information of bacterial strain BOVIS40

The WGS analysis of strain BOVIS40 yielded a sequence read count of 7,301524, the genome size (4,427,090 bp), G+C content (66.4%), and total bases of 1,102,530,124. The read length mean was 151 bp

while N_{50} and L_{50} values were 29,344 and 44 bp respectively and 252 contigs. The genome analysis revealed 4,160 coding sequences and 63 RNAs. The circular genome visualization of *S. indicatrix* strain BOVIS40 is presented in figure 2. The subsystem statistics show 27 subsystem feature counts of the coding protein into functional groups based on the annotated genome classification by SEED in RAST. The functional basis of 3,957 protein-coding genes (PCG) was assessed from the KEGG database and RAST online server. The 1,476 genes annotated by the SEED viewer were grouped into molecular function, cellular components, and biological processes. The topmost five groups were protein metabolism (177 genes), amino acids and derivatives (258 genes), cofactor vitamins, prosthetic groups, pigments (128 genes), carbohydrates (165 genes), and membrane transport (136 genes) (Figure 3).

3.3 Phylogeny analysis

The results of taxonomic identification of species and subspecies clusters from the phylogeny output on the genome server yielded an overall 10 species and 11 subspecies clusters, and 1 species and subspecies regarding the bacterial genome respectively. Also, 11 strains and 96.1% average branch support, and a delta statistic value of 0.131 were obtained from the genome blast distance *phylogeny* (GBDP) tree based on genome sequence datasets. The results output from the genome sequence data showed that strain BOVIS40 form a cluster clade of 100% with *S. indicatrix* WS40 and *S. lactitubi* M15 (Figure 4).

3.4 Functional genes annotation information of *S. indicatrix* BOVIS40

Various metabolic genes were detected in the bacterial genome (Tables 3, 4 and 5, Tables S1-S3, S4, S5-S13, S14-S16). Notable secretion systems genes found in strain BOVIS40 are presented in table S1. The expression of genes involved in carbohydrates such as trehalose, glucose, hexose, xylose, galactose, mannose, and fructose degradation pathways is presented in table S2. The carbohydrate metabolism pathways include the pentose phosphate pathway, glycolysis – Embden-Meyerhof pathway, and Leloir pathway. Other genes responsible for organic phosphate solubilization, sulfur metabolism, and nitrogen fixation and transport were detected in the genome (Table 3, Table S3 and S4). Similarly, functional genes involved in iron transport, enterobactin, and siderophore production were also found (Table 4). Bacterial colonization in the root endosphere mediated by several genes involved in chemotaxis, flagella, pilus and fimbriae biosynthesis is presented in table S5. The signaling molecules that aid bacterial biofilm production and genes responsible for their attachment were also detected (Table S6). Genes, such as *cyc*A, *gcv*A, *pst*J, *lld*R, and *bet*L coding for quorum-sensing in N-acyl-homoserine-lactone pathway were detected in the genome of strain BOVIS40 and presented in table S7. Plant protection against oxidative stress based on genes expression ability in strain BOVIS40 is presented in Table S8). Genes involved in organic acids metabolism, GABA, amino acid metabolism and transport, and phenolic gene, *phe*A coding for catechol metabolism were found in the strain BOVIS40 (Tables S9-S12). The detection of genes involved in lignin degradation and degradation against toxic peroxides and plant growth hormones were profound in strain BOVIS40 (Table 5, Table S13). Notable biocontrol, stress tolerance, and temperature resistance genes that stimulate an internal response in plants were presented in tables S14-S16.

3.5 Predicted secondary metabolite cluster genes by antiSMASH

The predicted secondary metabolite cluster genes from antiSMASH analysis of strain BOVIS40 are presented in table 6. The biosynthetic gene clusters identified include bacillibactin type non-ribosomal peptide siderophore and APE Vf type arylpolyene. Three (3) notable secondary metabolite cluster genes detected include transport-related genes, resistance, regulatory genes, additional biosynthetic genes, core biosynthetic genes, other genes, and TTA codons (Figures 5a and b). The genes cluster exhibiting 35% and 60% similarity for gene type arylpolyene and NRPS with the most similar known cluster for other and NRP: siderophore at node region 11.1 and 15.1 were further analyzed (Table 6).

3.6 Growth parameters

The yield parameters of inoculated and non-inoculated sunflower from the greenhouse experiment are presented in table 7. Except for taproot width, the aboveground parameters of inoculated plants compared to non-inoculated were statistically different. In terms of seed wet and dry weight, there was no significant difference compared to other belowground parameters. Inoculated plant yielded 348.04 g plant wet weight compared to un-inoculated with 332.04 g wet weight. Similar results were obtained on the shoot dry weight 57.28 g (un-inoculated) and 76.88 g (inoculated), un-inoculated head fresh and dry weight of 153.76 g and 41.55 g and inoculated head fresh and dry weight of 182.83 g and 50.48 g respectively. The soil variables tested recorded high magnesium content of 642 mg/kg, potassium content of 399 mg/kg, nitrogen content of 0.10 % and pH 7.4 (Table S17). The percentages of clay, sand, and silt were 22.20, 68.30, and 7.92 respectively

4.0 Discussion

The growing of plants for food production is affected by environmental factors (biotic and abiotic) (Dimkpa et al. 2009). Biotic factor such as microorganisms establishing mutualism or antagonism relationship with the host plants can significantly express their importance in mitigating ecological stressors (Yu et al. 2019). Our interest is targeted at cooperation between plants and microbes. Many beneficial microbes occupy different ecological niches, such as the rhizosphere, phyllosphere, spermatosphere, and endosphere (Adeleke and Babalola 2020). Endosphere represents the entire internal tissue of plants colonized by endophytic microbes with utmost beneficial effects on plant growth (Asaf et al. 2017). The beneficial endosphere inhabitants with PGP traits, as demonstrated by *in vivo* assay in this study, confirm their agricultural importance. The coevolution of endophytic microbes with the hosts has prompted complementary dynamics in their functions, such that, the host plant houses, protect, and supply nutrients to microbes. In return, these microorganisms enhance plant growth and survival (Omomowo and Babalola 2019).

The design of the study was to isolate and characterize endophytic bacteria strains from the sunflower root with PGP potentiality. Generally, *Stenotrophomonas* species are linked to human pathogens with opportunistic tendencies (Rojas-Solís et al. 2018). In recent times, their potential in plant growth promotion has significantly widened the research scope of this species, thus providing knowledge based

on their environmental and agricultural importance (Alexander et al. 2019). So far, whole-genome analysis for detecting diverse genes involved in cellular metabolism and phytohormone production from *S. indicatrix* has not been studied. Hence, we present the first report on the genomic information on *S. indicatrix* isolated from sunflower roots.

S. indicatrix was isolated on Luria Bertani medium, screened on growth-promoting media, and then sequenced. From the annotation results, it was revealed that the type of genes detected in strain BOVIS40 corroborate the *in vitro* screening. Nevertheless, other genes found aside from those screen *in vitro* were also detected. The results from plant growth-promoting tests showed that strain BOVIS40 could modulate plant growth in diverse ways. For instance, in phosphate solubilization, exopolysaccharide production, IAA, and siderophore biosynthesis. The results obtained corroborate with earlier documentation on plant growth-promoting attributes of *Stenotrophomonas* strains and other root-associated endophytic bacteria (Ngoma et al. 2013; Mukherjee and Roy 2016; Perez-Perez et al. 2020).

The genome-based phylogeny classification showed that strain BOVIS40 forms 100% identity to *S. indicatrix* WS40 and *S. lactitubi* M15 in a closely related cluster clade. The average nucleotide identity (ANI) of *S. indicatrix* WS40 (97.05%) further confirms that strain BOVIS40 belongs to *indicatrix* species based on high ANI value (Goris et al. 2007). As reported in this study, researchers have also demonstrated the phylogenetic relationship of novel *S. cyclobalanopsidis* with 100% similarity to *S. indicatrix* WS40 and *S. lactitubi* M15 in a close cluster clade (Bian et al. 2020).

The ability of microbes to fix atmospheric nitrogen for plant use depends on certain genes and enzymes produced by them (Valentine et al. 2018). The protein nature of enzyme coding genes such as *nif*H and *nif*DK involved in nitrogenase production in a bacterial cell has contributed to their functions in the biotransformation of bound nitrogen in the soil into absorbable form (Guerrieri et al. 2021). The enzyme cysteine desulfurase coding for gene *nif*S involving in the nitrogen fixation pathway was identified in strain BOVIS40. Other genes involved in nitrogen regulatory protein NR (I) (*ntr*C) and flavodoxin production (*nif*F), together with the ammonia transport gene *amt* identified, could contribute to an increase in nitrogen level in the soil. The presence of nitrogen fixation (NF) genes such as *nif*ABDEFHJKLMNSTUVWXYZ in the genome of *Klebsiella* species function in the formation of complex membrane structure and transport of molecules in the presence of nitrogenase have been employed as a model in the NF ability of *Rhodobacter capsulatus* (Jeong and Jouanneau 2000). The detection of *nif*F in the genome of rhizobacterium *Azospirillum brasilense* Sp7 and *K. variicola* UC4115, contributing to their nitrogen fixation ability for yield improvement in tomato, has been identified with success in recent findings (Guerrieri et al. 2021). A similar gene identified in this study could contribute to bacterial functions in improving sunflower yield. Also, the expression of *fix* genes such as *fix*ABCX in the genome of *Azorhizobium caulinodans* and *Bradyrhizobium japonicum* with alternate *nif*JF and their functions in the electron transport chain system have contributed to the transfer of proteins in rhizobia (Tsoy et al. 2016). Also, identification of flavodoxin and ammonia transport gene (*amt*B) in the genome of strain MSR2 has been reported (Nascimento et al. 2020a), with similar results documented in this study.

The number of *nif* genes produced by the bacterial cell may be due to their physiological nature and source of isolation. However, unidentified genes as reported in other literature might be responsible for the replacement of these genes with other *nif* products (Shen et al. 2013). Interestingly, information relating to the nitrogen fixation potential of *S. indicatrix* isolated from the root of oilseed sunflower based on their genome studies has not been documented.

Phosphate solubilizing potential of strain BOVIS40 under *in vitro* assay on tricalcium medium was validated by identification of copious genes involved in phosphate solubilization and transport. Gluconic acid (GA) is known as a precursor that facilitates phosphate solubilization in diverse bacterial phyla. The synthesis of GA due to the catalytic actions of enzyme glucose-1-dehydrogenase and its non-protein chemical compound pyrroloquinolone quinine (*pqq*) have contributed to microbial functions and applications (Ramachandran et al. 2006). Despite non-detection of *pqq* genes in strain BOVIS40, reports have shown the expression of heterologous *pqq* genes (*pqq*ABCDEF) that confer phosphate solubilization potential in diazotroph endophytic bacterium *Herbaspirillum seropedicae* Z67 associated with economic plants (Baldotto et al. 2011; Wagh et al. 2014). Similarly, the absence of *pqq* genes has been reported in the genome of *Klebsiella* sp. D5A (Guerrieri et al. 2021).

Phosphate uptake by strain BOVIS40 may be enhanced due to their high affinity and presence of phosphate transport genes, *pst*ABCS, which corroborate the findings earlier documented by Guerrieri et al. (2021) and Shariati et al. (2017) on the expression of phosphate transport genes, *pst*ABCS on the whole-genome analysis of *K. variicola* UC4115 and *Pantoea agglomerans* P5. The presence of *pst* may enhance phosphate bioavailability in the soil and uptake by plants. The detection of *pho*A gene coding for enzyme alkaline phosphatase involved in various phosphorus metabolism has been identified in *Burkholderia multivorans* WS-FJ9 (Liu et al. 2020). Furthermore, the detection of *pgl, ppx*, and *ppa* in the genome of BOVIS40 that code for 6-phosphogluconolactonase, exoployphosphatase, and inorganic pyrophosphatase for the synthesis of D-gluconate and degradation of inorganic pyrophosphatase could contribute to the ability of the bacterial strain to solubilize phosphate in the soil. Interestingly, our results corroborate the findings of Nascimento et al. (2020b), who reported *pho*AD genes, coding for alkaline phosphatase in the genome of *Bacillus megaterium* STB1, that enhance phosphate solubilization ability in the bacterial strain.

In line with the previous studies on the PGP properties of endophytic bacteria colonizing plant root (Stoltzfus et al. 1997; Tariq et al. 2014; Eida et al. 2021), expression of genes involved in secretion system, siderophore, IAA synthesis, acetoin, and 2,3-butanediol was detected in endophytic bacterial strain BOVIS40. The synthesis of IAA in plants and microorganisms has been postulated through multidimensional pathways, which include, indole-3-acetonitrile (IAN), tryptamine, indole-3-pyruvate (IPA), and indole-3-acetamide (Spaepen and Vanderleyden 2011). IAA, a major occurring auxin in plants can signally mediate gene expression and biosynthesis in microorganisms. Thus, plant stimuli against natural pathogenic microbes may be linked to the synthesis of auxin signal molecules by the microorganisms (Spaepen and Vanderleyden 2011). To this premise, two major pathways, namely IPA and IAN were identified in the genome of BOVIS40 as previously reported in the genome of *K. variicola*

UC4115 (Guerrieri et al. 2021). The biotransformation of IPyA - tryptophan to indole pyruvic acid in the presence of aminotransferases that convert IPyA to IAAld - indole acetaldehyde and gene *ipd*C encoding indole pyruvate decarboxylase in *Pantoea* spp has been documented (Nascimento et al. 2020a). Also, the presence of *ipd*C gene in *Enterobacter cloacae* with similar functions has been identified (Koga, et al. 1991). Furthermore, the presence of aldehyde dehydrogenase in the genome of strain BOVIS40 has been identified to facilitate the conversion of intermediate IAAid to IAA (Koga et al. 1991).

Interestingly, strain BOVIS40 harbors tryptophan synthase that reversibly catalyzes indole-3-glycerol phosphate to form glyceraldehyde-3-phosphate and irreversibly involved in pyridoxal phosphate pathway to form tryptophan through the condensation of serine and indole (Ireland et al. 2008). The presence of amidase enzymes could contribute to bacterial functions in the production of IAA along the IAN pathway. Additionally, the involvement of strain BOVIS40 in two different IAA metabolic pathways significantly revealed their IAA production ability to play a vital role in plant root formation and development.

Subsequently, *mia*AB genes involving cytokinin biosynthesis and transformation were detected in the genome of BOVIS40. Enzyme coding for tRNA dimethylallyltransferase genes in the bacterial genome could enhance iPR - N_6-(dimethylallyl)adenosine production. The expression of other cytokinin genes such as *mia*BE that convert iPR to 2-methylthio-N_6-(dimethylallyl) adenosine and further to 2-methylthio-cis-ribozeatin has been identified in the genome of *P. phytobeneficialis* MSR2 (Nascimento et al. 2020a). The direct effects of these genes in plant growth enhancement suggest that cytokinin synthesis could play a vital role in plant growth promotion and plant health (Wani et al. 2016). Nevertheless, further comparative research into agriculturally important *S. indicatrix* strains will help provide information on their novel genes, as reports on cytokinin genes from this endophytic bacterium strain BOVIS40 have not been documented.

The detection of siderophore genes in *Stenotrophomonas* strain BOVIS40 can enhance plant accessibility to soil mineral nutrients and contribute to their growth. Genes coding for iron transport found in strain BOVIS40 could play a vital role in the mineralization of insoluble iron and bioavailability for plant use. Furthermore, the synthesis of 2,3-butanediol by endophytic bacteria has been linked to their biocontrol ability, induction of systemic resistance, and improve plant tolerance to drought (Samaras et al. 2020). Reports on the biocontrol activity of endophytic bacteria such as *Pseudomonas* spp against *Phytophthora capsici* that cause rot and blight disease in pepper (Aravind et al. 2009), *Bacillus* spp against wilt disease in chili (Dowarah et al. 2021), *Burkholderia cenocepacia* against *Fusarium* wilt in banana (Ho et al. 2015), and *Stenotrophomonas* spp against *Sclerotium rolfsii* that cause collar rot in tomato (Sahu et al. 2019) have been studied.

Endophytic microbes have employed indirect mechanisms in the production of metabolic compounds in the control of phytopathogens, and their pathogen suppressive actions rely on their ability to produce biocontrol agents (Santoyo et al. 2016). The biocontrol ability of strain BOVIS40 may be due to siderophore synthesis that enhances their antibiosis activity against plant pathogens (Maheshwari et al. 2019). Notably, strain BOVIS40 harbors the siderophore gene *fiu.* Siderophore catecholate found in the

bacterial genome has been recognized to play an important role in bacterial adherence to the receptor surfaces, iron chelation and transport (Pedraza et al. 2010). Conversely, other related genes such as *ent*ABCDEFGHS and *fep*ABCDG are involved in siderophore production and transport that function in the conversion process of chorismate into enterobactin that was not detected in this study have been documented (Tortora et al. 2011; Hubrich et al. 2021).

Additionally, based on the beneficial effects on plant growth promotion, strain BOVIS40 harboring biocontrol genes could contribute to disease suppression promote plant growth. Also, detection of genes modulating the biological activity of strain BOVIS40 can stimulate the secretion of antimicrobial compounds such as g-aminobutyric acid (GABA) and 4-hydroxybenzoate, thus revealing their genome response in plant health and exploration as bioinoculants for the control of microbial infections.

The survival of endophytic microbes in the root endosphere is affected by the number of exudates released from the plant roots. Majorly, root exudate is composed of amino acids, sugars, phenolics, organic acids, etc. (Baudoin et al. 2003). Various organic compounds such as sugars serve as an energy source for microbial metabolism. The release of carbon-containing compounds from the rhizo-compartment as exudate could supply endo-rhizobiome the required energy for various cellular activities (Zhalnina et al. 2018). Sugar assimilation potential of strain BOVIS40, as reported in Table 1 was validated based on the detection of various genes involved in carbohydrate metabolism and transport. Carbohydrate utilization response of strain BOVIS40 can enhance their biofilm production and colonization in the host plants. Several genes involved in the degradative pathway of organic compounds such as carbohydrates may contribute to the biotransformation of various organic substrates (Nascimento et al. 2020a). Aside from carbohydrates, the expression of genes participating in organic acid metabolism may contribute to bacterial affinity with the host plant. Also, various genes involved in the degradation and transport of amino acids and their derivatives contributing to amino acid metabolism and bacterial colonization with the host plants have been documented (Mavrodi et al. 2021).

Prephenate hydratase gene (*phe*A) involved in catechol metabolism was found in strain BOVIS40. Genes involved in gallate catabolism were also detected in strain BOVIS40. The presence of these genes could suggest their ability to modulate phenolic degradation, which plays a major role in endophytic lifestyle. Phenolics are classified as vital compounds that regulate plant growth and stimulate resistance stimuli in plants (Nascimento et al. 2020a). Furthermore, phenolic metabolism can enhance the colonization efficiency of strain BOVIS40 in plants. The ability of bacteria to degrade lignin due to their inherent lignin-degrading enzymes is known to functionally contribute to bacterial infiltration and colonization in the host endosphere compartment (Shi et al. 2015).

Bacterial colonization within the plant endosphere to form biofilm can be engineered based on certain bacterial traits and the synthesis of biofilm production genes. Genes involved in bacterial attachment to plant surfaces such as *bcs*ABCFGZ and *yhj*Q as expressed in the genome of strain BOVIS40 have been identified in the genome of Ghats1 (Shastry et al. 2020). The expression of colonization genes in the genome of endophytic bacteria may enhance their colonization potential, host selection and

establishment of plant-bacterial interactions in a symbiotic manner (Eida et al. 2021). Furthermore, the presence of cellulose biosynthetic genes in strain BOVIS40 may help configure endophytic lifestyle for host-bacterium mutual dependence.

The presence of exopolysaccharide genes could enhance bacterial colonization to form a biofilm, mucilaginous active substances, and boost stress tolerance in plants (Santaella et al. 2008; Meneses et al. 2011). This suggests that bacterial strain BOVIS40 could form synergistic cooperation by colonizing the plant endosphere, thus benefiting the host plants. Endophytes can infiltrate the host plant either by tissue damage, natural opening or through enzyme action (Omomowo and Babalola 2019; Yadav et al. 2020). Hence, the ability of bacterial strain BOVIS40 to produces enzymes, and genes coding for exopolysaccharides and cellulose biosynthesis may mediate bacterial colonization and attachment for biofilm production in the root endosphere (Meneses et al. 2011).

Cellular organelles such as fimbriae, flagella, and pili play a vital role in bacterial attachment, movement, and response to chemicals (Zheng et al. 2015). The detection of chemotaxis and motility genes can mediate bacterial ability to infiltrate plant roots and establish a bacterial community for plant benefits. The results obtained from this study corroborate the findings of Shastry et al. (2020), who reported chemotaxis functional genes (*che*AVY) and other genes involved in flagella biosynthesis in *E. cloacae* Ghats1. Similarly, identification of *mot*AB and other genes responsible for chemotaxis actions such as *che*ABYWR in *P. phytobeneficialis* MSR2 have been reported (Nascimento et al. 2020a).

Plant-microbe interactions stimulating a lot of chemical reactions in the production of antioxidative and antimicrobial compounds such as phytoalexins, nitric oxide, and reactive oxygen species (ROS) have boost plant defense against osmotic stress (Gaber et al. 2006; Samaras et al. 2020). The expression of oxidative and osmotic stress genes in the bacterial genome could reveal their strong resistance tendencies to ROS, a chemical compound produced by bacteria in a stress environment (Scott et al. 2007; Nascimento et al. 2020b). Also, the expression of osmotic genes is known to boost host immune responses. The colonization and survival of endophytic bacteria in plants growing in an environment exposed to oxidative stress can be modulated by the synthesis of antioxidant lytic enzymes such as glutathione reductase, monodehydroascorbate reductase, superoxide dismutase, ascorbate peroxidase, and catalase. The function of these enzymes is paramount in scavenging free radicals in the plant endosphere and other regulators such as reduced glutathione and ascorbic acid (Das et al. 2015). Similar functional genes coding for catalase, superoxide dismutase, peroxiredoxin, glutathione peroxidase, alkyl hydroperoxide reductase, glutaredoxin 3, glutathione monothiol glutaredoxin, and glutathione-disulfide reductase were detected in strain BOVIS40. The detection of a catalase-peroxidase gene (*kat*E) and catalase gene (*kat*G) responsible for the degradation of toxic peroxide corroborates the findings of Nascimento et al. (2020a), who reported similar genes from *Pantoea phytobeneficialis* MSR2. Hence, their ability to stimulate defense against oxidative and osmotic stress in plants could be promising in the formulation of bioinoculants for plant benefit.

The presence of osmotic stress regulatory genes could enhance plant response to heat or cold shock. Similarly, the protein-coding genes that modulate cold and heat stressors can function through multiple gene families and differential regulation (Wani et al. 2016). Plant response to cold or heat stress has been attributed based on the expression of notable genes such *csp, hsp*, and a gene coding for the chaperone (Phadtare 2004). As presented in this study, these genes could contribute to the resistance and survival of sunflower in temperate environments. The presence of *csp* in the bacterial genome contributing to RNA and DNA stabilization for improved translational and transcriptional efficiency of plants under cold or heat conditions has been documented (Nascimento et al. 2020b). However, the expression of these genes in strain BOVIS40 corroborates the findings of Nascimento et al. (2020b), who had earlier reported chaperones and other heat stress genes such as *gro*EL, *gro*ES, *dna*K, *dna*J and *hsp* in the genome of *Bacillus megaterium* STBI.

Transcriptional regulator and amino acid transporter genes were found in train BOVIS40. These genes can mediate mechanisms involved in quorum-sensing and dynamics in the lifestyle and ecology of endophytic microbes. Various antimicrobial compounds secreted by bacterial strains can exert beneficial effects in sustaining plant health. For example, the role of bacteriocin produced by endophytic bacteria as a source of new antibacterial compounds have synergistically been employed to upsurge antibiotic resistance (Lopes et al. 2017; Oliveira et al. 2018). Organic compounds synthesized by bacteria naturally play a vital role in the adaptation and survival of the host plant. Although, *Stenotrophomonas* species are regarded as opportunistic pathogens that affect humans and plants, and in recent times, their biocontrol efficacy due to the ability to secrete vital secondary metabolite is promising. However, documentation on secondary metabolite gene clusters from *S. indicatrix* is rare. Interestingly, two prominent gene clusters such as arylpolyene (APE Vf) and bacillibactin NRP: siderophore with type arylpolyene and non-ribosomal peptides (NRPS) detected in the genome of strain BOVIS40 could contribute to plant protection against oxidative stress and other biological functions. For instance, the siderophore gene cluster detected in the genome of copious endophytic *Streptomyces kebangsaanensis* contributes to their biocontrol activity against pathogenic fungi *Fusarium oxysporum* and enhances plant survival (Remali et al. 2017; Siupka et al. 2020). The attribute of siderophore synthesis by bacterial strains can provide information on their ecological functions as biocontrol agents against plant pathogens. Hence, screening of secondary metabolites in the genome of strain BOVIS40 can enable researchers to understand their application in the control of phytopathogens.

Under greenhouse experimental conditions, an increase in yield parameters of inoculated sunflower compared to un-inoculated was observed. This can be linked to the pheno-genotypic attribute of endophytic bacteria to solubilize phosphate, synthesize IAA and siderophore, and detection of possible biocontrol genes in the bacterial genome, and this may contribute to bacterial activity in the improvement of below-and-aboveground crop yield. The presence of IAA genes seemed evident in the root formation in sunflower. Hence, results obtained as demonstrated significantly reveal the agricultural importance of *S. indicatrix* BOVIS40 upon inoculation. Nevertheless, the use of *S. indicatrix* under greenhouse experimental conditions in improving crop yield is rare in literature.

5.0 Conclusion

Detailed information on the notable genes with multiple functions of *S. indicatrix* BOVIS40 and evaluated sunflower yield parameters were documented in this study. The genome analysis highlighting important regulatory genes confirms some *in vitro* screening results. The plant growth-promoting genes exhibited by *S. indicatrix* BOVIS40 can be linked to their underlined promise in agriculture. The multiple genes identified that revealed their biotechnological significance in plant-microbe interactions, plant resistance to environmental stressors (such as temperature, oxidative and osmotic stress), degradation of organic substrate (carbohydrate), and organic compounds could enhance plant growth, soil health, and crop improvement. Importantly, the presence of these genes has provided insights into the genomic functions of *S. indicatrix*, with a promising outlook in developing sustainable agriculture. Also, based on the limited study on endophytic bacterium *S. indicatrix*, the information provided in this study will help harness their potential as a suitable candidate in the formulation of bioinoculants towards addressing agricultural problems.

Declarations

Authors' contributions

BSA and OOB designed the study; BSA managed the literature searches, carried out the laboratory work, interpreted the results, and wrote the manuscript's first draft. AOA assisted in the analysis while OOB, the principal investigator, provided academic input, thoroughly critiqued the manuscript, proofread the draft and secured funds for the research. All authors approved the article for publication.

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Declarations of interest

None

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Tables

Table 1: Biochemical, cultural characterization and plant growth-promoting features of *S. indicatrix* BOVIS40

Characteristics	Result	Plant growth-promoting tests		
		Isolate screening	**Qualitative**	**Quantitative**
Gram reaction	-	IAA	+	16.15±0.15[a]
Shape	Rod	Siderophore	++	77.54±0.42[c]
Catalase	+	Phosphate	+	32.93±0.08[b]
Starch hydrolysis	+	Exopolysaccharide	+	-
Nitrate	+	**Enzyme assay**		**ZOC (mm)**
Oxidase	+	Amylase	+	33.97±0.06[c]
Citrate	+	Cellulase	+	41.00±0.01[d]
Casein hydrolysis	+	Xylanase	+	20.00±0.01[b]
Salt tolerance (% v/v) (max.)	3.5	Mannanase	+	34.00±0.01[c]
Temperature range (°C)	25-40	Protease	+	3.00±0.01[a]
pH range	4-10			
Glucose	+			
Fructose	+			
Galactose	+			
Sucrose	+			
Mannitol	+			
Xylose	+			
Raffinose	+			
Maltose	+			
Arabinose	+			

Key: - = negative reaction, + = positive reaction, ZOC - zone of clearance measurement. Values are represented as means ± standard deviation in triplicate

Table 2: Genome characteristics and annotation information

Genome annotation features	*S. indicatrix* strain BOVIS40
Domain	Bacteria
Taxonomy	Bacteria; *S. indicatrix* strain BOVIS40
Size (bp)	4,427,090
G + C content (%)	66.4
N_{50}	29344
L_{50}	44
No. of contigs (with PEGs)	525
No. of subsystems	304
No. of coding sequences	4160
No. of RNAs	62
Plasmid	No

Table 3 - Genes involved in phosphate solubilization and transport.

Gene	Locus tag	Product	Pathway
Pgl	J0657_08070	6-phosphogluconolactonase	**D-gluconate production**
Ppx	J0657_02585	Exopolyphosphatase	**Degradation of inorganic polyphosphates**
Ppa	J0657_14755	inorganic pyrophosphatase	
Ppx	J0657_02585	exopolyphosphatase	**Organic phosphate solubilization**
***pho*U**	J0657_09385	phosphate signaling complex protein *Pho*U	
***pho*A**	J0657_04540	alkaline phosphatase	
***pst*A**	J0657_09395	phosphate ABC transporter (**P(ABC)T**) permease *Pst*A	**Phosphate transport**
***pst*C**	J0657_09400	P(ABC)T permease subunit *Pst*C	
***pst*S**	J0657_08775 J0657_08780	P(ABC)T substrate-binding protein *Pst*S	
***pst*B**	J0657_09390	P(ABC)T ATP-binding protein *Pst*B	

Table 4 - Genes involved in iron transport and siderophore production

Gene	Locus tag	Product	Pathway
***feo*B**	J0657_02445	ferrous iron transport (**FIT**) protein B	**Iron(II) transport**
***feo*A**	J0657_02450	FIT protein A	*feo*A
***fhu*E**	J0657_10270	ferric-rhodotorulic acid/ferric-coprogen receptor *Fhu*E	**Iron complex transport**
***ent*H**	J0657_11000	Thioesteras	**Enterobactin production**
***ent*E**	J0657_01105	2,3-dihydroxybenzoate-AMP ligase	
***ent*C**	J0657_01130	Isochorismate synthase	
Fiu	J0657_11665	catecholate siderophore receptor *Fiu*	**Siderophore receptor**

Table 5 - Genes involved in the modulation of plant hormones.

Gene	Locus tag	Product	Pathway
***Trp*D**	J0657_01675	anthranilate phosphoribosyltransferase	**L-tryptophan production; IAA production**
***Trp*B**	J0657_13265	tryptophan synthase (**TS**) subunit beta	
***Trp*A**	J0657_13275	TS subunit alpha	
***Trp*C**	J0657_01680	indole-3-glycerol phosphate synthase *Trp*C	
***Ald*H**	J0657_14110	aldehyde dehydrogenase	**IAA production, IPA pathway**
-	J0657_04850	Amidase	**IAA production, IAN pathway**
***Mia*B**	J0657_07115	tRNA (N6-isopentenyl adenosine(37)-C2)-*Mia*B methylthiotransferase	**CK biosynthesis and transformation**
-	J0657_16875	xanthine dehydrogenase family protein molybdopterin-binding subunit	
***Mia*A**	J0657_16145	*Mia*A tRNA (adenosine(37)-N6)-dimethylallyltransferase	
-	J0657_16870	xanthine dehydrogenase family protein subunit M	

Table 6: Estimate of secondary metabolite gene clusters in the genomes of *S. indicatrix* strain BOVIS40

Node Rg	Type	From	To	MSKC		Similarity
Rg 11.1	Arypolyene	1	38,874	APE Vf	Other	35%
Rg 15.1	NRPS	1	13,311	Bacillibactin	NRP:NRP siderophore	60%
Rg 87.1	RiPP-like	2,929	13,774			
Rg 156.1	RiPP-like	30,429	40,343			

Key: MSKC - Most similar known cluster, Rg – region

Table 7: Sunflower yield parameters

Growth parameter	Non-inoculated	Inoculated with *S. indicatrix* BOVIS40
Aboveground		
Tap root length (cm)	146.67±58.33[a]	177.33±28.31[b]
Tap root width (cm)	5.33±0.58[a]	5.33±1.15[a]
Root length (cm)	217±81.22[a]	257.33±48.44[b]
Lateral root number	25.67±1.53[a]	26.00±4.58[b]
Root wet weight (g)	44.09±16.12[a]	49.50±3.97[b]
Root dry weight (g)	8.41±1.55[a]	12.49±2.80[b]
Number of roots	935±11.30[a]	1236.00±616.83[b]
Belowground		
Seed wet weight (g)	0.05±0.01[a]	0.06±0.00[a]
Seed dry weight (g)	0.03±0.01[a]	0.03±0.01[a]
Head fresh weight (g)	153.76±13.94[a]	182.83±23.76[b]
Head dry weight (g)	41.55±4.27[a]	50.48±10.04[b]
Plant wet weight dry (g)	331.04±20.16[a]	348.04±30.28[b]
Shoot wet weight (g)	165.82±5.16[a]	216.44±57.21[b]
Shoot dry weight (g)	57.28±7.04[a]	76.88±29.91[b]

Values are represented as means ± standard deviation in triplicate

Figures

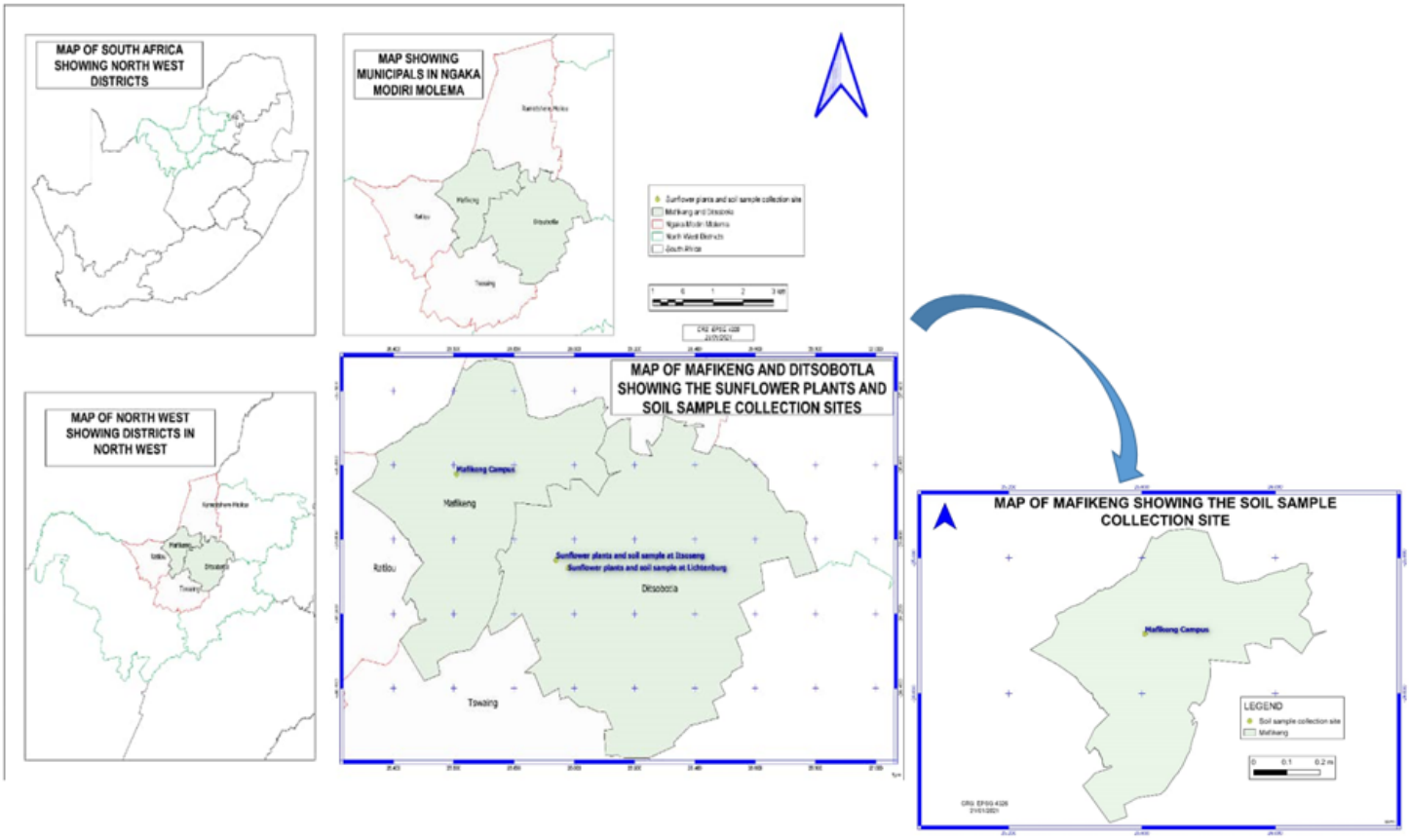

Figure 1

Map showing sample collection sites. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

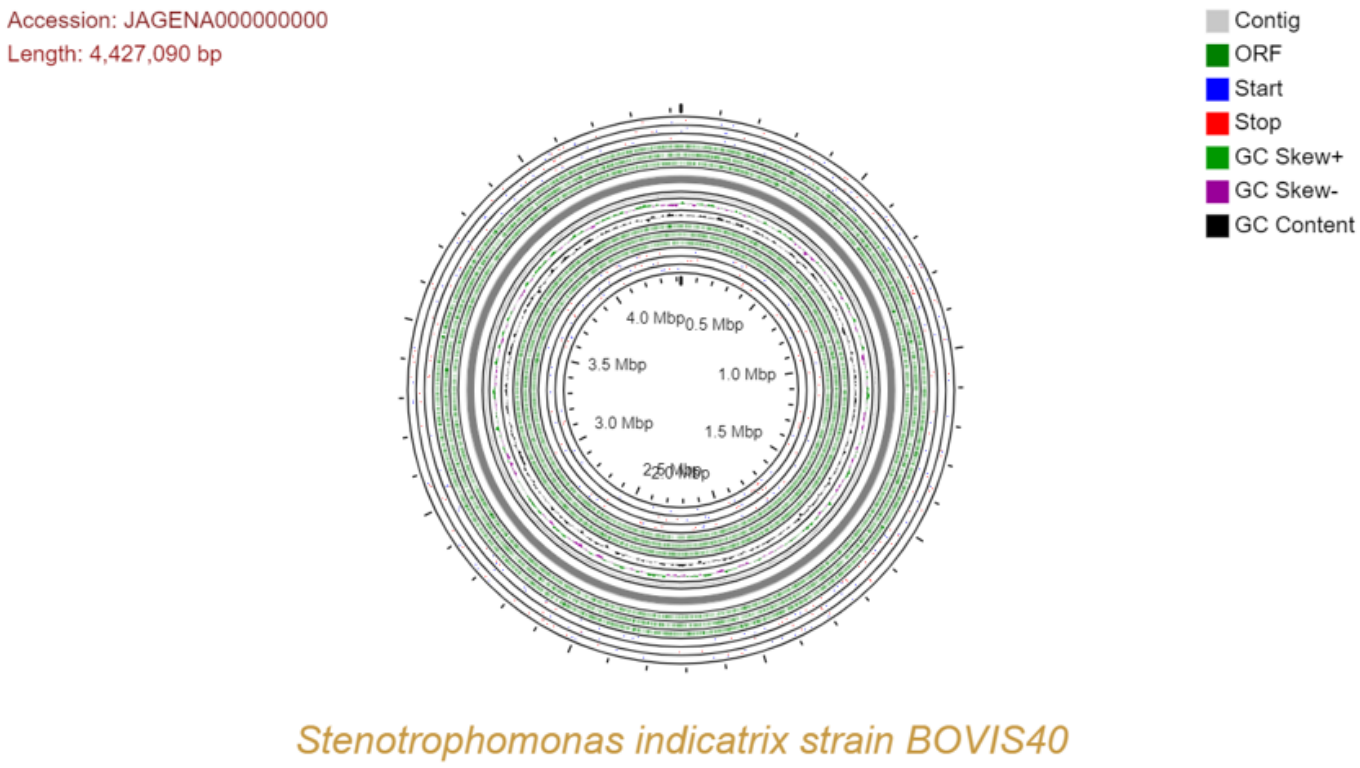

Figure 2

Circular genome visualization of whole-genome S. indicatrix strain BOVIS40. Each color from the external to internal circle depicts, green (ORF), red (stop codon), and blue (start codon). The ring black coloration (GC content) at the peak indicated higher or lower values than average GC content. The GC Skew (-/+) in purple/green peaks in/outside the circle indicated values greater or smaller than 1

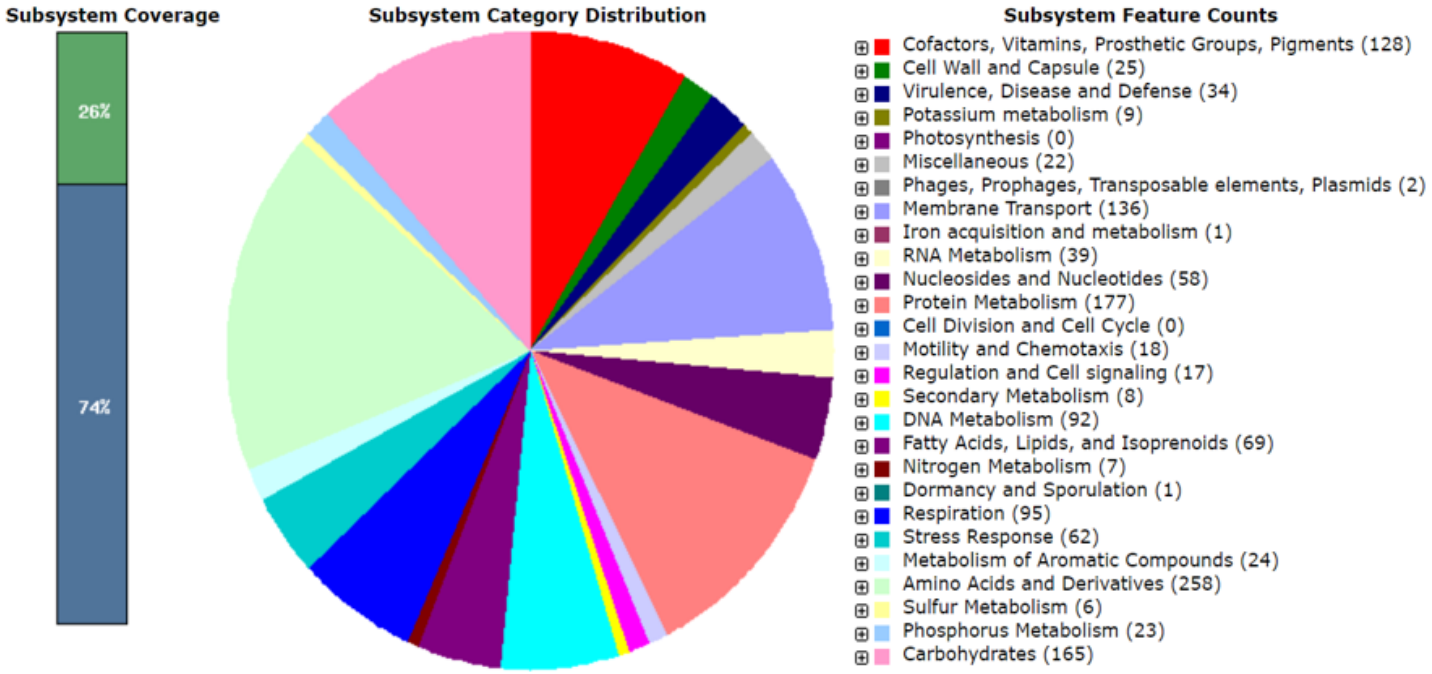

Figure 3

Subsystem category distribution of key PCG of S. indicatrix strain BOVIS40 annotated in the RAST SEED Viewer annotation online server. The green/blue bar shows the subsystem coverage in percentage. Blue bar correlates to the percentage (%) of proteins present

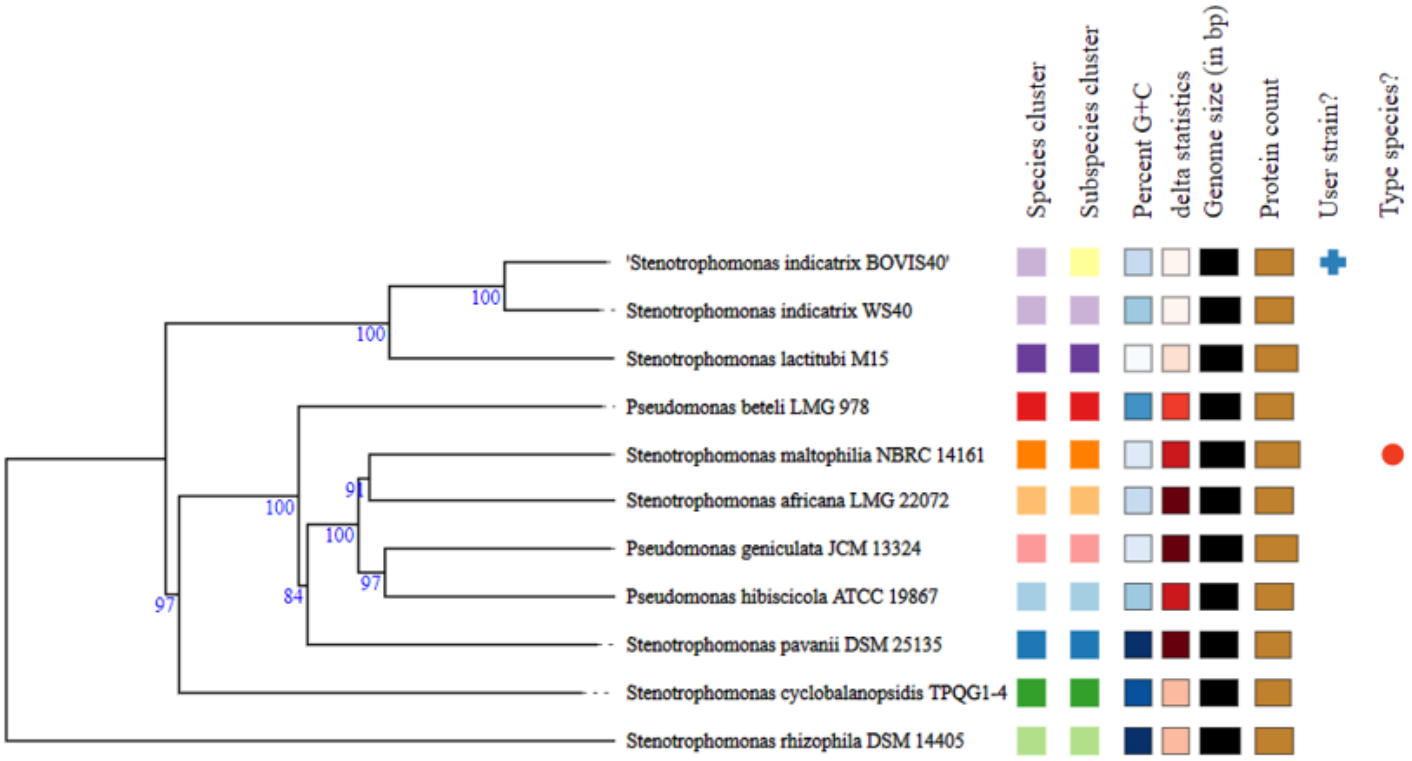

Figure 4

Genome blast distance phylogeny (GBDP) based on genome data The GBDP phylogeny based on genome data reveals percent G+C 65.89-67.39, delta statistics 0.078-0.18 (the lower the delta statistics, the higher the accuracy for the assessment of the phylogenetic accuracy in term of treelikeness (Holland et al. 2002), the genome size 4,212,838-4,936,723 bp, number of proteins 3,685-4,537, SSU lengths 1417-1,535 bp

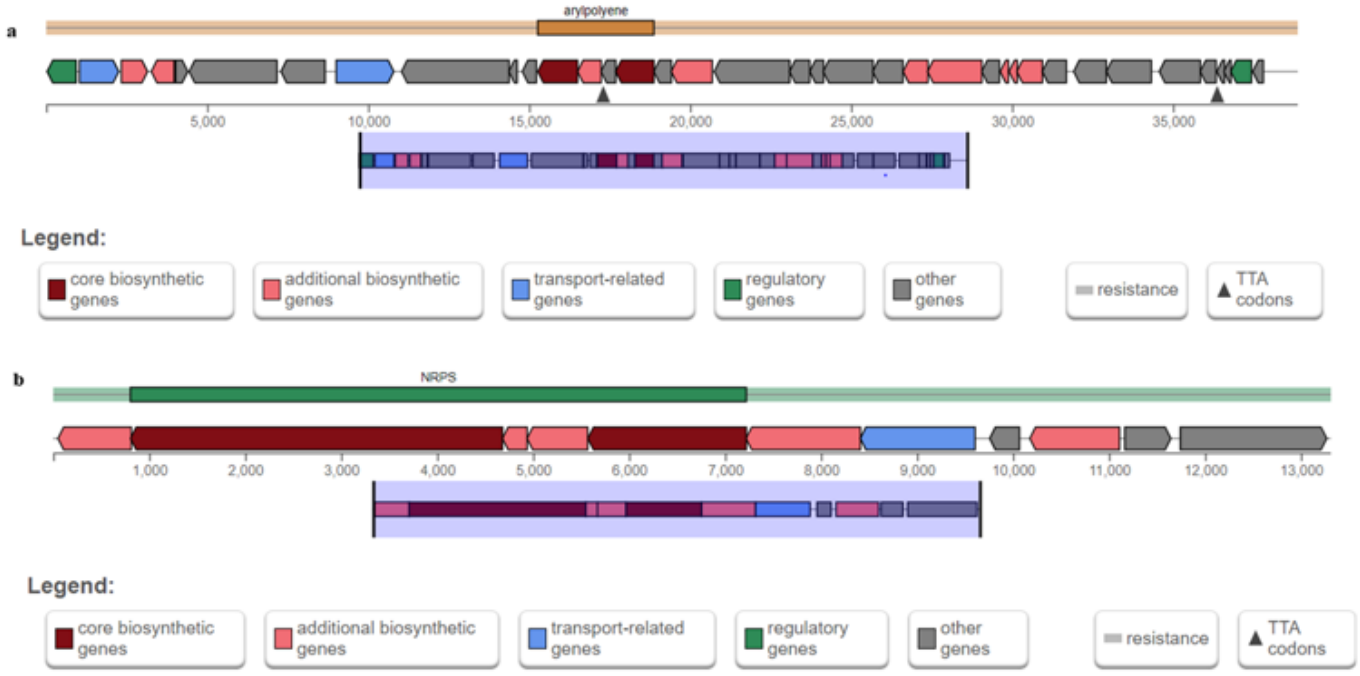

Figure 5

Arylpolyene-and-NRPS-encoding genes

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