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Bioactive secondary metabolites of *Bacillus toyonensis,* AVSW 2 an endophytic bacteria on Plant growth promotion of tomato

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Abstract:

This study focuses on the role of chilli root endophytic bacteria *Bacillus toyonensis* AVSW 2 in promoting plant growth of tomato and fungal antagonism against *Fusarium oxysporum* .AVSW 2 ability regarding fungal antagonism, SEM of root colonization , optimization of growth and enhancing production of Indole-3-acetic acid, phosphate solubilizing, Ammonia and siderophore production, subsequently followed by in vitro plant growth promotion of tomato using seed bacterization were evaluated . Potential volatile organic compounds and secondary metabolites production were studied using GC-MS and HPLC. AVSW 2 showed 22.17 µg/ml of Ammonia production, 18.23 µg of IAA production ,29 psu of siderophore and 51.33 ppm of phosphate solubilization under optimized growth conditions (40 ^{0} C, pH 7, 1% NaCl,1% Glucose ,1% Peptone and 60 hours of incubation). In AVSW 2 inoculated tomato plants , plant growth promoting traits such as root length, shoot length, no of leaves, no of lateral roots, biomass , protein and carbohydrate content and antifungal traits are higher compared to untreated control .2-[2-(2-Butoxyethoxy)ethoxy-trimethyl silane , 2-Ketoisocaproic acid, trimethylsilyl ester, O-methylisourea, 1-Butanamine , n-methyl-n-nitroso- are the significant compounds identified in GC-MS analysis of *B.toyonenis* AVSW 2 responsible for fungal antibiosis, root colonization and plant growth promotion.

KEYWORDS: *Lycopersicum esculentum*, Plant growth promotion, fungal antagonism
Bacillus toyonensis, GC-MS, *Fusarium oxysporum* .

Introduction:

Tomato (*Lycopersicon esculentum* Mill.) is one of the most pre dominant vegetable crops globally enriched with vitamins, minerals, organic acids, essential amino acids and dietary fibres. Productivity of tomatoes decreasing due to abiotic and biotic stresses Microbial diseases such as Fusarium wilt, early blight, Late blight ,damping off,leaf spots,bacterial fruit canker, Anthracnose,bacterial speck affects Tomato .Previous findings reported that nitrogen deficiency is the reason for low quality and productivity of the Tomato. Despite adeverse conditions,plant growth-promoting rhizobacteria (PGPR) has the potential to promote plant growth(Abdallah et al .,2018; Alexander et al ., 2019, Bandana Saikiam et al., 2022, Adeleke et al., 2022). Currently eco-friendly, cost-effective and easily replicable bioinoculants like plant growth promoting. Rhizobacteria are used more in agricultural ecosystems as they produce secondary metabolites promoting plant growth, stress tolerance and disease resistance(Rajnish P Singh et al., 2017, Kumar et al.,2018, Vipin Kumar et al., 2018, Ankita Alexander et al., 2020, Kristina Ulrich1 et al et al.,2021,Osman,N.H et al., 2022) .Bacterial endophytes produces IAA, Ammonia, Siderophore , secondary metabolites and solubilizes phosphate and maintain host cell wall integrity (Aeron et al.,2020,Cigdem etal.,2022).Being in symbiotic association with the host,endophytes synthesizes secondary metabolites. Under oxidative stress such secondary metabolites induce immunity and systemic resistance against microbial pathogens and promote plant growth (Quan et al., 2010, Deshmukh et al., 2018, Rojas-Solís et al., 2018, Aira Ali et al ., 2020, Alijani, Z et al., 2020, Raio et al., 2023). Siderophores produced by Plant growth promoting Rhizobacteria ,as bio control agents scavenge the available iron present in the pathogen surroundings and also involve in iron transportation(Atsushi Hisatomi et al.,2021).However, physiological attributes of metabolites from endophytic bacteria and metabolic profiling have not been much studied. The present study focussed on the metabolic profiling of an endophytic bacteria , *Bacillus toyonensis* isolate extracted from Leaves of chilli and its plant growth promoting features

Isolation of *Bacillus toyonensis* AVSW 2

In the present research , an endophytic bacterial isolate AVSW 2 isolated from leaves of the chilli plant , was collected from Guntur, Andhra Pradesh, India.It was collected from the corresponding author as a subject for the current research. AVSW 2 was purified by the streak plate method and grown in nutrient broth. After incubating for 48 hours , it was stored(Vacheron, J et al., 2013, Mahdi et al., 2014, Bastien Cochard et al ., 2022).

Materials and Methodology

Screening of fungal antagonism and PGP activities

AVSW 2 fungal antagonism was assessed by double culture method (Kumar et al.,2002) .Bacterial isolates were streaked at 3 cm from the pathogenic fungi inoculated at the centre on Sabouraud dextrose sugar (SDA) medium .After 4-7days of incubation, Antifungal activity was measured at room temperature. Inhibition levels were measured as I= 1-(a/b) X as described earlier (PattenCL, Glick , 2002 ; Sudhir Allu et al., 2014 ; Rania Aydi Ben Abdallah et al ., 2016, Sarbadhikary et al., 2017, Aktas et al., 2023)

Indole-3-acetic acid production

One ml of Bacteria were cultured for 72 hrs in filtered and supplemented with L- tryptophan (1 µg ml- l), and 2 ml Salkowski reagent and incubated at 28 ± 20° C for 30 min. Development of Pink colour indicates the presence of IAA (**AT Wahyudi, et al** .,2011)

Phosphate solubilisation ability:

Tricalcium phosphate solubilisation was detected in Pikovskaya's agar described (Vacheron et al.,2013). Bacterial isolates were streaked on the surface of the Pikovskaya agar medium, and after 1 to 5 days of incubation at room temperature,activity was estimated .A clear zone development surrounding the bacterial colony was a positive response for phosphate solubilization (Husen E, 2003).

Ammonia Production

Ammonia formation was tested with peptone water. Newly grown cultures were inoculated into 10 ml of peptone water and incubated at 36 ± 2° C for 48-72 hours. Nessler's reagent (0.5 mL) was added. A change from brown to yellow was a positive reaction for ammonia production (Desire et al.,2014).

Siderophore Production

The bacterial strain was grown on succinate medium and incubated for 24-30 h at 120 rpm and at 28 ºC. For every 20 minutes interval , 5 ml broth was centrifuged at 10,000 rpm, for 10 minutes at 40C , and cell-free supernatant was transfused with 0.5 ml CAS solution. Taking 0.5 ml uninoculated succinate medium and 0.5 ml CAS solution as control , colour was measured at 630 nm using a UV-visible spectrophotometer (Desire et al.,2014).

Biochemical Physiological and Molecular Identification

Biochemical tests such as IMViC test, Indole test, MRVP, Citrate utilization, hydrogen sulphide production, and sugar fermentation were done following the standard protocols (Aneja, K. R., 2003)

Physiological characteristics

Amylase,Cellulase, Catalase, Oxidase,Urease, Gelatin hydrolysis, Nitrate reduction, Haemolysis and Lecithinase were analysed as per the protocols (Cappuccino, J. G. et al., 1992; Aneja, K. R., 2003 ; Eman F. Sharaf et al., 2014).

Molecular Characterization

It was done according to Bergey's manual of determinative bacteriology for tentative identification of Genus (Holt,J.et al., 1994).After that 16 S RNA partial gene sequencing analysis done by using universal primer 1492R(5´-TACGGYTACCTTGTTACGACTT-3') and 27F(5' AGAGTTTGATCMTGGCTCAG-3').Phylogenetic tree analysis was done by Neighbour-joining method with 1000 bootstrap replicates. Isolate was deposited in Genbank NCBI (N Saitou and M Nei, 1987; Holt,J.et al., 1994)

Optimization studies

Physical parameters such as temperature, pH, incubation period, and chemical parameters NaCl , carbon and nitrogen sources were studied in 10ml of broth inoculated with 100 µl of bacterial culture and incubated for 48 h .O.D of the culture broth was read at 600 nm .Growth optimization of endophytic bacteria was analyzed at various emperatures ranging from 20-45°C at an interval of 5°C , pH value range [3-9] ,saline concentration range 1-6 % , 1% carbon sources (Glucose, Fructose, Sucrose, Lactose, Maltose, Glycerol, Mannitol, Starch, and Cellulose) , 0.5%nitrogen sources (KNO3, NaNO3, NH4SO2, Urea, Peptone, Beef extract, Yeast extract, Casein, and Malt extract) and incubation periods (24 h, 36 h, 48 h, 60 h and 72 h).Bacterial Growth Curve for 24 h bacterial culture was also determined at an interval of 4 h .

PGP activity of endophyte by Pot Assay Studies:

Tomato seeds were treated with 48 h bacterial culture for 30 min and shade-dried for 1 h. These seeds were sowed into coco peat and grown under greenhouse conditions. Germination % of seeds was evaluated at an interval of 4, 6 and 8 weeks. Physiological parameters root length, shoot height , biomass, no of leaves, no of lateral roots were estimated along with chemical constituents such as Protein content by Lowry's and carbohydrate content by DNS method .

Metabolite profile Finger print of endophytic bacteria

Secondary metabolite finger print of endophyte was analysed in ethyl acetate extract using Fourier transforms infrared spectroscopy (FTIR) and GC-MS (JyotiPrakashMaity et al., 2013; Serban C et al., 2018 ; Haron et al., 2022; Muhammad Ramzan et al .,2023)

Scanning electron microscopy

Root colonization ability was observed using SEM. One cm root pieces were fixed and processed with the PATOTO method. The prepared samples were mounted on aluminium stubs with Scotch TM double-sided tape, coated with gold in a sputtering Hummer II (Technics,Springfield,VA) and examined in a Cambridge S360 Scanning Electron Microscope.

Results and Discussion

Fungal antagonism and PGP Traits

AVSW 2 pure culture was screened for fungal antagonism against *Fusarium oxysporum* by dual culture method as shown in Plate 1. AVSW 2 showed significant inhibition of *Fusarium oxysporum* compared t o control .and positive to PGP traits such as IAA, Ammonia, Phosphate solubilisation and siderophore production in qualitative screening.

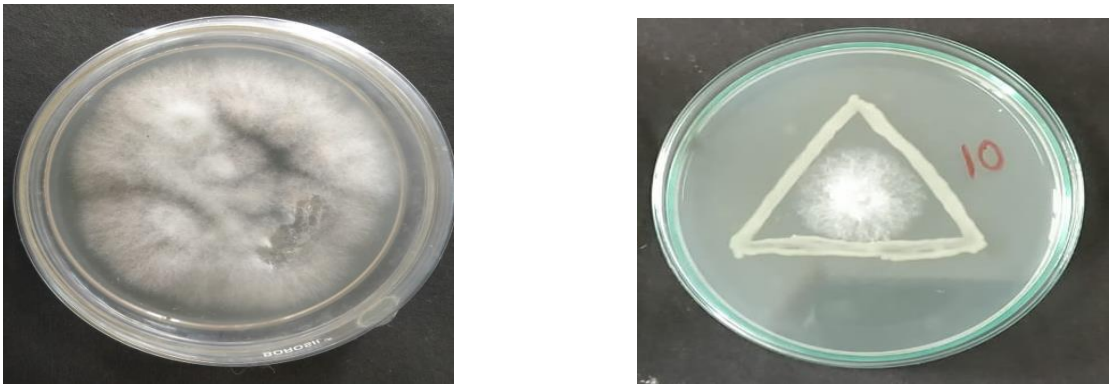


Plate1 Screening of Fungal antagonism of AVSW 2 against *F. oxysporum*

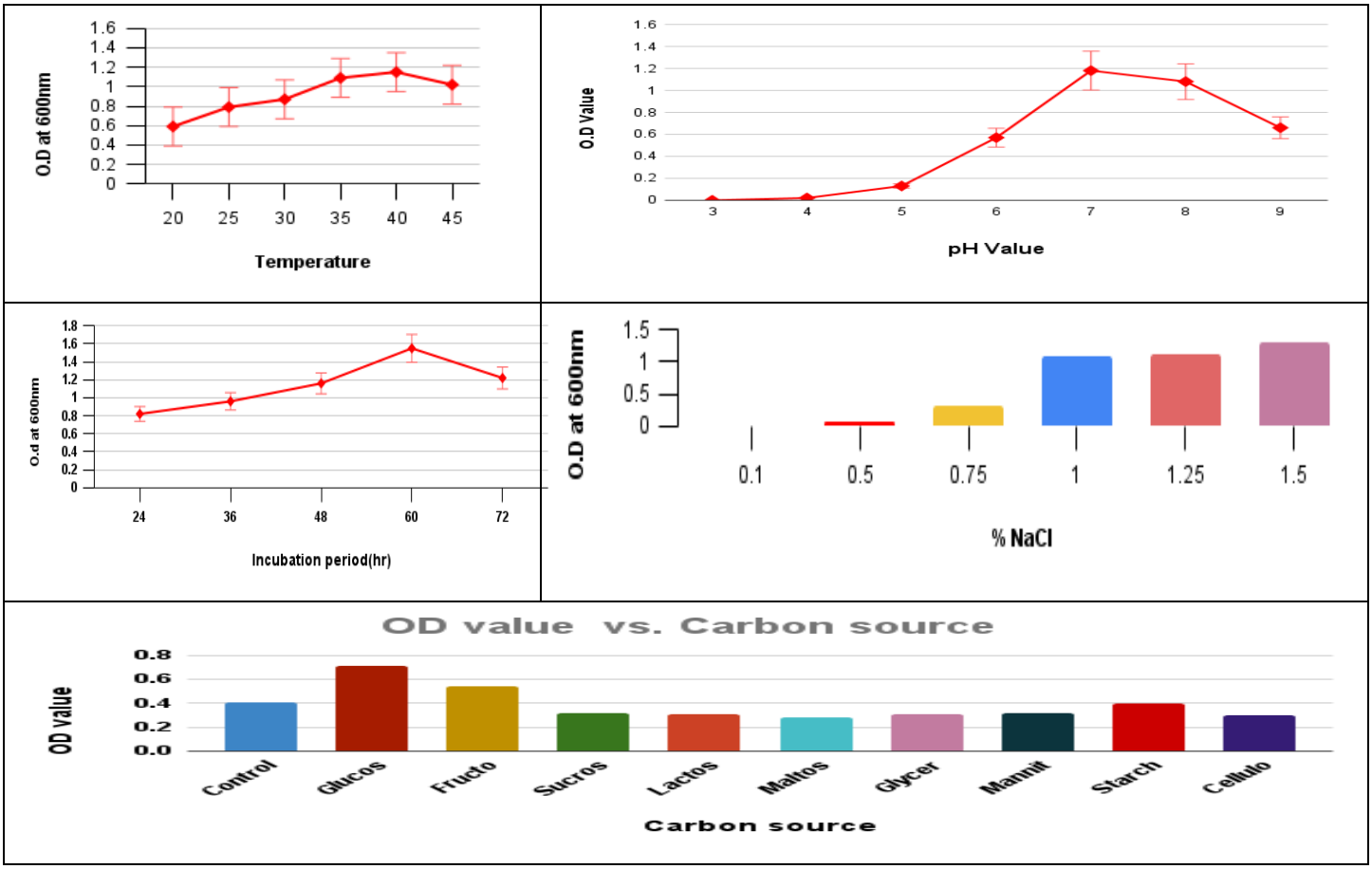
***F. Oxysporum* on NAM as control** **Dual culture of *F.oxysporum* and AVSW 2**

Effect of Physical and chemical parameters on Bacterial Growth:

Physicochemical parameters were analysed on a one factor one -factor one- time factor (OFOT) basis to optimize he growth and maximise the production of PGP traits. Optimization studies

revealed that AVSW 2 showed maximum growth at 40 ^{0} C, 1% salinity and 60 hrs incubation period with minimal medium ameliorated with 1% Glucose and 1 %Peptone (Fig 1).

The growth curve of *B. toyonensis* AVSW 2 displayed 4-8 hours Lag phase, 12-44 hours Exponential phase, and 44-68 hours stationary phase. After 68 hours , decline phase was observed (Fig2).



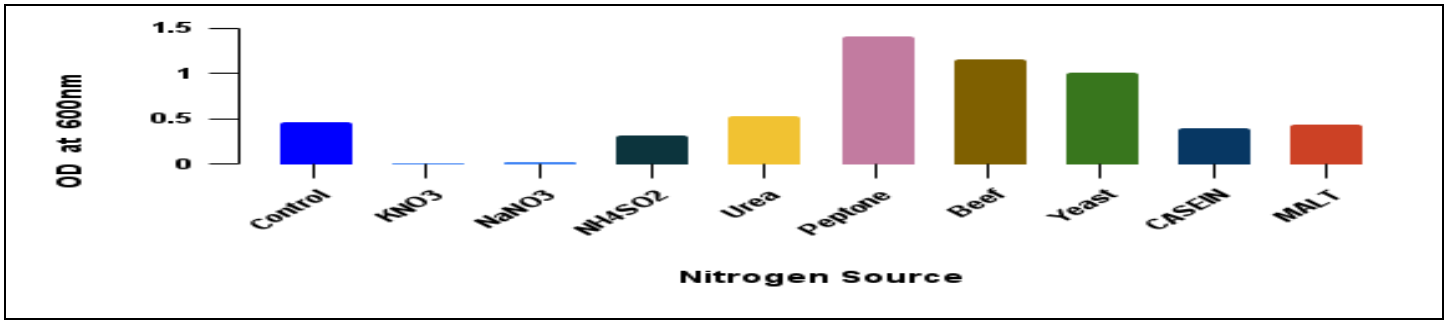


Fig .1 Optimization of Physical and Chemical parameters on growth of AVSW 2

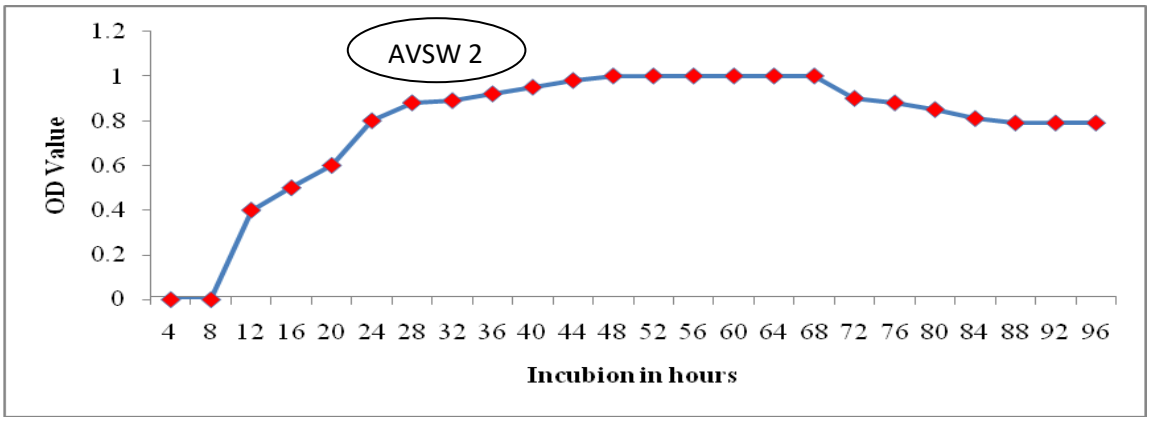


Fig 2: Growth analysis of AVSW 2 from 4 - 96 hrs of incubation in optimized medium

PGP traits of AVSW 2

Plant growth promotion (PGP) traits in *B.toyonensis* AVSW 2, such as IAA, Ammonia, PO4-solubilisation and siderophore production, were evaluated after optimization. (Table1 and fig 2) . In *B.toyonenis* , AVSW 2 after optimization ,IAA production increased by 25.06 % , Ammonia production increased by 38.13 % ,Phosphate solubilisation increased by 41.75 % and siderophore production increased by 13.89 % whereas in earlier findings, *B.toyonensis* reported IAA (20.49 µg ml- ^{1})phosphate solubilisation(830.00 ppm)Nitrate solubilisation(175474.67ppm) after optimization (Mohammad, M et al.,2023).Previous studies reported that in a bacterial endophyte , *B.toyonensis* HAPH8 isolated from tissues of black saxaul *Haloxylon aphyllum* Minkw , after optimization reported IAA (119.03 µg/ml) and 25 % increase in phosphate solubilisation, siderophore production and in Ammonia production (Shurigin et al., 2022)

Similarly, *B. toyonensis* CR 30 isolated from roots of *Physalis ixocarpa* reported IAA (51.275 µg /ml), 25-50 %increase in phosphate solubilisation and in siderophoreproduction . *B.toyonensis* isolated from stems reported IAA (3.9455 µg /ml) ,50-75 % increase in Phosphate solubilisation and 25-50 % increase in siderophore production (Hernández-Pacheco et al., 2021) . In the same

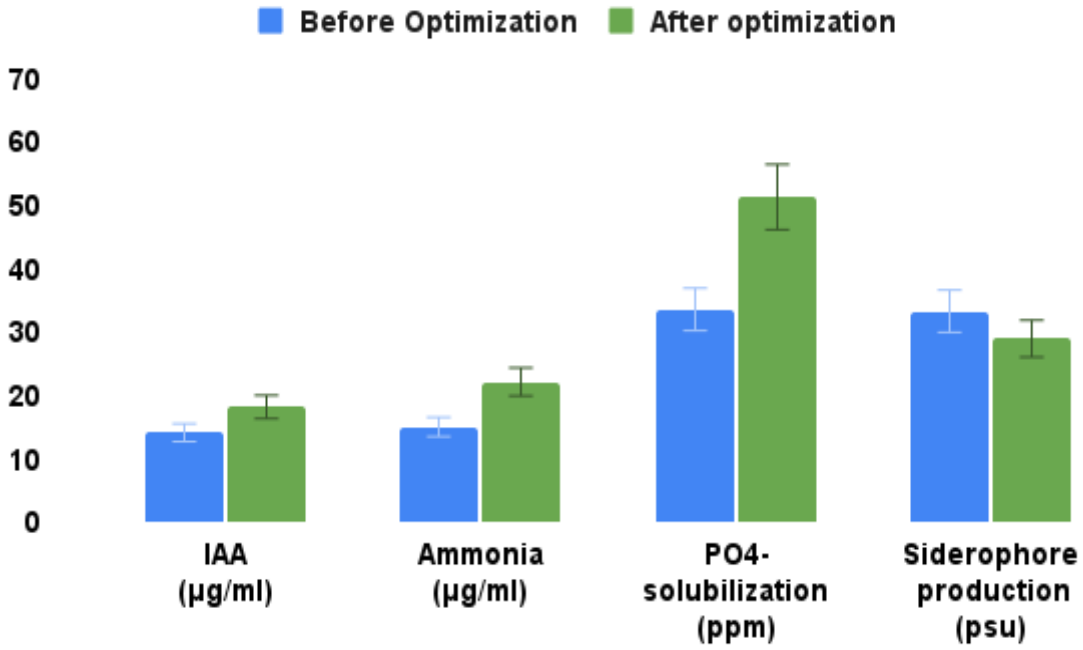
manner *Bacillus toyonensis isolate* EPL1.1.3 reported IAA (34.6 µg ml-1) and promoted the plant growth rate of tomato (Yanti et al., 2017). Previously *Bacillus* sps HBS –VII *i*solated from the rhizosphere of tomato plants reported IAA (150 µg ml- ^{1}) and increase in phosphate solubilisation and siderophore production by 25 % each (Agrawal et al., 2013)

Previously two Species of Bacillus namely *B. cereus* (So3II) and B. *subtilis* (Mt3b) isolated from rhizosphere of *Solanum nigrum and Malvastrum tricuspidatum* reported IAA production (18 µg ml-1 and 20 µg ml-1) , enhancement of phosphate solubilisation by 73% and 78 % and enhancement of Siderophore production by 74.2 % and 64.4 % at pH 7, temperature 37 and 25 ^{0} C , and broth incubated for 24 and 48 hrs respectively(Wagi et al., 2019) .In the same manner , our results also revealed that *B.toyonensis* AVSW 2 is specific to host in expression of PGP traits and varies with endospheric and rhizospheric habitats.Ability of inorganic phosphate solubilisation is less in endophytic strain compared to rhizosphere strain which might be a marker attribute to distinguish endophytic PGP strain from PGPR. As per this study *B.toyonensis* could be a promising strain for further exploration as it shows fungal antagonism and promote plant growth.

Table 1 Quantitative analysis of Plant growth promotion (PGP) traits in AVSW 2

Optimization	IAA (µg/ml)	Ammonia (µg/ml)	PO_4^- solubilisation (ppm)	Siderophore (psu)
Before (control)	14.17	15.07	33.6	33.33
After	18.23	22.17	51.33	39

Fig 3 Impact of optimization on Plant growth promotion (PGP) traits of AVSW 2



10.4 **Identification of AVSW 2**

Based on colony morphological, and physiological characteristics (Table 2) followed by 16s rRNA partial gene sequencing analysis (Fig 4), AVSW 2 was identified as *Bacillus toyonensis* and deposited in NCBI GENBANK with the name *Bacillus toyonensis* AVSW 2 with the accession no OQ293976

Table 2: Colony morphology and Morphological, Biochemical and physiological characteristics of AVSW 2

Morphological Characteristics	
Colony Morphology	Small, circular ,Light Brown
Gram's reaction	Negative
Cell shape	Rod
Biochemical characteristics	
Indole , VP	Negative

MR/Citrate	positive
Sugar fermentations	
Glucose/Sucrose/Fructose/Lactose/Arabinose/ Inositol/Sorbitol/Dulcitol	A^+/G^-
Maltose/Mannitol	A^-/G^-
Physiological chaacteristics	
Decarboxylation reactions	
Lysine	Positive
Ornithine/ Arginine / Creatinine/ Malonate	Negative
Enzymatic reactions	
Amylase/Lecithinase/Catalase/Super oxide dismutase/ Gelatin hydrolysis	Positive
Urease/ Cellulase/ peroxidises/ Poly phenyl oxidase /Phenyl alanine deaminase/oxidase/ Nitrate reduction	Negative
Haemolysis	Negative
Spore formation	Negative

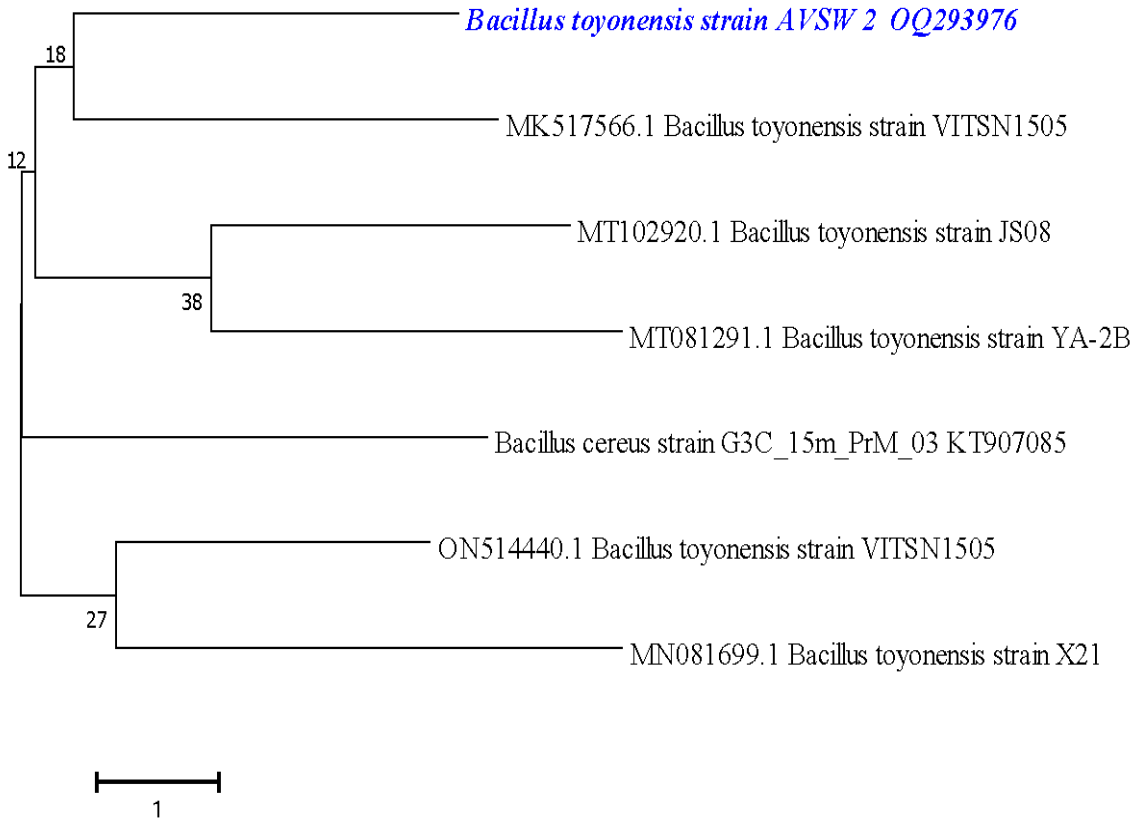


Fig: 4 Phylogenetic distance tree of *Bacillus toyonensis* AVSW 2 (OQ293976**) constructed by the neighbour-joining method using BlastN of NCBI with 1000 bootstraps**

10.5 Greenhouse studies

The plant growth promotion potential of *Bacillus toyonensis* AVSW 2 was tested on tomato seedlings under green house conditions using the seed bacterization method followed by inoculation of culture at the collar region . Root colonization efficacy of AVSW 2 inoculum was observed on 4,6 and 8week old seedlings treated with AVSW 2 under scanning electron microscope (Fig 5.1 & 5.2)

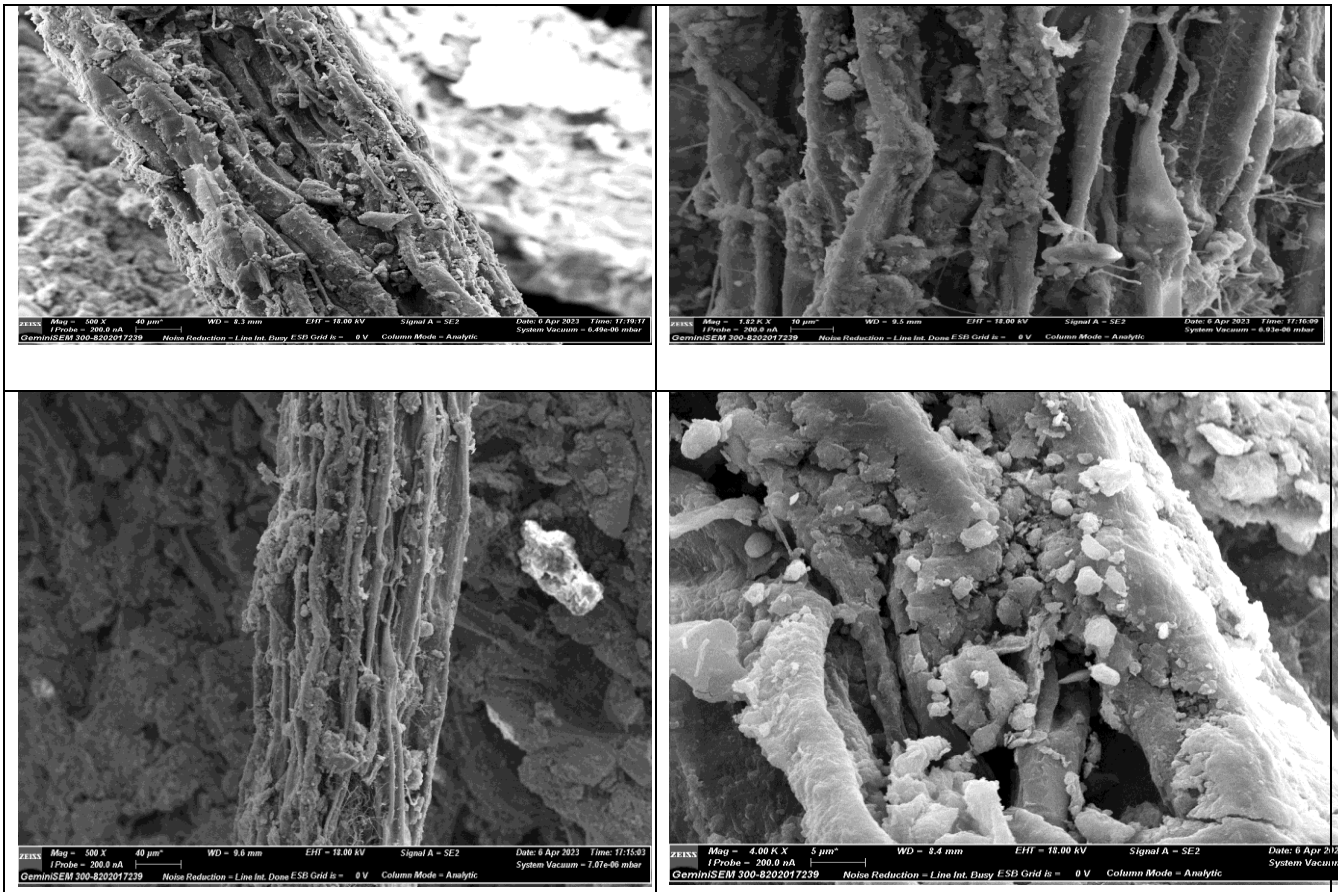


Figure 5.1.Scanning electron micrograph of root of tomato seedling treated with AVSW 2

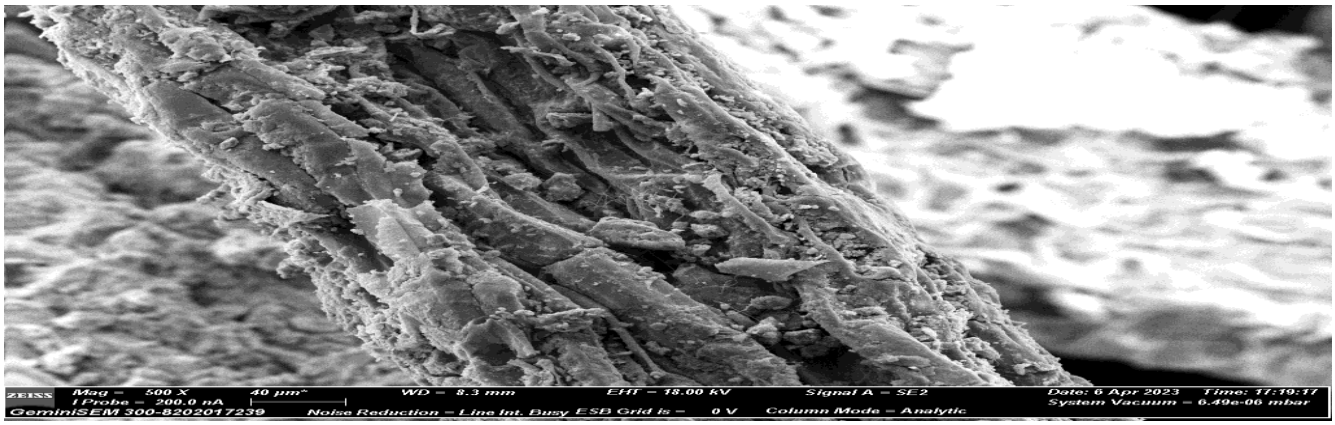


Figure 5.2. Scanning electron micrograph showing clear root Colonization in AVSW 2 treated tomato roots. Bacteria seen forming a third dimensional net together the strands of mucous Scale bar = 10 µm.

Plant growth parameters such as root length, shoot height, root shoot ratio, No of leaves and fresh weight, and nutritional metabolites (carbohydrates and proteins) were studied at intervals of two weeks from 4th week onwards using un inoculated tomato seedlings as control.

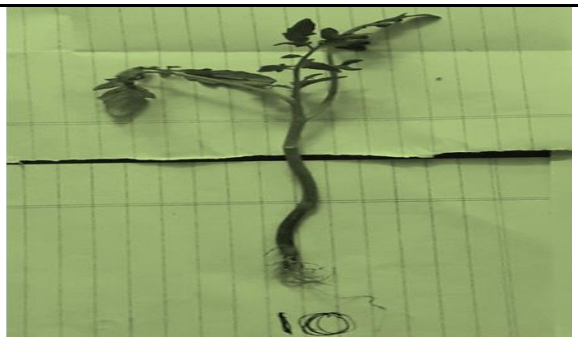
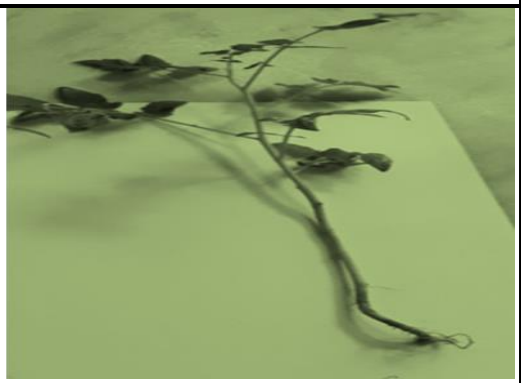
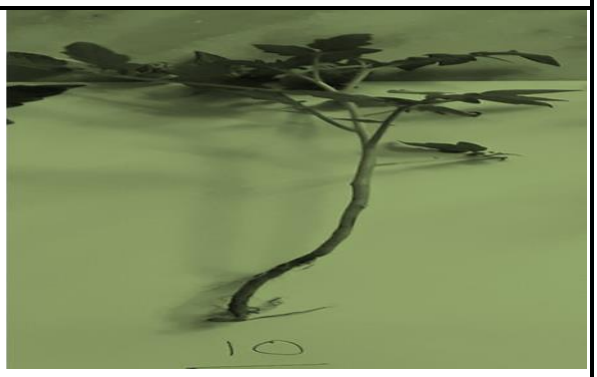
Week	Control Plant	AVSW 2 Treated plant
IV		
VIII		

Figure 6. Greenhouse studies of tomato seedlings treated with AVSW 2

In previous findings , *B.toyonensis* HAPH8 a bacterial endophyte isolated from tissues of black saxaul (*Haloxylon aphyllum* Minkw.) showed 23.09 % enhancement of shoot weight and 27.52 % root weight of tomato seedlings(Shurigin et al., 2022) .Previously endophytic bacterial strain of *Bacillus* sps Cr-71 , isolated from the rhizosphere of soyabean (*Glycine max*) showed nearly 143 % enhancement of shoot height , 70.89 % root length and 85.41 % enhancement of lateral roots of soya bean seedlings under greenhouse conditions (Wahyudi et al., 2011) Similarly, *Bacillus* sps HBR –VII *i*solated from rhizosphere of tomato plants showed 26.08 % ,26.31 % enhancement of shoot length in 6 days and 14.06 % ,30 % enhancement of root length in 16 days respectively [48] .In previous findings *Bacillus cereus* MH778713 isolated from root nodules of *Prosopis laevigata* reported 193.7% enhancement of shoot length and 510 % enhancement of root length in tomato (Verónica Ramírez et al., 2022) .

In the present study AVSW 2 treated tomato seedlings showed significant response in terms of the growth attributes and nutritive metabolites (fig 7.1 to 7.3). Compared to control clear increase of values in shoot length (19.54% 1.38 % , 16.25 %) root length (3.6 % ,5.7 % , 20.20 %). , number of leaves (37.18 %, 52.35 %,29.75 %),lateral roots (10.34 %,10.8 %, 0.48 %),fresh

weight of Plant (50.74 %, 62.56 %, 34.13 %)were observed in 4^{th} , 6^{th} and 8^{th} weeks respectively .AVSW 2 treated plants also showed significant enhancement of protein (22.22 %, 8.40 %, 15.62 %) and carbohydrate (22.22 %, 19.67 %,11.59 %) in 4^{th} , 6^{th} and 8^{th} weeks respectively. Our results emphasized that the isolate AVSW 2 can promote plant growth, nutritional health and its antifungal activity against *Fusarium oxysporum*. Based on the study, When compared with control, in AVSW2 treated seeds , there is a significant increase in root and shoot length, number of leaves ,lateral roots along with protein , carbohydrate content and fresh weight . When compared with untreated control tomato seedlings, treated seeds with AVSW 2 inoculum exhibited better antifungal activity against *Fusarium oxysporum*

Fig 7. 1 Root length and shoot length on 4^{th} ,6^{th} and 8^{th} week in treated and control plants

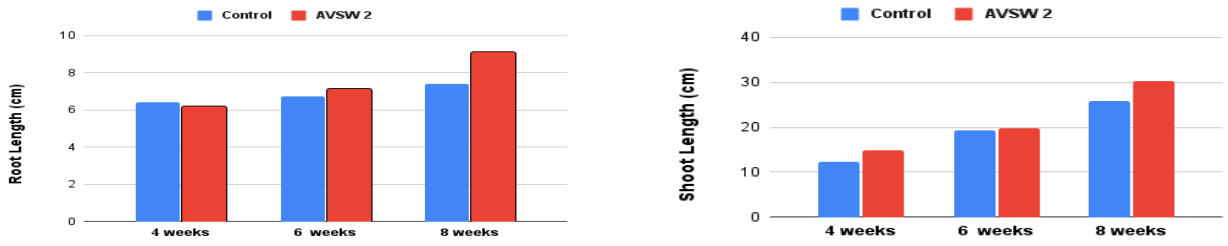


Fig 7.2 Fresh weight and number of leaves on 4^{th}, 6th and 8^{th} week in treated and control plant

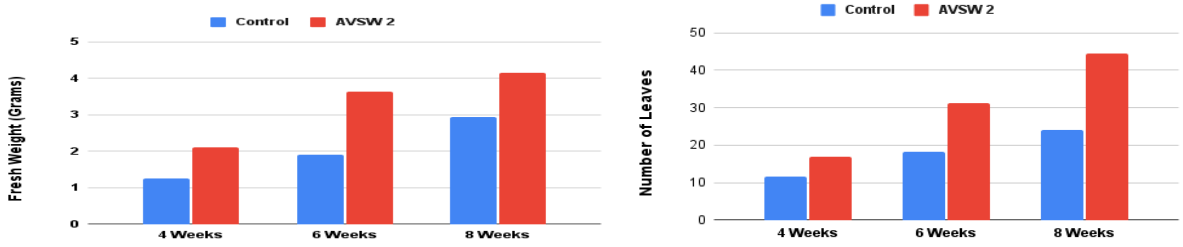


Fig 7.3 Lateral roots, Protein and carbohydrate on 4^{th}, 6th and 8^{th} week in treated and control

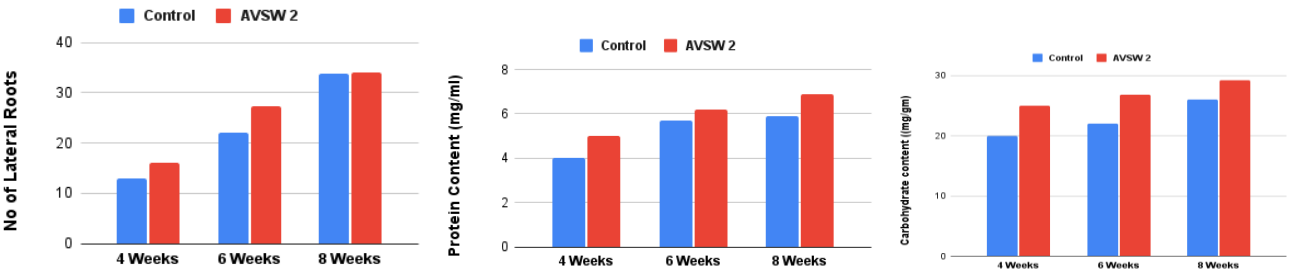


Fig 7. 1 Root length and shoot length on 4th ,6th and 8th week in treated and control

10.6 FT – IR Analysis

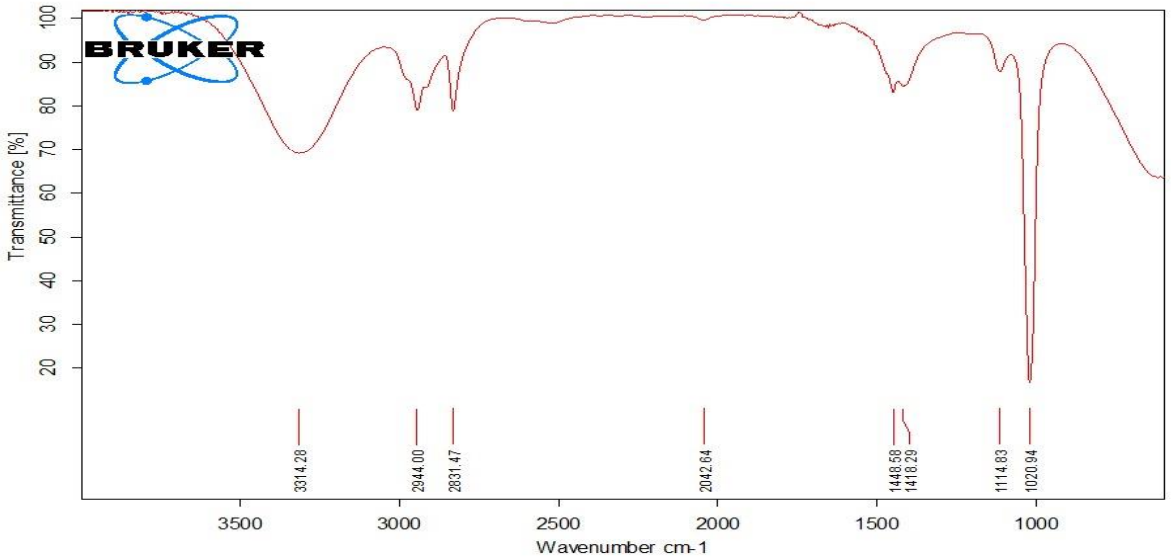


Figure 8. FTIR spectral analysis and interpretation of bioactive metabolites of *Bacillus toyonensis* AVSW 2

FTIR Spectral Data Analysis of *Bacillus toyonenis* AVSW 2

Wavelength range: cm $^{-1}$	**Functional groups and their bonds**
3314.25 *	C-H stretch, Alkyne (strong, sharp)
3303.73	OH stretch normal polymeric
2944.98	C-H stretch, Alkane (medium)
2832.35	N-H stretch, Alcohol (weak, broad)
2042.92 *	N=C=N stretch, Isothiocyanate (strong)
1659.67	C=C stretch, Alkene (medium)
1449.21	C-H bend, Alkane (medium)
1414.57 *	S=O stretch, Sulfate (strong)
1114.16 *	C-O stretch, secondary alcohol (strong)
1019.84 *	C-F stretch, Fluoro compound (strong)

10. 7 GC-MS Analysis

High-resolution analysis was performed by using the Thermo Scientific Q Exactive HF Orbitrap mass spectrometry system 6 min 8sec with the retention factor value of 4,3.00 with the molecular weight of 42.9582g/mol and the chemical formula C6H12O2. Compared to the NIST library, the

primary compound was identified as 2-[2-(2-Butoxyethoxy)ethoxy-trimethyl silane , which is confirmed by the FTIR results also . Similarly, the other compounds identified in the GC-MS analysis are confirmed to be the same as those in the FTIR analysis. As per the results of GC-MS, AVSW 2 extract contains several volatile organic compounds and secondary metabolites, which can efficiently control phytopathogens, increase plant growth, and induce systemic resistance.

Fig: 9 Chromatogram of GC-MS Analysis of *Bacillus toyonenis* AVSW 2

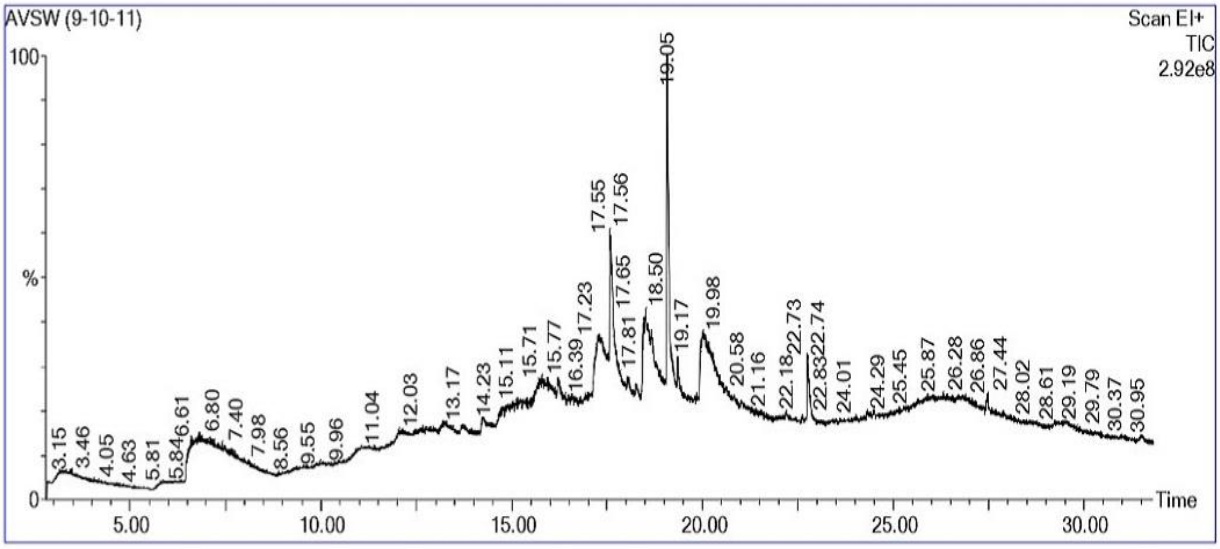


Fig.10 Chromatogram of GC-MS analysis of RT: 19.05 Bacterial extract of *Bacillus toyonensis* with the highest peaks .Spectrum RT images from GC-MS Analysis

CFL 10 showed 2-[2-(2-Butoxy Ethoxy) Ethoxy] EthoxtTrimethylSilane with mol. weight (278.46 gm/mol)and 12.275 % area at RT 17.274,2-ketoisocaproic acid,trimethylsilylester with mol. weight (202 gm/mol)and 13.507 % area at RT 17.559 ,O-Methyl isourea with mol. weight (74.08 gm/mol)and 7.883 % area at RT 18.500 , 2-1-Butanamine,N-Methyl-N-Nitroso with mol. weight (116.1616 gm/mol)and 3.423 % area at RT 18.640 (Figure 10)

Table 3 : Metabolic Profile of Bacterial extract of AVSW 2 *Bacillus toyonenis*

Isolate	RT	Height	Mol.Formula	Mol. weight gm/mol	%Area	Norm	Compound
CFL 10	17.274	40.798.592	C13H30O4Si	278.46	12.275	60.20	2-[2-(2-Butoxy Ethoxy) Ethoxy]EthoxtTrimethylSilane
CFL 10	17.559	106,691,120	C9H18O3Si	202	13.507	66.24	2-ketoisocaproic acid,trimethylsilylester
CFL 10	18.500	52,147,852	C_2H_6 **N** $_2O$	74.08	7.883	38.66	O-Methyl isourea
CFL 10	18.640	35,413,672	C5H12N2O	116.1616	3.423	16.79	1-Butanamine,N-Methyl-N-Nitroso

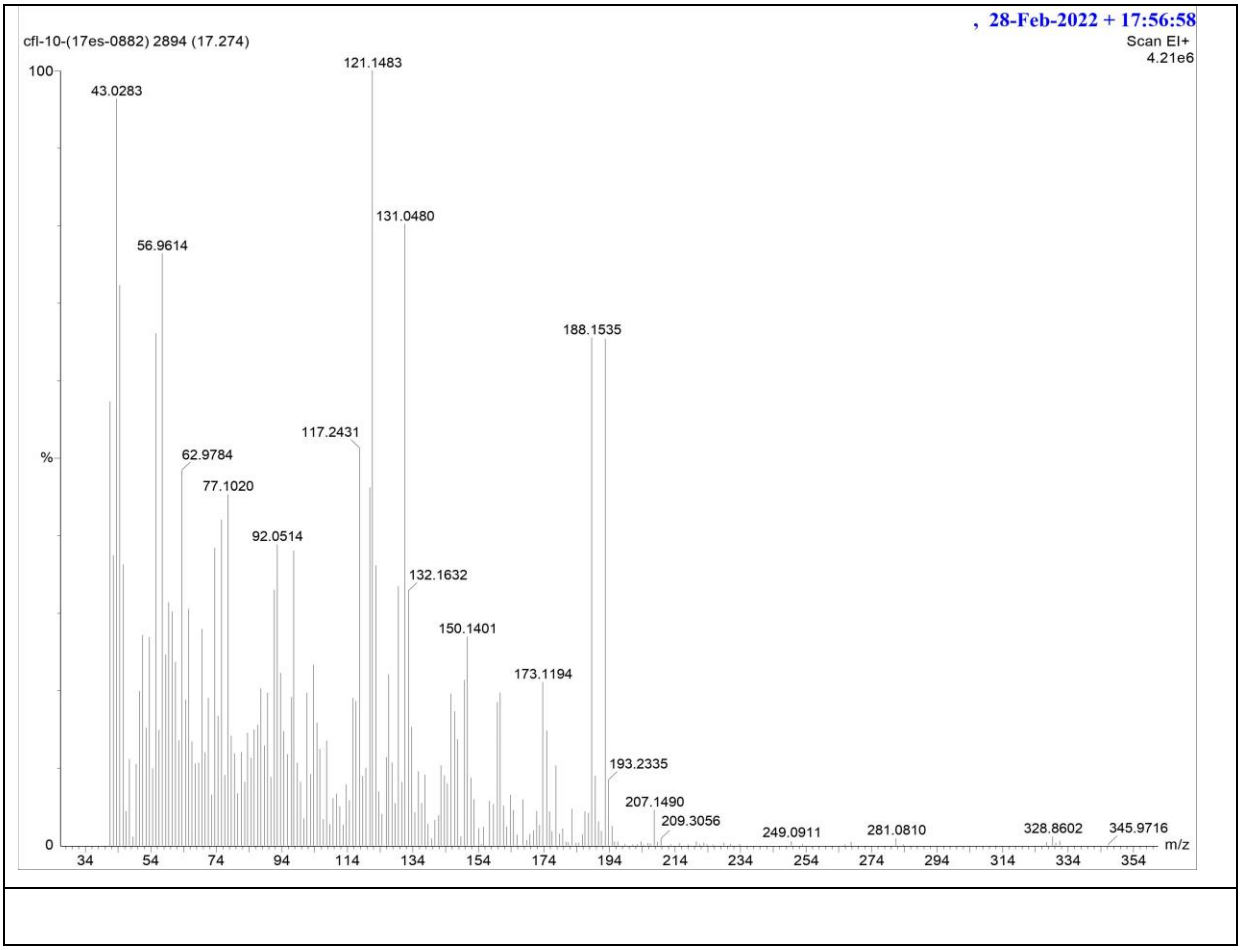


Fig.11 Chromatogram of GC-MS analysis of RT: 19.05 Bacterial extract of *Bacillus toyonensis* with the highest peak .Spectrum RT images from GC-MS Analysis

Metabolic Profile of Bacterial Extract of AVSW 2 *Bacillus toyonensis*

As per the NIST Database bioactive metabolites such as 2-[2-(2-Butoxyethoxy)ethoxy-tri methyl silane ,2-Ketoisocaproic acid, tri methyl silyl ester, O-methyl isourea,1-Butanamine , n-methyl-n-nitroso, propanedioic acid, propyl, 2- Propanamine ,N- Hydroxy, N-N Di methyl formamide di propyl acetal,carbamic acid,n aminoc arbonyl methyl,-iso butyl ester,Hexanoic acid 1-methylethyl ester, trifluoroacetyl -diisopropyl phosphine,1- Butanamine, n-methyl , n-nitroso-, N-N-Di methyl formamide dipropyl aceta,3-iso propoxy alanine, Beta methyl xyloside, Cycloserine, Beta-D –Ribo pyranoside, methyl, octyl-beta–Gluco pyranoside, Alpha -D- Gluco pyranoside, 1-o-methyl-4-hexyl-, alph ,beta ,D- Gluco pyranoside,4-o-hexyl are the pivotal compounds which showed PGP features like root colonization, antifungal, high salinity and stress tolerance features, production of secondary metabolites and agrochemicals ,signalling of molecules,enhanced microbial tolerance,anti-oxidation,cryo protection,immune response, regulating bacterial glycogen metabolism, gluconeogenesis, anti-inflammatory properties ,inducing plant defence mechanisms and there by promotes plant growth and metabolism.

Some of these compounds play a very significant role in several biological and physiological processes such as 2-[2-(2-Butoxy ethoxy)ethoxy-trimethyl silane used as a solvent ,diluent, coalescing agent and its products used as microbial pesticide .Tri methyl silyl (TMS) derivatives are the protecting groups of alcohols in chemical reactions. O-methyl iso urea hydrochloride is an organic intermediates used to prepare 1-chloro-2-methyl-isourea. O-Methyl isourea sulphate **is** used in the synthesis of pesticides and pharmaceuticals (fluoro pyrimidine antitumor drugs for malignant tumors).

In 1-Butamine,N-methyl-N-nitroso,1-Butanamine there is an ingredient which is used in pesticides like thiocarbazides, pharmaceuticals,emulsifiers and a precursor for synthesizing N,N′-dibutylthiourea.N-methyl-N-nitroso MNU used as a research chemical for developing mutants with improved growth parameters, yield potential, and physicochemical properties. Propanoic acid and propyl/propyl malonic acid are considered as Bio protectants with anti microbial potential and antioxidant properties. Di methyl formamide acetals and Bredereck's reagent (tert-butoxy-bis(dimethylamino) methane) are building blocks of aldehydes, ketones, enones, enol ethers, methyl groups and also act as alkylating agents. Carbamic acid ,n amino carbonyl methyl,-isobutyl ester is a biopesticide and derivatives of carbamic acid like ethylene bisdithiocarbamates(EBDCs)are fungicides which are used practically as ecofriendly insecticides and ecto parasiticides and increase Agricultural productivity . Hexanoic acid 1-methylethyl ester is a GC-MS standard that provides aroma and flavor to fruits, flowers and foods. Beta-methyl-xyloside induces xylanase in bacteria there by increasing the quality,purity and yield of products. Cycloserine shows antibacterial ,antiviral and antifungal properties . Beta D ribopyranoside , methyl / β-Mercaptoethanol is an Enzyme, Protein reactivator and potent reducing agent used in cell culture media ,Electrophoresis ,amino acid detection and in distinguishing ssDNA/dsDNA. Octyl beta-D-gluco pyranoside is a beta-D-glucoside and a plant metabolite which solubilize membrane bound proteins Hexanoic acid- 1- methyl ethyl ester is a nutrient ,surfactant, emulsifier, membrane stabilizer, flavoring agent and plays a significant role in Lipid transport,peroxidation , Fatty acid metabolism and in Cell signaling. alpha beta D gluco pyranoside 4-O- hexyl is a flavonoid , antioxidant , non ionic detergent useful for solubilization of lipids and proteins and also in isolation and denaturation of pigment proteins and facilitate cellular uptake and reduce cytotoxicity.

Similar to our results, in previous findings , GC-MS analysis of *Bacillus toyonensis* FORT 102 (BSS 121) revealed that Acetone , 2,3-Butanedione ,2,3- Pentanedione, Acetoin, 3-Pentanol, 2-methyl-, 6 Oxirane, (methoxymethyl)- .7 Nonanal, Acetic acid, 9 1-Hexanol, 2-ethyl- , E-3-

Pentadecen-2-ol, 2,3-Butanediol, [S-(R*,R*)-, Formic acid, octyl ester, Propanoic acid, 2-methyl-,2-Octanol ,(S)-(+)-6-Methyl-1-octanol,1-Nonanol,Butanoic acid, 2-methyl-, Dodecanal , Oxime-, methoxy-phenyl-, 2,4-Decadienal, (E,E)- ,Pentanoic acid, 3-Buten-2-one, 4-(1- cyclopenten-1-yl)-,(E)-, Hexanoic acid, 2-ethyl- ,1-Dodecanol ,Phenol,Octanoic acid, Nonanoic acid , Hexadecanoic acid, methyl ester, 1,4-Benzenediol, 2,6-bis(1,1- dimethylethyl)-, Decanoic acid, Benzoic acid, heptyl ester ,Benzoicacid , 1,2-Benzene dicarboxylic acid bis(2-methylpropyl) ester, Dibutyl phthalate,Hexadecanoic acid ,Oleic Acid ,9,12-Octadecadienoic acid (Z,Z)- are produced by *Bacillus toyonensis* FORT 102 (BSS 121) and were reported to have antibacterial, anti fungal, antioxidant , anti microbial, signalling properties and also significantly enhances the fresh weight and length of roots and shoots. (Moldir koilybayeva et al , 2023).

B.toyonensis BMC10 showed many VOCs like Dodecane,3-hydroxy-2–butanone,2,3-butanediol,2,5-dimethylpyrazine,Benzaldehyde,Hexadecane,2-ethyl-1-hexanol,cyclopropyl carbinol,2-propanamine,4-methyldecane,2,6-dimethylundecane,ethylene,ethyleneoxide,cyclo butanol showing antifungal properties and plant growth promoting features (Heenan-Daly et al.,2021). In particular, DMS has been demonstrated to have antifungal activity both *in vitro* (Wang et al., 2013) and *in vivo* when fumigated to plants (Li et al., 2010).

Mono terpenes from *S. rhizophila* Ep2.2 play biological and ecological roles in bacteria to cope with abiotic and biotic stresses and act as info chemicals in mediating microbial interactions (Avalos et al., 2022). Bacterial terpenes i.e. β-pinene can also show inhibitory activity against fungi (Song et al., 2015; Aida Raio et al., 2023)

11.0 Discussion:

Presently, utilizing PGP bacteria in agriculture as Bio fertilizers and Bio inoculants is more optimistic. By producing various regulatory chemicals, volatile organic compounds and secondary metabolites in the vicinity of the rhizosphere, PGPR protects plants from diseases , biotic and abiotic stress conditions . Majority of the bacterial endophytes are potential bio control agents against diseases as they can colonize an ecological niche. To meet the phosphate demands of plants in sustainable agriculture, one of the alternative bio technological solutions is using phosphate solubilizing PGPR as inoculants. In regulating plant growth IAA is one of the most crucial signal molecules. Common abiotic stress factor , soil salinity reduces plant growth, photosynthetic capacity, protein synthesis, energy, lipid metabolism, and total nitrogen content.

PGP bacteria are a potential bio resource for improving crop productivity as they can face abiotic salinity stress tolerance effectively. As soil pH is another limiting factor for the growth of plants, PGPR tolerates a wide range of pH and thus confirms its ability to survive both in acidic and alkaline soil and promote plant growth .Another limiting factor that affects PGPR is Temperature . The strain, identified by 16S rRNA as *Bacillus toyonensis*, exhibited many plant-growth-promoting attributes, both direct (IAA, phosphate solubilisation) and indirect (ACC deaminase, siderophore)and enhanced the growth of tomato plants in greenhouse trials.

It was found out from the present research on AVSW 2 that this can produce IAA, ammonia, and HCN, solubilize insoluble phosphorus, and tolerates biotic and abiotic stress (salt) conditions. When plant growth parameters were analyzed, this *Bacillus toyonensis* strain AVSW 2 showed enormous seedling growth potential (**35**%), compared with the control, and a solid antagonistic nature against the plant pathogenic fungus *Fusarium oxysporum.* Thus, they can be used as bio inoculants or, bio-fertilizers and bio control agents instead of agrochemicals for improving crop productivity. Hence, this promising *Bacillus toyonensis* AVSW 2 species can be further formulated for greenhouse and field applications.

12.0 Conclusion:

In conclusion, Tomato plants inoculated with AVSW 2, an endophytic plant growth promoting bacteria isolated from chill roots exhibited significant growth-promoting traits and growth enhancement compared to untreated control and effectively suppressed fungal (*Fusarium oxysporum*) growth .

PGPR isolate AVSW 2 can be used as a plant growth promoter in several vegetable or fruit crops as in this study AVSW 2 showed maximum PGP traits in in-vitro conditions. Probably through phosphate solubilisation, production of secondary metabolites, volatile organic compounds, and growth hormones including nitrogen fixation AVSW 2 improves plant growth directly. By sequestration of iron, secretion of siderophores, release of volatiles AVSW 2 reduces phytopathogens .As in farther, the plant pathogens growth may be directly inhibited by antibiosis. By analysing the potential of volatile organic compounds in GC-MS Profile, AVSW 2 may also induce systemic resistance in the host by activating the plant defence mechanism against pathogen attack.

The results indicate that AVSW 2 Plant growth-promoting rhizobacteria isolated from chilli roots aid the Plant morphologically, physiologically, biochemically and also can help the tomato plant withstand stress. Directly or indirectly it can potentially promote tomato growth .To confirm commercial significance of AVSW 2 as a bio inoculant, further exploration can be tested at the field level. To understand its bio control potential, mode of action, regulatory mechanisms, and plant-microbe interaction in detail, Genomic, proteomic and metabolomics of holobiome (Plant and associated micro biome) and interdisciplinary research findings of AVSW 2 will be beneficial.

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Author's contribution

Gadala Swapna carried out the laboratory work, conceptualization, wrote the manuscript text and methodology. Amrutha V. Audipudi was the supervisor of the research work

Data Availability Statement

The manuscript incorporates all datasets produced throughout the research study

Ethics Approval Statement

Not applicable

Conflict of Interest:

The authors declare that there is no conflict of interest

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