

Inhibitory action of mVOCs from *Shewanella* algae Sg8 against phytopathogenic fungi and transcriptional elicitation of PR genes in tomato

Radhesh S Krishnan

T Stanes and Company Ltd

SENGALI RAGUNATH KUMARAVEL

T Stanes and Company Ltd

R Srinivasan

T Stanes and Company Ltd

N. Prabakaran

T Stanes and Company Ltd

Keerthana Ponni

T Stanes and Company Ltd

G. Balaji

T Stanes and Company Ltd

Latha K (✉ testinglab@t-stanes.com)

T Stanes and Company Ltd <https://orcid.org/0000-0001-8456-0512>

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Abstract

The analysis of Microbial volatile organic compounds (mVOCs) is an emerging research field with huge impact in the fields of medical & agricultural biotechnology, Microbial volatile organic compounds (mVOCs) are being considered as imminent eco-friendly alternatives to chemical pesticides and fertilizers in sustainable agriculture. In this study, we characterized the effect of volatiles emitted from *Shewanella algae* (Sg8) isolated from a marine ecosystem in promoting plant growth, in controlling the activity of *Fusarium oxysporum* through mVOCs and its antagonistic activity against other phytopathogenic fungus. Sg8 also inhibited the growth of four other agronomically important foliar and soil plant pathogens: *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Magnoporthae oryzae* and *Macrophomina sp.* The effect of microbial volatiles (mVOCs) produced by the bacterium Sg8, on plant growth were investigated on tomato plants under *in vivo* conditions. The VOCs emitted from Sg8 up regulated the Thaumatin-like antifungal (PR-5) gene (9-fold) and *Glutamine synthetase* (GS) gene (0.96-fold) in tomato plants. Sg8 effectively inhibited the growth of *F. oxysporum* and possessed plant growth promoting (PGP) activity. Our results show that Sg8 generates bioactive volatiles that induces the regulation of Pathogenesis related (PR) genes, & stimulation of the growth of the plants and also suppresses the growth of other agriculturally important foliar and soil phyto-pathogenic fungus.

Introduction

Volatile organic compounds of microbial origin, have a high potential in agriculture in influencing plant health and overcoming the economic loss caused by the pathogens & pests. The mVOCs apart from having biological and ecological roles also have application as markers for phenotyping and differentiation of phylogenetically closely related species of microorganisms. The volatiles are released during the growth of the microbe along with metabolic products and secondary metabolites that are used for protection against antagonists, competitors, or as signaling molecules in cell-to-cell communication (Ryu et al. 2004; Groenhagen et al. 2013; Zhang et al. 2013; Garbeva and Weiskopf 2020; Krishnan et al. 2021). These compounds have antifungal, anti-nematicidal, plant growth modulating activities and a few also function as semio-chemicals. In contrast to macromolecules, these low molecular weight molecules are highly volatile and act not only at the site of their production but also due to the high vapor pressure, they are able to traverse rapidly to longer distances and inflict cellular damage in the pests or pathogens.

The most commonly emitted chemicals belong to alcohols, ketones and carboxylic acids classes. Meldau et al (Meldau et al. 2013) reported the antimicrobial effect of 2-Phenylethanol, 3-methylbutan-1-ol, dimethyl-disulfide and dimethyl-trisulfide. Sulfur based volatiles such as dimethyl disulfide and dimethyl tri-sulfide are mainly emitted by soil bacteria and are reported to effectively inhibit biofilm formation as well as the growth of several pathogenic bacteria, fungi, nematodes and positively influence plant growth.

Crop productivity is strongly affected by biotic stresses in the plant rhizosphere (HYAKUMACHI 1994; Shivanna et al. 1994). The mVOCs from plant growth promoting microbes (PGPM), serves as an alternate disease control agent for the farmers to curtail the use of chemicals (Bailly and Weiskopf 2017).

Numerous in vitro studies have proven that mVOCs contribute specifically by inhibiting the growth and development of several phytopathogenic fungal members of *Alternaria* (Aldehydes et al. 1994; Chaurasia et al. 2005; Trivedi et al. 2008; Zhao et al. 2011; Groenhagen et al. 2013), *Aspergillus* (Vespermann et al. 2007; Hua et al. 2014; Chaves-López et al. 2015; Gong et al. 2015), *Botrytis* (Huang et al. 2011; Li et al. 2012; Rouissi et al. 2013; Wang et al. 2013; Parafati et al. 2015), ***Penicillium*** (Rouissi et al., 2013), ***Rhizoctonia*** (Fiddaman and Rossall 1993, 1994; Kai et al. 2007; Vespermann et al. 2007; LIU et al. 2008), ***Pythium*** (Chaurasia et al. 2005; Sánchez-Fernández et al. 2016), ***Sclerotinia*** (Fiddaman and Rossall 1993, 1994; Fernando et al. 2005; Vespermann et al. 2007; Giorgio et al. 2015), and ***Fusarium*** (Vespermann et al. 2007; Minerdi et al. 2009; Yuan et al. 2012; Tenorio-Salgado et al. 2013; Wang et al. 2013; Cordero et al. 2014)(Vespermann et al., 2007; Minerdi et al., 2009; Yuan et al., 2012; Tenorio-Salgado et al., 2013; Wang et al., 2013; Cordero et al., 2014), and *Phytophthora* (Zhao et al. 2011; Yap Chin Ann 2012; Sharma et al. 2015).

Numerous studies have proven that these mVOCs could be replaced as a potent basal supplement to induce systemic resistance (ISR) in plants. Previous studies have shown overexpression of stress responsive genes in plant and improvement in its adaption to various stresses and increase the yield (Li et al. 2014; Shi et al. 2014).

The plant PR proteins have been studied to combat numerous biotic and abiotic stress that are classified into 17 classes based on their amino acid sequence, serological relationship, and biological activities (van Loon et al. 1994; Van Loon and Van Strien 1999).

Three of the PR gene families, **PR-1**, **PR-2** (β -1, 3-glucanases), and **PR-5** (Thaumatococin), have been reported to encode proteins that can convey increased resistance to phytopathogenic fungi when over expressed in plants (Broglie et al. 1991; Alexander et al. 1993). PR-1 family members with a molecular weight of 14 to 17 kDa, are mostly basic in nature, show induction with SA or pathogen and are commonly used as a marker for SA dependent SAR (Mitsuhara et al. 2008). PR2 encodes β -1, 3-glucanases are pathogenesis-related (PR) proteins, and play an important role in plant defense responses to pathogen infection. PR5 gene encodes osmotin-like proteins that play important role in both biotic and abiotic stress. These proteins have assorted functions in development, protection against osmotic & cold stress and also antifungal activity. The above reports imply that PR1, 2, and 5 play pivotal roles in multiple stress tolerance.

In the current study, the effect of mVOCs emitted by the marine isolate *S. algae* strain Sg8 on the growth of agro-economically important fungal phytopathogens (*Fusarium oxysporum*) were explored along with the expression/elicitation of PR genes in the leaves of treated tomato plants. The isolate collected from marine resources was identified as *Shewanella algae* strain - Sg8 (NCBI accession number - MK121204.1) was analyzed for the inhibitory effect of the volatiles produced, followed by evaluation of its antifungal and PGP activity. Based on the literature survey, it can be mentioned that this is the first report on the induction of defense responsive genes (PR genes) and plant growth promoting (PGP) activity by *S. algae* strain (Sg8).

Materials And Methods

Isolation and Identification

The isolate S. algae (Sg8), was isolated from the seaweed *Sargassum* collected from Munaikadu, Mandapam coast, Rameshwaram district of India (9° 16' 32.56" N, 79° 07' 25.03" E). Isolates were streaked at least thrice to ensure purity of the culture. The isolated bacteria were routinely cultured on nutrient agar or nutrient broth (Fang et al. 2014) media. The generated VOCs from the bacterial culture were evaluated by inoculating on Zobell Marine Agar (ZMA) plates (Gong et al. 2015). Bacterial cultures were maintained as 40% glycerol stocks at -80°C for long-term storage. The identity of the isolate (Sg-8) was confirmed (based on the microscopy, morphological, biochemical and molecular analysis) as *Shewanella algae* (Kandasamy et al. 2020)

Inoculation experiments

Culture medium and growth conditions:

The culture (Sg8) was inoculated in Zobell marine broth and incubated at 30°C overnight in orbital shaker at 150 rpm and used as a seed inoculum for testing its inhibitory activity against phyto-pathogenic fungi.

Effect of *Shewanella algae* (Sg8) against *phytopathogenic* fungi by *in vitro* assay

The antagonistic potential of the isolate against fungal phytopathogens mainly *Fusarium oxysporum* & others such as *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Magnoporthae oryzae* and *Macrophomina phaseolina*) were tested by dual-culture technique (Fernando et al. 2005) using Zobel Marine Agar (ZMA) for Sg8. The radial growth of the pathogens in dual culture and control plates was measured after 7 days of incubation at 28±1 °C. The average growth of the pathogens in presence of the Sg8 strain was compared with that of the pathogens grown in absence of the strains (control) and the percentage of inhibition was determined (Fernando et al. 2005).

Effect of the volatile organic compounds produced by *Shewanella algae* (Sg8) on the growth of pathogenic fungi by *in vitro* assay:

The antagonistic activity of SG8 against the mycelial growth of phytopathogenic fungi (*Fusarium oxysporum*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Magnoporthae oryzae* and *Macrophomina phaseolina*) was tested following the method of Fernando et al (Fernando et al. 2005) by inverse plate assay. Aliquots of the suspension of the Sg8 isolate (10⁸ cfu/ml) was inoculated on Zobel Marine Agar (ZMA) plates. A disc (5mm) of fully grown phyto-pathogenic fungi (7 day old) was placed on the fresh PDA plate. The two petri dishes were sealed with cello tape (ensuring that no air or volatile leakage is occurred), and incubated at 30°C for 5 days. Fungal disc inoculated on (PDA) plates were co-cultured on ZMA plates smeared with ZMB medium was used as a control. The inhibition rate was calculated as follows:

Inhibition rate (%) = [Diameter of control-Diameter of antagonistic treatment]/the diameter of control] × 100.

Evaluation of the inhibitory effect of microbial volatile organic compounds (mVOC) emitted by *Shewanella algae* (Sg8) on the growth of *Fusarium oxysporum*

Effect of the mVOC emitted by *Shewanella*Sg8 strain on the growth of *Fusarium oxysporum* was tested as per the Tahir et al. (2017). *Fusarium oxysporum* (1.0×10^6 CFU/mL) was inoculated in double sterilized garden soil. The container with garden soil was placed above the tissue culture jar inoculated with Sg8 (1.0×10^8 CFU/mL) on ZMA media. The container and the jar were sealed with scotch tape (ensuring no air exchange) and incubated at 28°C for 14 days. Un-inoculated experimental setup served as control.

Effect of mVOCs on the growth tomato plants by *in vivo* lab assay

Healthy seeds of tomato were surface sterilized and sown aseptically in the double sterilized garden soil, and the experimental set up (as mentioned above) was placed in a climate controlled green house at 28°C for 14 days under a photoperiod of 12 h light/12 h dark. The VOCs produced by Sg8 were entrapped in a tissue culture bottle fixed with the plant set up. Tomato saplings were exposed to the VOCs produced by the isolate Sg8. Two weeks after exposure to mVOC's, biometric parameters like plant height, root length, girth, fresh and dry weight were recorded.

Induction of metabolic and defenselated genes in tomato treated with mVOC's of *Shewanella algae* (Sg8)

Gene expression of metabolic and PR genes (*Glutamine synthetase*, *Citrate synthase*, defense responsive genes such as PR1, 2 and 5) was carried out after exposure to the volatile compounds emitted by the isolate Sg8 and was compared with the expression of the genes of untreated plants.

Induction of defenselated genes in tomato leaves treated with *Shewanella algae* (Sg8) in the presence of the pathogen

PR genes expression in response to inoculation with *Fusarium oxysporum* in Tomato plants

A study was carried out to test the direct and indirect antagonistic effect against *F. oxysporum* (FOC) at green house in pot culture. Pot mixture was prepared by mixing garden sand and farm yard manure at 2:1 (w/w) and was filled (3 kg) in 15 inch plastic pots followed by inoculation with FOC inoculum (20% of pot weight, 200 g per pot; two weeks before sowing). FOC inoculum was mass-multiplied on chickpea grains (variety Co 03; highly susceptible to Fusarium wilt, acquired from Farm Aid Service, TNAU, Coimbatore). Inoculum was thoroughly mixed with the pot mixture and the pots were covered with polythene sheets, in order to maintain the moisture in the soil, & left for 15days for the development of pathogen & to induce disease condition. Two weeks later, the seedlings of Tomato (variety Shivam) were transplanted to the pots and treated with respective treatments *viz.*, Sg8 (10^8 CFU/mL), Salicylic acid and Acibenzolar-S-

methyl at a concentration of 10 mM (Khiareddine and El-Mohamedy 2015) and 0.5 mM (Małolepsza 2006) solutions respectively.

Gene expression (in PR genes) was monitored 0h, 6h, 24h, 48h, 7th day and 14th day after treatment, and was compared with the gene expression in untreated plants. (The primer details are given in Supplementary Table 1)

Results

Effect of *Shewanella algae* (Sg8) against *phytopathogenic* fungi by dual plate *in vitro* assay:

The inhibition of fungal pathogens in dual-culture test showed the efficacy of Sg8 against all tested fungi (60% to 87 %). Among the five different pathogens tested maximum growth inhibition and antagonistic effect of Sg8 was observed against *Macrophomina phaseolina* (88%) which causes charcoal rot in different plant hosts and *Magnoporthae oryzae* (77%) which is a rice blast pathogen (**Supplementary Figure 1**). Similarly Sg8 showed effect against *Botrytis cinerea* (causal agent of gray mold), *Colletotrichum gloeosporioides* (causal agent of bitter rot in variety of crops) and *Fusarium oxysporum* (causal agent of wilt disease in variety of crops). The inhibitory effect observed in dual plate that the isolate could have arrested the growth of the fungal pathogens through nutritive competition or antibiosis or mycoparasitism or due to the cumulative effect of all.

Effect of Sg8 mVOCs produced by *Shewanella algae* (Sg8) on the growth of pathogenic fungi by *in vitro* assay (VOC):

The inhibitory effect of the mVOCs emitted by Sg8 was prominently observed on *Macrophomina phaseolina* (by 87.8 % which causes charcoal rot, collar rot, and stem rot, in deferent plant hosts) and *Magnoporthae oryzae* (a rice blast pathogen with 76.67 % inhibition) measured after four days of incubation. Fungal growth inhibition was also observed in *Botrytis cinerea* (73.17 %) which causes grey mould diseases in different horticulture crops, *Colletotrichum gloeosporioides* (67.03 %) which causes bitter rot disease in different crops, and *Fusarium oxysporum* (60%) which causes wilt diseases in various hosts.

Evaluation of the inhibitory effect of microbial volatile organic compounds (mVOC) by *Shewanella algae* (Sg8) on the growth of *Fusarium oxysporum* (*In vitro* assay)

The growth of *F. oxysporum* was observed to be reduced in the soil treated with Sg8 (**Table 1**). A similar growth was observed in the soil treated with *F. oxysporum* that was exposed to Sg8 VOCs (Table 2). Inhibitory action of Sg8 against mycelial growth of *F. oxysporum* was noted in the experiment wherein mVOCs were allowed to pass through the soil from the bottom of the tissue culture jar (**Supplementary Figure 2**).

Growth-Promoting Activity of Sg8 VOCs in Tomato plants (*In vitro* assay-in tissue culture jars)

The plant growth promoting potential of VOCs produced by *S. algae* Sg8 was examined by growing plants in tissue culture bottles exposed to Sg8 VOCs for 14 days. No visual deleterious effect was observed on the leaves or to the parts of the plants that were directly exposed to the microbial volatiles throughout the experimental period (**Figure 1**).

The data revealed a significant enhancement in the growth of tomato seedlings in terms of fresh weight, dry weight, root and shoot length. The observations recorded on plant height (15.45%), root length (8.47%), stem girth (40%), fresh weight (55.26%), dry weight (52.17%) showed that there was a significant increase in the above said parameters in the plants that were exposed to Sg8 in comparison to the untreated control (**Figure 2-4**).

The biometric parameters of the plants exposed to the volatiles generated by SG8 were enhanced when compared to the plants grown in an un-inoculated set up (plant height, root length, girth fresh and dry weight).

Induction of metabolic and defenselated genes in plants exposed to mVOC's of *Shewanella algae* (Sg8) (Plate assay- Lab):

VOCs of SG8 did not elicit significant effect on the expression of *GS* gene (0.96-fold) though the biomass of the plants exposed to Voc was higher compared to the un-inoculated plants. The expression of the defense responsive genes (PR-5 gene) showed a 9-fold enhancement in its expression compared to un-inoculated control, whereas the other pathogen related gene (PR-2) was not stimulated significantly (0.5 fold) in the presence of the pathogen compared to control that did not show any change in the expression of PR-1 gene (**Figure 2-4**).

Induction of defenselated genes in tomato treated with *Shewanella algae* (Sg8) in the presence of the pathogen inoculated in the soil (Climate controlled Green house studies).

The levels of the transcripts were analyzed for 14 days after infecting the soil with *F. oxysporum*. The plants treated with the biocontrol agent & standard synthetic substances induced elicitation of the PR genes within 48h after treatment. The up-regulation of the expression of the defense responsive genes was observed in the Sg8 treated tomato plants till 14days, which is an indication of its effectiveness in the control of pathogen. There was considerable elicitation of the genes (PR 1, 2, & 5) in plants treated with Sg8 (soil application), in the presence of the pathogen compared to the control plants (un-inoculated). The genes encoding the pathogen related proteins PR1 was up regulated six-fold, followed by the PR genes (2 and 5) that showed accentuation of 7 and 5-fold respectively. The **Figure (7A & B) shows that both the activity of β -1,3-glucanase (PR2) & Thaumatin (PR5) genes in the inoculated plants reached its maximum activity at 7th day post inoculation (dpi) as compared to the pathogen and mock-inoculated controls. On the other hand both the synthetic chemicals that are well established elicitors could trigger the PR genes in tomato plants till 14th day (**Figure 5A and B**). Thus it is evident that the isolate Sg8 has the potential to stimulate the PR genes in the plants exposed to *F. oxysporum* more effectively in soil compared to its invitro inhibitory potential.**

Discussion

Diverse & rapidly evolving pathogens and global climate changes threaten crop yield and food security. The increased use of synthetic pesticides and fertilizers provides immediate solutions for the alleviating plant disease and crop yield but drastically affect human and environment health. Although bio-pesticides, bio-fertilizers, and bio-control agents derived from living microbes are becoming suitable replacements for the hazardous synthetic pesticides and fertilizers, their reduced efficiency, high costs and inconsistent field performance generally relegate them to niche products.

Bacterial inoculants are reported to modulate plant growth, metabolic & defense gene expression and development through the emission of mVOCs. The impact of VOCs produced by rhizospheric bacteria on plants and their important role in plant-bacterial interactions has been well-documented. It is reported that VOCs act both as plant growth promoters and inhibitors, of which 2,3-butanediol and acetoin (3-hydroxy-2-butanone) produced by *Bacillus* spp., are the best-known growth-promoting VOCs (Rath et al.,2018). In addition, VOCs such as 2-pentylfuran, 13-tetradecadien-1-ol, 2-butanone, 2-methyl-n-1-tridecene, from various bacterial species have also been reported to promote plant growth, whereas hydrogen cyanide (HCN), dimethyl sulfide and inorganic volatiles, are reported to be phytotoxic to plants. VOCs also trigger induced systemic resistance in several plant species in response to pathogen challenge.

The present study reports the growth promoting; antagonistic; & gene elicitation potential of Sg8, through its ability to produce VOCs. The data recorded on the inhibition of fungal pathogens through the production of volatile and non-volatile metabolites by Sg8, show that it significantly inhibited the growth of all the pathogens tested. Maximum inhibition of mycelia growth of *M. phaseolina* was observed followed by *M. oryzae*, *B. cinerea*, *Colletotrichum* sp., and *F. oxysporum*. The inhibitory effects observed can be mainly attributed to the antibiosis effect of the volatile metabolites and induction of defense responsive genes in plants. Thus Sg8 can be considered as a promising bio-control agent for control of root-rot/wilt diseases, as it is able to colonize in advance to the pathogens and stimulate PR genes & promote plant growth under biotic stress

The test pathogen *B. cinerea* is a non-specific, necrotrophic pathogen that reportedly cause heavy crop loss due high disease incidence in most vegetable and oil crops (Elad et al. 1994; Cowan et al. 2005; Swartzberg et al. 2008; Petrasch et al. 2019). *In vitro* evaluation of the mVOC's released from Sg8 showed that the inhibitory effect of the isolate from marine algae was substantial (73%) and cogitates its evaluation in open field.

The inhibitory activity of mVOC's released by Sg8 was effective in controlling the growth (60%) of *F. oxysporum*, a fungal pathogen, which is a wide spread threat to a variety of economical important crops like cotton, chickpea, banana, melon and tomato(Michielse and Rep 2009; Gawehns et al. 2015).

The mVOC's of Sg8 showed significant inhibitory effect (67%), on the phyto pathogen *Colletotrichum geosporioides*, a causal agent of anthracnose disease in vegetables, fruits, legumes and ornamental plants (Luis Fernando Zepeda-Giraud et al. 2016). Similarly the mVOCs showed a significant effect on the

mycelial growth of *Magnaporthe oryzae* (a causative agent for rice blast) and *M. phaseolina* (a causative agent of seedling blight, collar rot, stem rot & root rot). Review of the literature show that this is the first report on the inhibitory potential of *Shewanella algae* strain Sg8 against *M. oryzae* (77%) and *M. phaseolina* (88%), thus underlining the potential of the strain as a biological input in Agriculture. This is also the first study investigating the growth promoting potential of MVOC's of SG8 and it demonstrates considerable growth modulation effect on tomato seedlings when the bacterium is grown on culture media beneath the plant system.

GS enzyme is responsible for the production of glutamine in the leaf and generally GS activity is lower during fruit development, due to low metabolic activity, high requirement of carbohydrate, sugar and protein demand for the fruit formation. Glutamine, the major transported amino acid, generally increases in the senescing leaves compared to the early phase of the harvest, as the GS that synthesize, glutamine, is transferred to the growing tissues in plants. The expression of leaf GS activity in the leaves of treated & control plants was monitored to assess if the VOCs produced by Sg8 could activate metabolic machinery, through up-regulation of the glutamine synthase.

The genes encoding for GS and CS did not show any increased expression in the plants exposed to volatiles emitted by Sg8.

The genes coding for pathogen resistance (1, 2 and 5) showed higher levels of expression after 14 days in plants induced with FOC infection. The accumulation of PR RNAs correlates with the synthesis of PR proteins that are commonly observed to escalate in response to pathogen attack by the plant (Van Loon et al. 2006). Reports show an abundance of specific tomato proteins, including PR proteins, with changes in the xylem sap in *Fusarium* infected plants (Rep et al. 2002; Houterman et al. 2007).

Plants have evolved the ability to systemically defend to pathogens, through systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Berendsen et al. 2015; Radhesh et al. 2020). A set of PR proteins, such as PR1, PR2 and PR5, were identified as the basic characteristic of SAR signaling pathway (Molinari et al. 2014), and PR3, as the marker of ISR signaling pathway (Yu et al. 2018). The marker genes, PR1, PR2 and PR5 induced by validamycin to enhance plant resistance are considered as markers of SAR (Khunnamwong et al. 2020).

Sg8 mVOC-induced defense responses in tomato in terms of the expression of salicylic acid (SA) dependent marker genes were analysed by qPCR. The expression level of the SA inducible gene PR-1 (unknown anti-fungal function), PR-2 (β -1,3-glucanase) and PR-5 (thaumatin-like protein) genes (Cao Hui et al. 1994; Cao et al. 1997, 1998), showed higher expression (9.5 fold) of PR-5 gene in plants with the application of mVOCs from Sg8 which could induce enhanced disease-resistance. This investigation indicates that the reported *S. algae* strain Sg8 has the potential to control *Fusarium* wilt disease and promote growth and induce resistance to pathogens in tomato.

Several studies demonstrate mVOCs can inhibit a range of plant pathogens, highlighting their suitability as a potential sustainable alternative to pesticides. One of the first examples demonstrating an inhibitory

role for mVOCs against plant pathogens were those produced by *Pseudomonas* species isolated from soybean and canola, in the inhibition of *Sclerotinia sclerotiorum*; a fungal pathogen with a broad host range of over 400 plant species (Fernando et al. 2005). Of 23 VOCs identified from *Pseudomonas* species, six significantly reduced mycelial growth of *S. sclerotiorum*. Similarly, VOC production by two strains of *Bacillus* endophytes significantly reduced the weight and number of the vegetative, long-term survival structures (sclerotia) of *S. sclerotiorum* (Tahir et al. 2017a). VOCs from *Burkholderia ambifaria* and a range of other rhizobacterial isolates (Groenhagen et al. 2013) have also demonstrated the ability to inhibit growth of the ubiquitous soil-borne pathogen *Rhizoctonia solani*. MVOCs can also display inhibitory activity against bacterial pathogens. Exposure of *Clavibacter michiganensis*, the causal agent of bacterial ring rot of potato, to VOCs from *Bacillus subtilis* led to significant inhibition of pathogen growth, with benzaldehyde, nonanal, benzothiazole and acetophenone specifically demonstrating inhibitory activities (Tahir et al. 2017b). *Bacillus* VOCs also inhibited the growth of *Xanthomonas oryzae*, the causal agent of bacterial leaf blight of rice, with decyl alcohol and 3,5,5-trimethylhexanol specifically inhibiting pathogen growth (Fernando et al. 2005; Srinivasan et al. 2017).

Recent advances on the research aspects of microbial volatile & its interactions with plants has on the whole unfolded the understanding of the dynamic nature of mVOCs, & their potential role in enhancing crop protection and productivity in a sustainable way. It is observed that exposing plants to mVOCs, results in a significant modulation of plant metabolomics, physiology, and transcriptional status, which confirms that plants have the ability to perceive and respond to microbial volatile compounds. Most of the studies have, however, been conducted under lab conditions, though recently few studies been performed in open field conditions to demonstrate efficient adoption of mVOCs for sustainable crop protection and production (Gong et al. 2015; Tahir et al. 2017b; Fincheira and Quiroz 2018). These studies clearly demonstrate the need for implementation of MVOCs application in Agriculture taking advantage of the multiple functions exhibited such as increase in pathogen resistance, protection against herbivores ; enhancement in plant growth and control of disease & pests of plants. In addition the application of mVOCs in agriculture ensures a sustainable crop protection and production strategy as a possible substitute for hazardous and synthetic chemical pesticides and fertilizers.

According to Lemfack et al., (Lemfack et al. 2014), the application of mVOCs as plant defense and growth modulators is yet to be established, as out of the 10,000 microbial species described the mVOCs released by 400 bacteria and fungi have been described in the literature.

Conclusions

Microbial volatile organic compounds form a bioactive interface between plants and a myriad of microorganisms above and below ground where most of the interactions take place. MVOCs are intriguingly complex and dynamic and understanding their ecology and evolution is the key to bioprospecting suitable tools for crop protection and production for sustainable agriculture perspective. Application of the Sg8 to tomato plants primed the expression of genes encoding for basic PR-proteins; nitrogen & carbon metabolism and inhibited the growth of plant pathogens, supporting the significance

of VOCs that it can trigger growth and defense response similar to induced systemic resistance (ISR). The antagonistic & PGPR activity of SG8 and the effect of the emitted VOCs will be further studied in open field conditions to validate the results obtained in vitro conditions and to assess its application as a microbial biostimulant or as biocontrol agent & develop it as a cost effective, eco-friendly & sustainable tool for crop protection.

Declarations

Author statements

Authors and contributors

Contributor Role	Author name	Role Definition
Conceptualization	Radhesh Krishnan S Latha K	Ideas; formulation or evolution of overarching research goals and aims.
Methodology	Radhesh Krishnan S Sengali Ragunath K Latha K	Development or design of methodology; creation of models.
Software	Radhesh Krishnan S	In-silico analysis, molecular confirmation of the organisms,
Validation	Radhesh Krishnan S Latha K	Verification, whether as a part of the activity or separate, of the overall replication/reproducibility of results/experiments and other research outputs.
Formal Analysis	Radhesh Krishnan S Prabhakaran N Latha K	Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesis of study data.
Investigation	Radhesh Krishnan S Srinivasan R Sengali Ragunath K Latha K	Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection.

Conflicts of interest

The author(s) declare that there are no conflicts of interest

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Ethical approval

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Consent for publication

Not applicable

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Repositories

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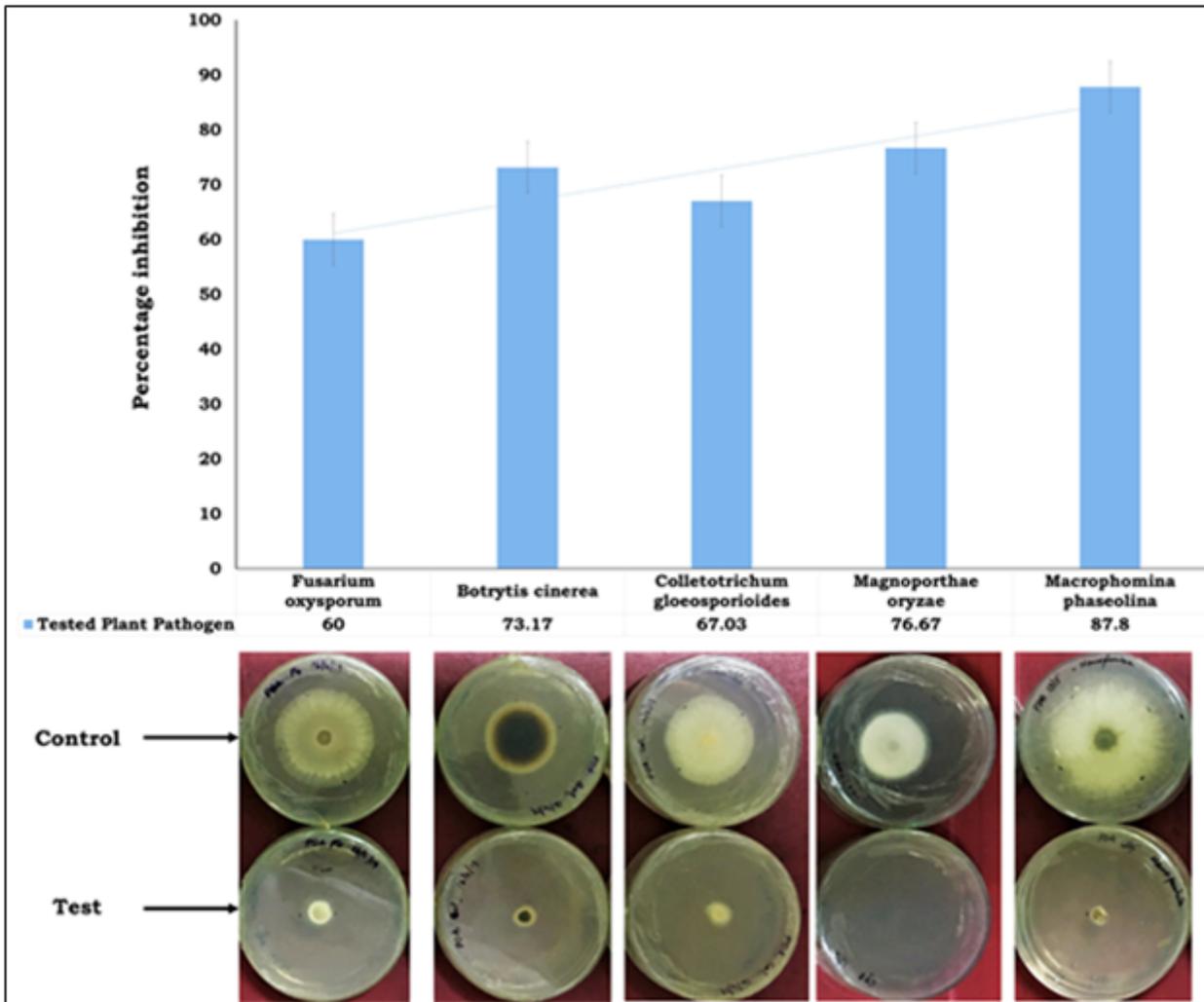


Figure 1

Paired plate Plate Assay for evaluating the effect of the volatile Compounds (VCs) of Sg8 on phytopathogenic fungi

Effect of the volatile organic compounds produced by *Shewanella algae* (Sg8) on the growth of pathogenic fungi.



Figure 2

Effects of Sg8 mVOCs on seedling growth of tomato (Tissue culture jar method).

14 days old tomato plants **A)** control plants and **B)** treated plants exposed to mVOCs from Sg8

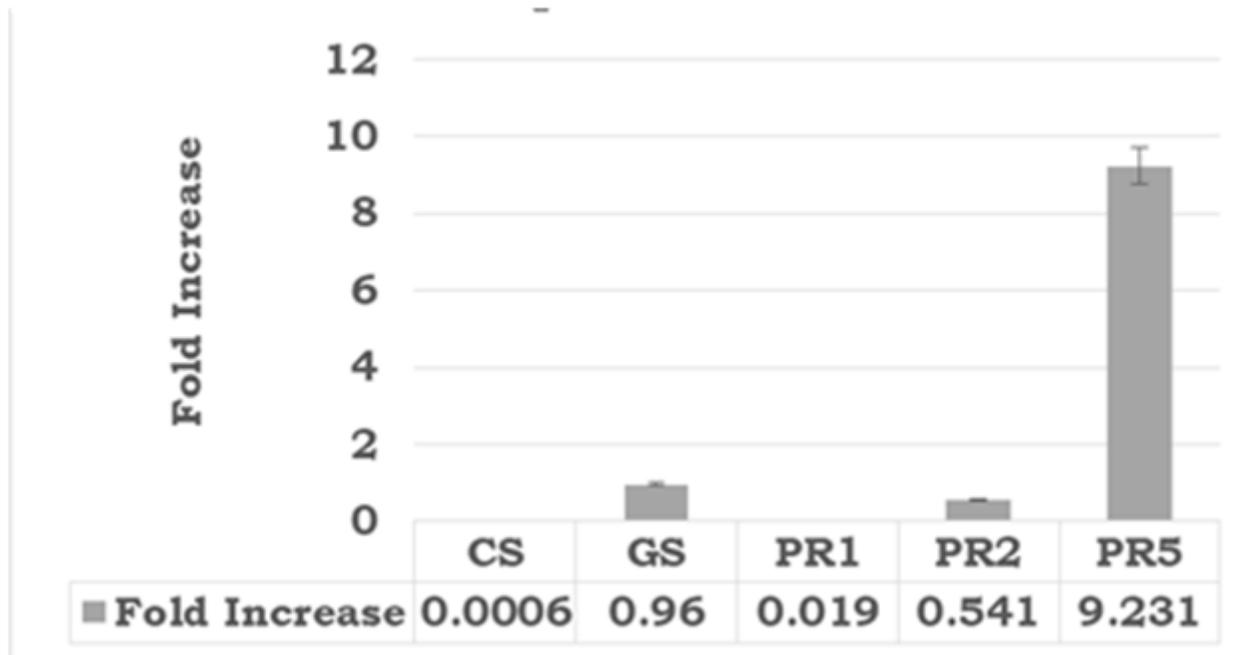


Figure 3

Growth parameters observed in the tomato plants exposed to mVOCs from Sg8

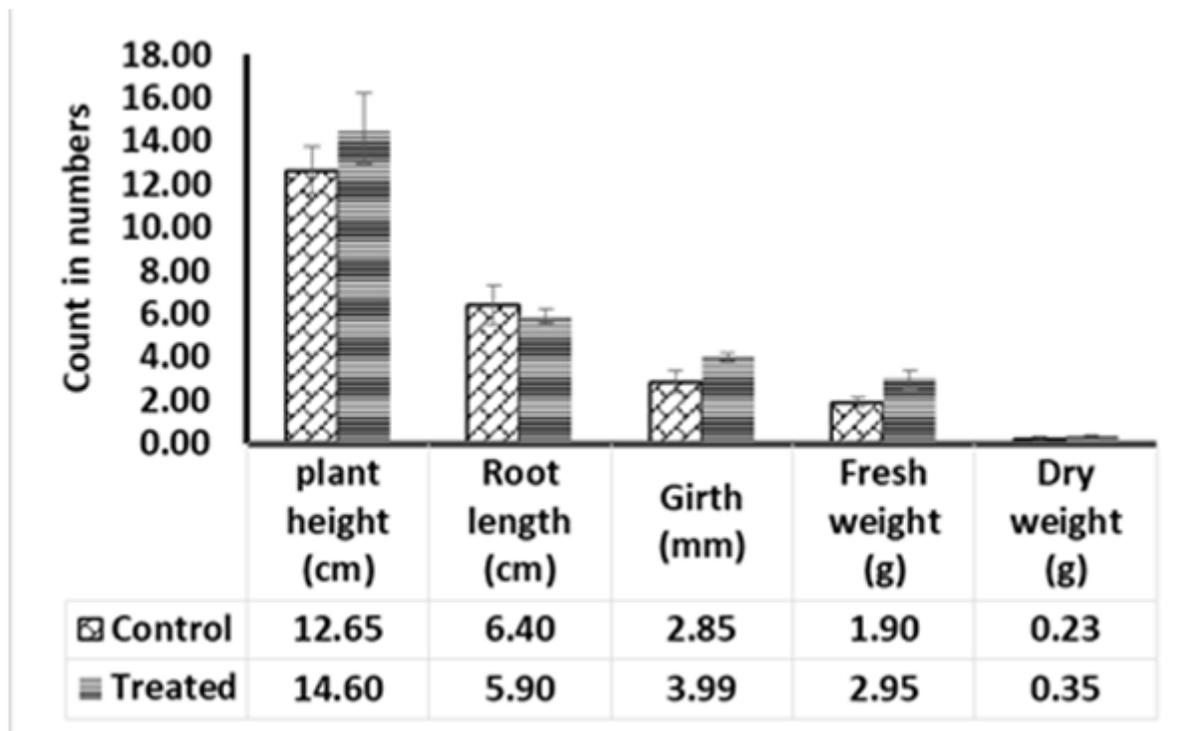
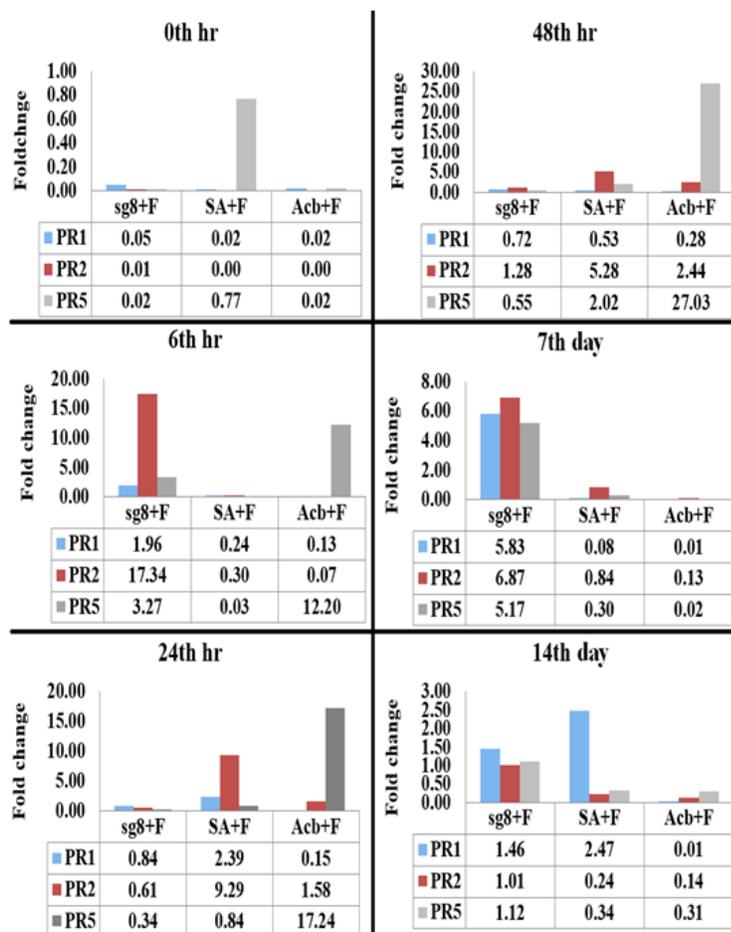
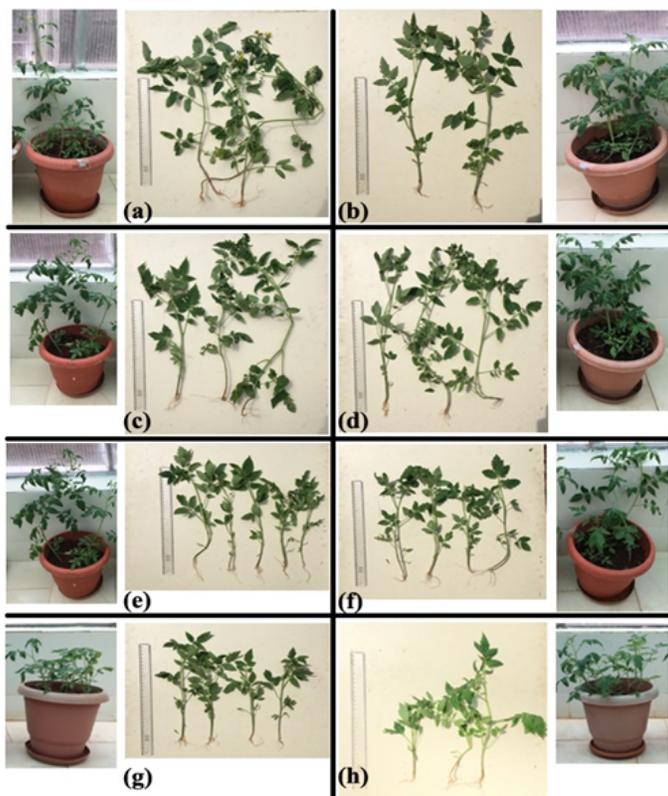


Figure 4

Metabolic and PR genes expression pattern observed in tomato leaves of plants exposed to Sg8 mVOCs

Metabolic and pathogen responsive gene expression in control and treated plants (exposed to Sg8 mVOCs)



A

B

Figure 5

a. Pot culture experiments conducted to evaluate antifungal and plant growth promoting activity of Sg8 at climate controlled green house. a) Sg8 treated, b) Sg8 + FOC, c) SA treated, d) SA+FOC, e) BTH treated, f) BTH+FOC, g) FOC alone and h) Water control

(*Shewanella algae* – Sg8, SA-Salicylic acid, BTH- Acibenzolar S Methyl)

b. Pathogenesis related gene elicitation in tomato plants grown in soil infected with *Fusarium oxysporum*

Supplementary Files

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