



The influence of *Streptomyces violaceoruber* and *Kocuria rhizophila* strains on *Origanum vulgare* L. growth promotion and bioactive metabolite composition

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ABSTRACT

Microbial strains belonging to the Actinomycetota phylum are known as plant growth-promoting (PGP) bacteria and are considered promising candidates for the development of biofertilizers due to their metabolic ability to produce diverse bioactive compounds. In this work, the actinomycetotal strains *Kocuria rhizophila* PA220 and *Streptomyces violaceoruber* DSM40783 were investigated using a two-step experimental approach: the first step focused on seed germination and provided visual evidence of initial seedling surface attachment via scanning electron microscopy (SEM); the second assessed PGP-associated responses in oregano rooted cuttings and relative differences in metabolite profiles, characterized through gas chromatography–mass spectrometry analysis of flowers volatilome. Seeds and rooted cuttings were inoculated (by priming or direct application, respectively) with the two individual strains or their consortium, alongside a non-inoculated control. For comparison, a commercial biofertilizer was included in the field experiment. Morpho-physiological parameters, including plant elongation and chlorophyll content, were monitored throughout growth. Both strains adhered to seedling surfaces, and *S. violaceoruber* was associated with higher seed germination rates. Under field conditions, *S. violaceoruber* was associated with higher chlorophyll content, whereas *K. rhizophila* was associated with increased shoot elongation at blooming. Increased relative abundances of p-cymene and terpinolene, monoterpene with relevant bioactive properties, occurred alongside the actinobacterial consortium. Overall, these findings indicate that the tested actinomycetotal strains improved specific plant traits under both controlled and open-field conditions, and moreover align with changes on metabolite profiles. These results support further investigation of Actinomycetota-based biofertilizers as tools for sustainable management of Mediterranean aromatic crops.

1. Introduction

The intensive use of chemical fertilizers and pesticides in modern

agriculture has led to a progressive soil degradation, triggering a vicious circle that requires increasing inputs to maintain productivity (Yasir et al., 2025). In this scenario, it becomes urgent to identify alternative

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and sustainable strategies capable of restoring soil fertility and biodiversity while achieving high crop yields and quality. One of the most promising outcomes in this direction is boosting soil microbial diversity, an often-neglected factor in ecological assessments (Li et al., 2025), yet fundamental for agroecosystems. Soil microorganisms contribute to the stability of the soil structure, participate in the cycling of essential nutrients, and give resilience against environmental stressors. In this context, plant growth promoting bacteria (PGPB) are beneficial microorganisms that enhance plant growth and development by different mechanisms - including mitigation of biotic and abiotic stresses, and improvement of nutrient uptake in most crop species (Oyebamiji et al., 2024).

In particular, PGPB can tolerate drought and salinity (Ebrahimi-Zarandi et al., 2023), colonize the rhizosphere, solubilize nutrients, and produce bioactive metabolites such as plant hormones (i. e. auxins, gibberellins, cytokinins), antibiotics, antifungals and siderophores (Rangseekeaw and Pathom-aree, 2019; Lee et al., 2020). Moreover, different members of actinomycetota (formerly actinobacteria) phylum are known as promising PGPB and, therefore, are considered to develop biofertilizers, due to their peculiar metabolic versatility and characteristic ability to produce bioactive compounds with significant potential for agricultural applications (Faddetta et al., 2023).

Beyond plant growth promoting (PGP) effects, the potential role of beneficial actinomycetota in modulating the biosynthesis of plant metabolites — including bioactive compounds with possible applications in the nutraceutical and agro-industrial sectors — remains a relatively unexplored and emerging area of research. In particular, the actinomycetotal valuable production of bioactive compounds may have a potential role in influencing plant metabolite profiles (Semenzato et al., 2022). This aspect might be particularly interesting for Mediterranean aromatic plants, such as *Origanum vulgare* L. (commonly known as oregano), that thrive in arid and nutrient-poor environments, showing remarkable drought tolerance (Emami Bistgani et al., 2024). This adaptability allows aromatics to survive adverse conditions, making them a rich source in bioactive metabolites, including terpenoids and flavonoids (Khan et al., 2025). These compounds, which are essential for increasing plant resistance to environmental stresses, confer antioxidant, antibacterial and anti-inflammatory effects, and are particularly concentrated in the essential oils (EOs) that can be obtained by steam distillation processes. In addition, aromatic plants synthesize a wide variety of chemically and biologically active low molecular weight volatile compounds that act in human and plant-environment interactions (Schulz-Bohm et al., 2017; Nurzyńska-Wierdak, 2023). In oregano, the production of these compounds is responsible for the organoleptic properties, which are critical for its use as a culinary herb.

Understanding the relationship between oregano's adaptive characteristics, its biochemical profile and the interplay with PGP microorganisms might open the way for novel biotechnological applications in food science. Indeed, the relevance of the use of PGP microorganisms in Mediterranean aromatic species has already been documented. For example, inoculation at the pre-germinative stage with a *Pseudomonas* strain has been shown to improve growth parameters and essential oil yield in *Origanum majorana* L. (Banchio et al., 2008). Similarly, multiple strains of the Bacillaceae family significantly enhanced overall plant development, including plant height but also macro and micronutrient absorption, essential oil content and oregano essential oil yield in *Origanum onites* L. (Çakmakçı et al., 2023a). Thus, ongoing research into the environmental stresses experienced by oregano can facilitate the development of cultivation strategies that optimize its bioactive compound production, ensuring both culinary and potential modulating nutraceutical applications (La Scala et al., 2024). In this work, *Streptomyces violaceoruber* DSM40783 and *Kocuria rhizophila* PA220, two actinomycetota strains that were previously known to have PGP effects on tomato (*Solanum lycopersicum* L.) plants (Faddetta et al., 2023; Mauceri et al., 2024; Ricciardi et al., 2026), were investigated for their plant

growth-promoting effects on oregano plants and their influence on plant metabolite production. To evaluate the biostimulant potential of the two Actinomycetota, two separate trials were performed. First, a controlled experiment assessed early growth from seeds to seedlings, concluding 20 days after sowing. Second, a comprehensive trial was conducted using rooted cuttings to evaluate growth stimulation and potential effects on plant volatiles: inoculated and uninoculated plants were cultivated in greenhouse for 90 days after planting (DAP) and then transferred in open field until final harvest (151 DAP), with the aim of confirming their efficacy in modulating the plant productivity and potentially their nutraceutical quality.

2. Materials and methods

2.1. Bacteria and growth conditions

K. rhizophila PA220 strain is maintained in the SUS-MIRRI.IT collection at the University of Palermo. *S. violaceoruber* DSM 40783 strain was obtained from the DSMZ (German Collection of Microorganisms and Cell Cultures GmbH). *S. violaceoruber* spore production was obtained as described in (Kieser et al., 2000). The cell biomass of *K. rhizophila* was obtained in cultures from a single colony inoculated into Tryptone Soy Broth (TSB) (Condalab, Spain) and incubated for 24–48 h at 30 °C and 180 rpm. For a long storage time, spores or cells of each bacterial strain were stored at –80 °C in a 20% glycerol solution. Spore and cell concentrations were evaluated using the colony-forming unit (CFU) method on a tryptone soy agar (TSA) medium. *S. violaceoruber* and *K. rhizophila* cultures were performed in R5A medium (K₂SO₄, 0,25 g/liter; MgCl₂*6H₂O, 10,12 g/liter; glucose, 10 g/liter; Casamino Acids (Difco), 0,1 g/liter; yeast extract 5 g/liter; MOPS 21 g/liter; trace elements* 2 mL/liter; 6.8 final pH, adjusted with KOH; *trace elements per liter solution: ZnCl₂ 40 mg; FeCl₃*6H₂O, 200 mg; CuCl₂*2H₂O, 10 mg; MnCl₂*4H₂O, 10 mg; Na₂B₄O₇*10H₂O, 10 mg; (NH₄)₆Mo₇O₂₄*4H₂O 10 mg) at 30 °C for 72 h, 180 rpm by inoculating an initial concentration of 10⁷ spores mL⁻¹ for *S. violaceoruber* and 10⁷ CFU mL⁻¹ for *K. rhizophila*. At the end of the growth, the final concentration of PGP actinomycetotal cultures was about 3% v/v packed mycelium and 10⁹–10¹⁰ CFU mL⁻¹ for *S. violaceoruber* and *K. rhizophila*, respectively. For plant inoculations, *S. violaceoruber* and *K. rhizophila* cultures were diluted respectively 1:10 and 1:100 in sterile water. The consortium was obtained by combining equal proportions of each diluted monoaxenic culture. In addition to the actinomycetotal inoculations, a commercial biofertilizer was added as positive control for oregano explants (composition: 28% of crude inoculum including mycorrhized and ground roots, spores and composted organic matter, other beneficial microorganisms such as rhizosphere bacteria and saprophyte fungi) and applied according to the manufacturer recommendations (5 g/L solution of wet powder in water). Sterile distilled water and uninoculated actinomycetotal growth medium were employed as negative controls for both greenhouse and field experiments. As no significant differences (p < 0.05) were observed among these two control groups, only the sterile distilled water is reported as uninoculated control. In pot, actinomycetotal inoculations were applied by pouring 5 mL of actinomycetotal diluted cultures or commercial biofertilizer in the soil (at the stem base). All the applications were repeated three times every fifteen days in the pots precisely, at 30, 45, and 60 days after planting (DAP).

2.2. Genome sequencing and analysis of *Kocuria rhizophila* strain

Whole-genome sequencing of *K. rhizophila* was performed to accurately determine the taxonomic identity and ensure the correct interpretation of the biological results obtained in this study. Genomic DNA was obtained using salting out protocol according to (Pospiech, 1995). Quantitative and qualitative DNA analysis was performed using nanodrop. Additionally, the extracted DNA was used as template for 16S rRNA gene amplification by

PCR reactions, performed using 1X PCR RxN Reaction Buffer (Invitrogen), 1.5 mM MgCl₂, 200 μM dNTPs (Invitrogen), 0.2 μM universal primers 27 F (5'-AGAGTTTGATCTMTGGCTCAG-3') and 1492 R (5'-TACGGYTACCTTGTTACGACTT-3') and 1 U of Taq DNA Polymerase Recombinant (Invitrogen). Thermal cycling conditions were 98 °C for 3 min, followed by 30 cycles of 98 °C for 45 s, 50 °C for 1 min and 72 °C for 90 s, and finally 72 °C for 10 min. Reaction products were analyzed by agarose (1.5% w/v) gel electrophoresis in Tris-Acetate EDTA buffer (TAE) containing a safe DNA-Staining dye. The genome sequencing was conducted using the Illumina platform in outsource service by BMR Genomics SRL obtaining 847,000 of 300 bp paired-end reads. The genome sequence (BioProject PRJNA1423029) was characterized through comparative analysis by Mash similarity to identify its closest phylogenetically related *Kocuria* species. Read mapping and SNP calling were performed using Snippy, which aligns sequencing reads to the reference genome and identifies variants using a pipeline based on BWA-MEM and FreeBayes. Variant functional effects, including synonymous, missense, frameshift, and intergenic classifications, were obtained directly from Snippy annotations. Operons and transcriptional terminators were predicted using ProPr v2. Variant coordinates were intersected with predicted operons and promoter-associated regions using BEDTools to assign variants to coding sequences, transcriptional units, or intergenic regions. SNP density was calculated using fixed-size genomic windows, and mutational hotspots were defined as windows exceeding the 95th percentile of the genome-wide SNP density distribution. Variant data processing, summarization, and visualization were performed in R using standard data-wrangling and plotting workflows. Variants were grouped by functional impact and genomic context, and figures were generated using ggplot2 and related visualization tools.

2.3. Genome screening for BGCs and PGP-associated genetic determinants

Genome sequences of *K. rhizophila* NCTC8340 (chromosome LR134409) and *S. coelicolor* A3(2) (chromosome NC_003888) were retrieved from the MicroScope Annotation & Genome Environment (MaGe) platform (Vallenet et al., 2019). Both genomes were automatically annotated within MaGe and the resulting annotations, including gene names (Gene field) and functional descriptions (Product field), were used as input for all downstream analyses. Putative genetic determinants associated with PGP capacities were systematically identified using both the "Search by Keyword" and "Blast and Pattern Searches" tools implemented in MaGe, following established genome-mining frameworks PGP traits (Cheminat et al., 2025; Leontidou et al., 2020). In particular, nine functional macro-categories were screened: (i) polymer degradation, (ii) phosphate solubilisation, (iii) siderophore biosynthesis, (iv) indole-3-acetic acid (IAA) synthesis, (v) cytokinin biosynthesis, (vi) quorum sensing, (vii) putrescine and polyamine metabolism, (viii) nitrogen fixation, and (ix) sugar transport and metabolism. For each macrocategory, a curated set of regular expression patterns was applied case-insensitively to the concatenated Gene and Product annotation fields exported from MaGe as tab-separated value (TSV) files. Patterns included both canonical gene names (e.g., *gdh*, *pqqA-F*, *rhbEB*, *barS*, *pataA/patD*, *miaA*) and functional descriptions derived from the respective biochemical roles. Each locus tag was counted only once per subcategory to avoid redundancy arising from identical gene copies. Locus tags matching multiple subcategories were assigned to all matching entries independently. To detect biosynthetic gene clusters (BGCs) for bioactive or specialized metabolites genome mining analysis by antiSMASH was performed (Blin et al., 2025).

All analyses and visualisations were performed in R (v4.3+) using the ggplot2, dplyr, tidyr, stringr, and ggtext packages. For each macro-category, a presence/absence heatmap was generated showing the number of distinct gene copies detected per genome. Copy counts were binned into four levels (absent; 1–2 copies; 3–5 copies; ≥6 copies) and rendered using a sequential blue colour scale to facilitate visual comparison. All figures were exported at 300 dpi for inclusion in the manuscript.

2.4. Plant material and experimental design

O. vulgare L. commercial seeds (70% germination rate, 95% purity) were used for germination experiments, whereas *O. vulgare* L. subsp. *viridulum* (= *O. heracleoticum*) rooted cuttings, were used for the experiment in open field and obtained from adult plants of a local 3-year old oregano cultivation (Napoli et al., 2020). *O. vulgare* L. commercial seeds were surface sterilized with 70% (v/v) ethanol for 1 min, followed by a treatment with 2.5% (w/v) NaOCl solution for 5 min. Afterwards, three rinses in sterile distilled water (5 min each) were carried out. Sterile seeds were primed for 45 min by immersion into actinomycetotal diluted cultures or their mixtures at room temperature in agitation. Then, seeds were dried for 30 min on sterile filter paper in a laminar air flow cabinet to remove excess liquid from seed surface. Ten seeds were aseptically sown onto a 0.7% water-agar plate, in triplicate. Plates were incubated four days in the dark and then maintained in a climate chamber at 25 ± 1 °C under a 16 h day length, and a photosynthetic photon flux of 50 μmol m⁻²s⁻¹ provided by Osram cool-white 18 W fluorescent lamps for further 20 days when seedlings were collected. A total of ten seedlings per condition were harvested: five for SEM observations and five for the assessment of dry weight. For the field experiment one hundred rooted cuttings, 15–20 cm long and uniform in development and number of leaves, were individually transplanted in 0.45 liters pots filled with a commercial soil substrate (composition: baltic peat 0–30 mm, black peat, coconut fiber, ecofiber, pumice; features: pH (H₂O) 5.8 – 6.8, EC 0.15 – 0.25 dS/m). The individuals were inoculated as described in the paragraph "Bacteria and growth condition" and grown in greenhouse for the first 90 days watering with tap water every three days. At 90 DAP, twenty plants of each treatment were divided as follows: fifteen were transferred to the open field to continue their growth, while the remaining five were subjected to destructive measurements to obtain the average dry weight prior to transplantation. Field layout was arranged using a row-based experimental design (15 plants per row). Plants in the same row were spaced 50 cm apart, whereas the inter-row distance was set to one meter to minimize the risk of cross-contamination among the different treatments. Irrigation was supplied according to plants' necessity using a drip irrigation system, ensuring uniform water distribution over the entire experimental area. As plants reached the full blooming stage (BBCH 65) (Souza et al., 2016), recorded at 140 DAP, fresh flowers from each treatment were collected and sealed in headspace vials to perform volatilome analyses as described below. In parallel, five plants were harvested and dried at 70 °C in an oven to constant dry weight for 48 h for dry weight assessment. The field experiment was considered over at 151 DAP, when the remaining plants were air-dried under room conditions to obtain biomass for essential oil extraction via hydro-distillation procedures. Oregano EOs were obtained by hydro-distillation in Clevenger-type apparatus as described below.

2.5. Monitoring of oregano seed germination and seedling growth

O. vulgare L. seed germination rate was calculated at 4, 7, and 12 days after sowing (DAS) and expressed as a percentage of the total seed count. Both oregano seedlings and oregano plants were weighed at the end of their growing cycles. Weekly monitoring of shoot elongation began at 45 DAP, coinciding with the second inoculum administration. Full bloom was reached at 141 DAP, when half of the plants reached the blooming stage. Plants were monitored throughout the entire field trial until the final harvest at 151 DAP.

2.6. Relative chlorophyll content measurement in oregano leaves

The measures of the relative chlorophyll content in plant leaves were quantified using a Soil Plant Analysis Development (SPAD) meter (Uddling et al., 2007). SPAD units were measured during the pot and field growth of plants using a portable double wavelength meter (SPAD

502, Minolta Camera Co., Ltd., Osaka, Japan). Nineteen measurements (from 30 DAP until 151 DAP) were taken on healthy, fully expanded uniform leaves ($n = 20$) between 10:00 and 12:00 GMT on inoculated and non-inoculated plants. Only statistically significant data are shown in the results section.

2.7. Scanning electron microscopy characterization of seedling root and leaf surfaces

Scanning electron microscopy (SEM) was adopted to reveal the presence of inoculated actinomycetota on oregano seedling tissues. Roots and leaves from 20 DAS seedlings were dehydrated and mounted on aluminum stubs with double adhesive carbon tape. Subsequently, the sample were vacuum-coated with a 5-nm thick layer of gold (Sputter Coater LuxorAu, Luxor Tech, Nazareth BELGIUM) and observed by a Phenom PRO X SEM (Alfatest, Italy).

2.8. Chemical characterization of oregano flower volatile organic compounds and essential oils

Two distinct gas chromatography-mass spectrometry (GC-MS) techniques were employed to characterize the chemical profile of the samples: headspace-syringe injection for the analysis of flowers volatiles and liquid injection for the extracted EOs.

To minimize the impact of individual biological variability in the field condition and ensure a representative volatilome profile, samples were pooled by combining flowers from three individual plants per condition. Two technical replicates per condition were analyzed. For the flowers' volatiles, headspace (HS) vials were partially filled with 1 g of germination experiment oregano flowers, sealed and immediately analyzed. HS vials were conditioned at 40 °C for 20 min before extraction. Volatile organic compounds (VOCs) produced by the flowers were extracted from the vial headspace with a 2.5 mL Syringe-HS (0.64–57-R-H, PTFE, GERSTEL) conditioned and held at 40 °C from sample collection to injection and splitless injected into the Gas chromatograph (GC). Chromatographic analysis was carried out by modifying a previously described procedure (Semenzato et al., 2024). In particular, an Agilent 7890 B GC (Agilent Technologies, Inc., Santa Clara, CA, USA) was used to perform the gas chromatography analysis, equipped with a split/splitless injector, fitted with an Agilent HP5-MS UI capillary column (30 m $\times$ 250 μ m; 0.25 μ m film thickness), coupled to an Agilent 7000 C triple quadrupole (Agilent Technologies, Inc., Santa Clara, CA, USA), with ionization voltage 70 eV; electron multiplier energy 2000 V; transfer line temperature, 270 °C; solvent Delay: 6 min. Helium was used as the carrier gas (1 mL/min). Concerning the oven program, the temperature was initially kept at 40 °C for 5 min, then gradually increased to 250 °C at a 2 °C/min rate; this temperature was held for 15 min and finally raised to 270 °C at a 10 °C/min rate. Samples were injected at 250 °C automatically. Interval scan: 35–450 m/z ; Scan speed of 10,000 $amu \cdot s^{-1}$ (25 Hz). The GC-MS mass spectrum data were analyzed using Mass Hunter Qualitative Analysis B.06.00 and the database of the National Institute Standard and Technology (NIST) was used to interpret the analyzed data. A comparison between the mass spectrum of the unidentified components released by the oregano flowers VOCs and the mass spectrum of already known components available in the NIST 17 MS library was performed. The software assessed the molecular weight and peak area of unidentified chemicals in flower VOCs and in the EOs. The relative abundances were expressed as relative peak areas, providing a semi-quantitative assessment of the chemical profiles. Identifications were accomplished by comparing their mass spectra and retention times (RT) against a set of published standardized data (Wei et al., 2014; Fincheira et al., 2023; Hendel et al., 2024).

Following flowers' VOCs characterization, the analysis of EO was conducted using the same GC-MS instrument previously described. To give account of biological variability, 50 g of dried stems were obtained from a pooled sample of 15 individual plants from each condition. The

extraction procedure was carried out until increases in EOs concentrations were no longer observed (approximately 3 h). The EOs samples were stored at 4 °C. An amount of 1 μ L of each essential oil solution (EO/hexane v/v, 1/100) was injected, with a solvent delay of 4 min. The oven program was as follows: temperature was initially held at 40 °C for 5 min at a rate of 2 °C/min up to 270 °C, and then isothermal amplification for 20 min. Helium was used as the carrier gas (1 mL/min). The injector and detector temperatures were set at 250 °C and 290 °C, respectively. Linear retention indices (LRI) were determined by using retention times of n-alkanes (C₈–C₄₀), and the peaks were identified by comparison with mass spectra and by comparison to their relative retention indices NIST 17. Internal standards were not used.

2.9. Statistical analysis

Statistical analysis was performed using Minitab® version 17.1.0 (Minitab Inc., State College, PA, USA, 2013) for Windows. To validate adherence to the ANOVA assumptions, normality and variance homogeneity of all data were assessed using the Ryan–Joiner test ($\alpha = 0.05$) and Levene's test ($\alpha = 0.05$), respectively (Gomez, K. A. and Gomez, A., 1984). Significant ANOVA results were further analyzed using Tukey's HSD test ($p \leq 0.05$). The volatiles analysis was conducted on pooled samples, with two technical replicates per treatment, each consisting of three plants. In this context, statistical comparisons reflect variability among composite samples rather than independent biological replicates. Relative variations in volatile compounds were evaluated using normalized peak areas and expressed as log₂ ratios relative to uninoculated controls. In this context, statistical analyses were conducted on pooled samples, representing comparisons among composite experimental units under field conditions, where sampling integrates variability across individual plants.

3. Results

3.1. Comparative genomic analysis of *K. rhizophila* PA220

As a preliminary but essential step, comparative genomic analysis was performed to unambiguously verify the identity of the *K. rhizophila* strain used. This analysis revealed contrasting patterns of sequence variation depending on the reference genome selected. In particular, two reference genomes were chosen based on Mash similarity to the PA220 strain: *K. rhizophila* NBC 01227 (NCBI reference genome ASM3599006) showed lower similarity (Mash score 0.923264; 187/1000 shared hashes), whereas *K. rhizophila* NCTC 8340 (NCBI Reference Sequence NZLR134409.1) exhibited near identical similarity (Mash score 0.99957; 991/1000 shared hashes) to PA220. Consistent with these metrics, comparison with *K. rhizophila* NBC 01227 (Figure S1A) showed extensive genomic divergence, with ~70,000 SNPs across the genome. Most variants were synonymous (65.9%) and 29.5% were missense, with heterogeneous SNP density and a distinct substitution spectrum indicating broad genomic divergence. SNP density analysis identified mutational hotspots in the top 5% of the distribution involving 115 genes (Table S1). In contrast, comparison with *K. rhizophila* NCTC 8340 (Figure S1B) revealed only 15 variants, indicating high genomic similarity. These mutations were enriched in genes with essential cellular functions. Frameshift mutations were found in genes encoding a HEATrepeat-containing protein and in the MtrAB response regulator MtrA. The remaining missense variants affected genes involved in DNA repair (UvrB), proteolysis (S1C family serine protease), translation (30S ribosomal protein S12), metal homeostasis (VIT1/CCC1 transporter), and transcriptional regulation (Table S2). Additional intergenic variants may influence regulatory elements. Overall, despite minimal divergence from NCTC 8340 strain, a small set of functionally relevant mutations distinguishes the analyzed strain.

3.2. Genome-based assessment of PGP traits

Based on the genome similarity analysis described above, the *K. rhizophila* PA220 showed high similarity to *K. rhizophila* NCTC8340; therefore, the NCTC8340 genome was used as a proxy to infer its functional potential at the genome level. The *Streptomyces* strain DSM 40783 is associated with the historical designation A3(2) in DSMZ or JCM culture collections and corresponds to *S. coelicolor* A3(2) - as explicitly reported in the BacDive webpage (<https://www.bacdive.dsmz.de/strain/16081>) - for which a complete and well-annotated reference genome is available (Bentley et al., 2002). Accordingly, to assess the PGP potential at the genome level, the genomes of *K. rhizophila* NCTC8340 and *S. coelicolor* A3(2) were subjected to a

systematic *in silico* screening of nine functional macrocategories using the MicroScope Annotation & Genome Environment (MaGe) platform, combining keyword-based queries and BLAST searches. In particular, according to this analysis (Fig. 1, Table S3), the *S. violaceoruber* displayed a markedly richer polymer degradation repertoire, most notably an expanded chitinolytic arsenal of 14 chitinase and 4 chitosanase/chitin-binding genes — consistent with its role as a chitin-degrading soil actinomycete — alongside 4 cellulases and 5 xylanases absent in *K. rhizophila*. Both strains encoded gluconate dehydrogenase homologs (namely *gdh/gno*, 4 vs. 2 copies) and 6-phosphogluconate dehydrogenase (namely *gnd*; 3 vs. 1 copies), supporting mineral phosphate solubilization via gluconic acid production in both organisms; *K. rhizophila* also carried two *phoU* coding for phosphate

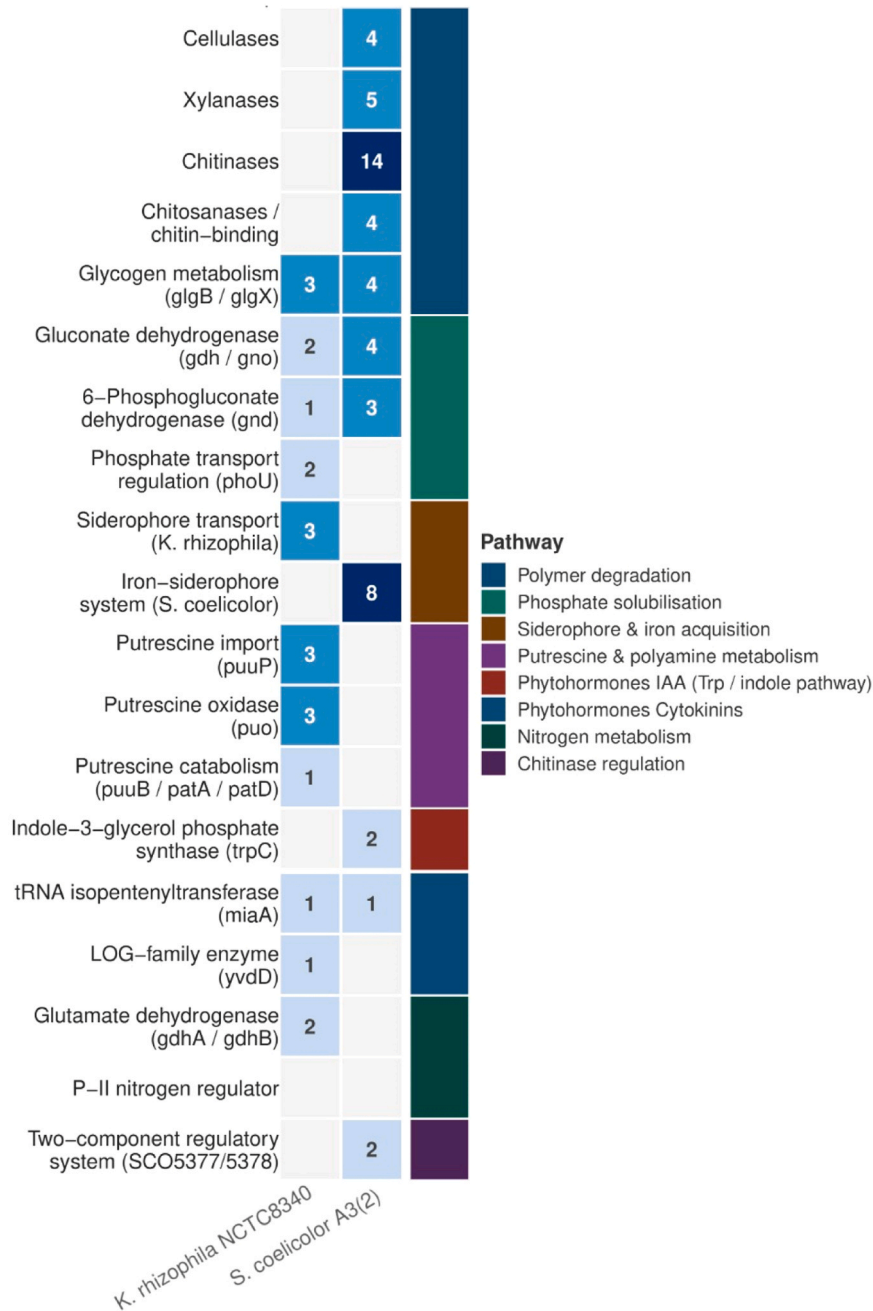


Fig. 1. Genomic screening for PGP-associated genes in *K. rhizophila* NCTC8340 and *S. coelicolor* A3(2). Presence/absence heatmaps for eight representative macrocategories as reported in figure. Cell color indicates the number of distinct locus tags detected per genome, binned into four copy-number classes (i.e. absent, grey; 1–2 copies, light blue; 3–5 copies, mid blue; ≥6 copies, dark navy). Numbers inside colored cells report the exact copy count. Genes were identified by keyword and pattern matching against MaGe genome annotations using curated regular expressions targeting both canonical gene names and functional product descriptions.

transport regulators. Both genomes carried a single *miaA* (i.e. isopentenyltransferase) relevant to cytokinin biosynthesis, with *K. rhizophila* additionally possessing the *yvdD* gene encoding a LOG-family enzyme. *K. rhizophila* uniquely showed evidence of active polyamine metabolism, with 3 putrescine importers (*puuP*), 3 putrescine oxidases (*puo*), and a *puuB* catabolism gene, while *S. coelicolor* encoded 2 *trpC* (i.e. indole-3-glycerol phosphate synthase) genes, positioning it upstream of the IAA biosynthetic pathway. Additionally, although the MicroScope Annotation & Genome Environment (MaGe) platform, combining keyword-based queries and BLAST searches, did not identify other genes annotated as involved in IAA biosynthesis, a direct BLAST analysis revealed the presence of *iaaM*- and *iaaH*-like genes corresponding to the entries SCO1454 and SCO1455, encoding the tryptophan-2-monooxygenase and indoleacetamide hydrolase, respectively. Siderophore-mediated iron acquisition was strain-specific: *K. rhizophila* encoded 3 siderophore transport genes, while *S. coelicolor* harboured a larger iron-siderophore system of 8 genes (SCO0494–0497, SCO1315, SCO1785–1787). Neither genome harboured nitrogenase structural genes, respectively.

Comparative antiSMASH analysis revealed a markedly higher BGC diversity in *S. coelicolor* A3(2) compared to *K. rhizophila* NCTC8340 (Fig. 2). In total, 29 BGCs were identified in *S. coelicolor* versus only 6 in *K. rhizophila*, with the latter showing a limited distribution across a few metabolite classes, including terpene-related clusters.

3.3. Effect of actinomycetotal inoculation on oregano seed germination

The biostimulatory potential of *S. violaceoruber*, *K. rhizophila* and their consortium was tested on oregano seed germination under laboratory conditions. Seed germination was significantly enhanced by inoculation with *S. violaceoruber* in respect to the uninoculated control already 7 DAS (Fig. 3). At 12 DAS, 76% of the *S. violaceoruber* primed seeds were germinated, significantly resulting in the highest rate above all. Since no more significant change was recorded on the twelfth DAS, the plants were then transferred to half-MS medium (Murashige and Skoog, 1962) to enable their growth until 20 DAS for SEM observations.

3.4. Early surface adherence of the two actinomycetotal strains on oregano seedling surfaces

The presence of the inoculated actinomycetotal strains on plant surfaces was qualitatively observed at 20 DAS by SEM (Fig. 4). Inoculated seedlings showed microbial structures associated with plant tissues, including filamentous mycelium (Fig. 4B, E, and G) and spherical cells likely organized in tetrad clusters (Fig. 4C and F). These features are consistent with the typical morphology of *Streptomyces* and *Kocuria* genus members, respectively (Hopwood and Glauert, 1961; Kovács et al., 1999). Bacterial structures remained detectable on plant surfaces following sample preparation and dehydration and were also observed in proximity to stomatal regions (Fig. 4E). In contrast, uninoculated seedlings showed smooth epidermal surfaces in both root tissues (Fig. 4A) and stomatal regions (Fig. 4D). The observed morphological features were consistent with the known characteristics of the inoculated strains, including filamentous structures and spores of approximately 800 nm for *S. violaceoruber* and tetrad arrangements for *K. rhizophila* (Raphel and Halami, 2024).

3.5. Biomass yield of oregano seedlings following actinomycetotal inoculations

Oregano cultivation is generally carried out using vegetative propagation methods, such as rooted or unrooted stem cuttings, rather than direct sowing, not recommended for commercial production due to low germination rates and the resulting heterogeneity of young plant (Skoufogianni et al., 2019). In this study, a seed-based approach was nonetheless essential to evaluate the capacity of actinomycetotal inoculants both on seed germination and on early-stage microbial colonization; for this purpose, seedlings were grown until 20 DAS. At the end of the experiment, at 20 DAS, oregano seedling biomass dry weight was measured, and data are shown in Table 1. Oregano seedlings inoculated with *S. violaceoruber*, or the consortium showed a slight increase ($p < 0.05$) in dried biomass in comparison to the control.

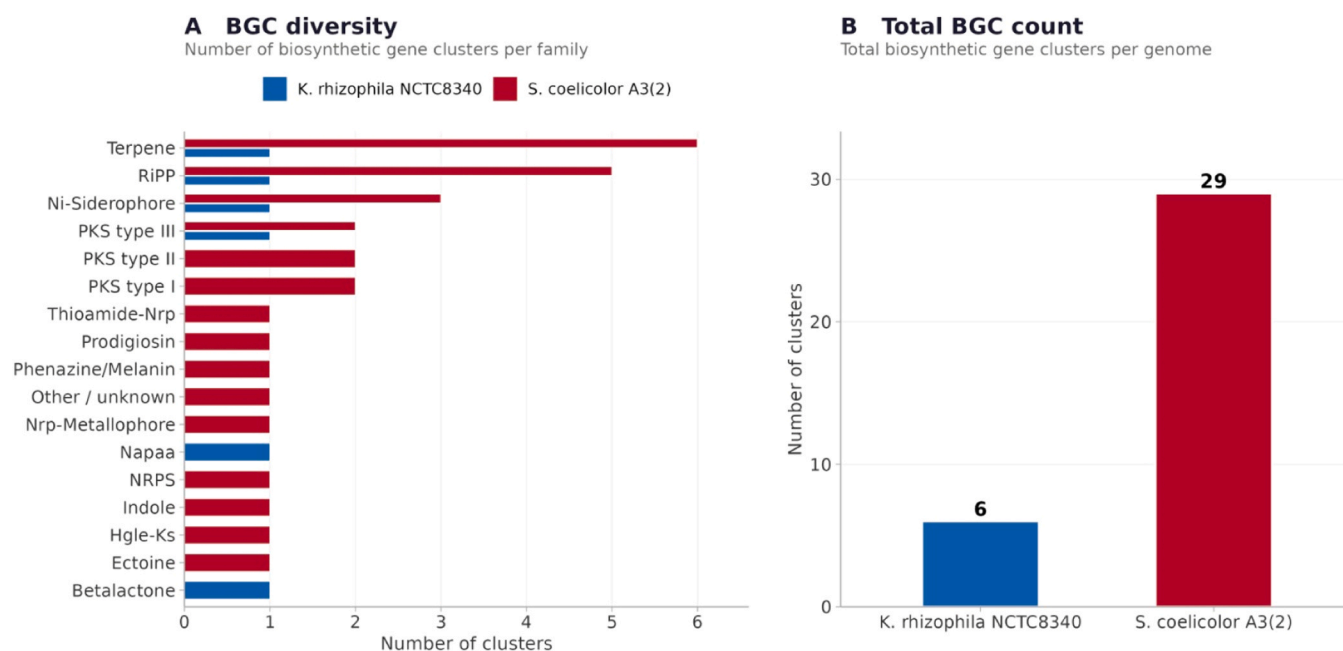


Fig. 2. Comparative antiSMASH analysis of BGCs in *K. rhizophila* NCTC8340 and *S. coelicolor* A3(2). (A) Distribution of BGC families, showing the number of clusters identified per metabolite class in each genome. *S. coelicolor* displays a broader and more diverse repertoire, including multiple terpenes, RiPP, PKS, and siderophore clusters, whereas *K. rhizophila* exhibits a reduced and more limited set of BGCs. (B) Total number of BGCs detected per genome, highlighting a markedly higher cluster count in *S. coelicolor* (29) compared to *K. rhizophila* (6).

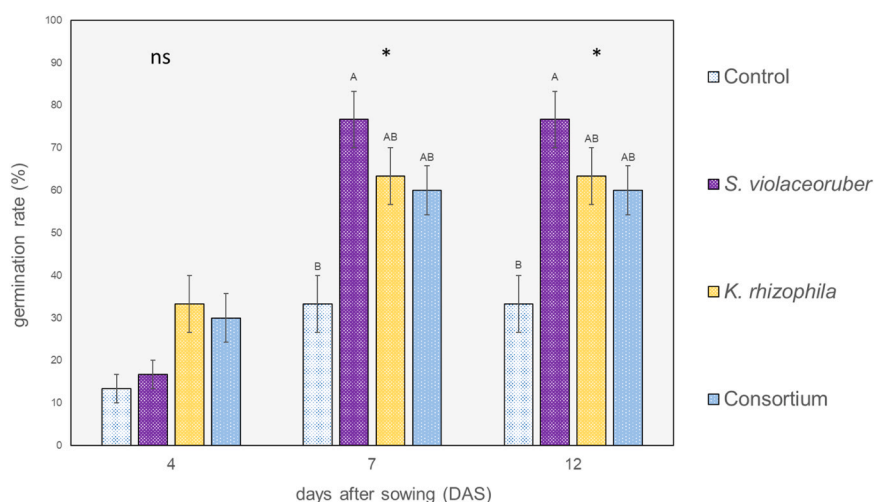


Fig. 3. Seed germination rate (%) following inoculation with the two actinomycetotal inoculants and their consortium at 4, 7, and 12 DAS. Data are presented as mean $\pm$ standard error ($n = 3$ plates with 10 seeds each). Different letters above bars indicate significant differences (Tukey's HSD test, $p < 0.05$).

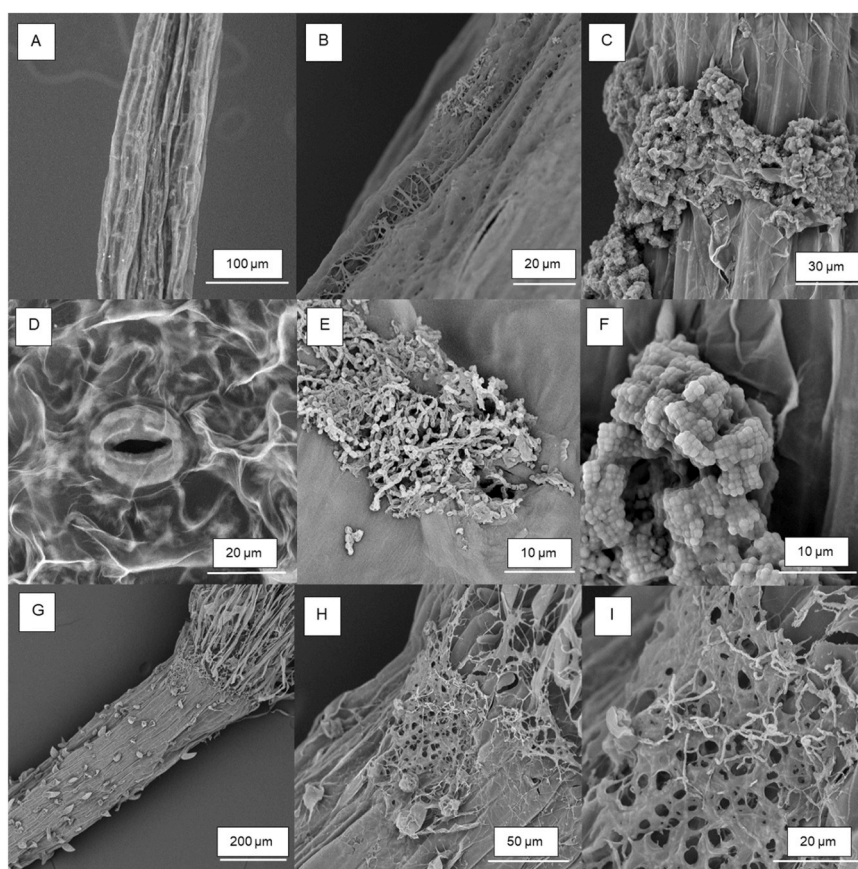


Fig. 4. Scanning electron microscopy micrographs of oregano seedlings showing the complex networks formed on plant sections by actinomycetotal inoculants 20 DAS. Overview of *O. vulgare* roots: uninoculated (A), inoculated with *S. violaceoruber* (B) or *K. rhizophila* (C); stomata from oregano leaves uninoculated (D) or inoculated with *S. violaceoruber* (E); other plant sections colonized by *K. rhizophila* (F); biofilm-forming actinomycetota over the primary root inoculated with *S. violaceoruber* and *K. rhizophila* consortium (G, H, I).

3.6. Chlorophyll content in oregano field inoculated and non-inoculated plants

To assess the reliability of the actinomycetotal effects across different environments, a field-based approach was used to confirm whether the growth-promoting benefits persist under realistic cultivation conditions.

As a key indicator of physiological performance and photosynthetic efficiency, SPAD values were recorded to provide an indirect measure of the chlorophyll content in the leaves. Chlorophyll content from all leaves was monitored throughout the growth period in both greenhouse and field conditions (Figure S2). *S. violaceoruber* inoculated plants consistently showed higher SPAD values among all treatments over a 36-

Table 1

Oregano biomass (g, DW) of seedlings treated with the two actinomycetotal inoculants and their consortium at 20 DAS. Data are presented as means ($n = 5$) $\pm$ standard error; letters indicate Tukey's HSD test results ($p < 0.05$). Treatments that share the same letters are not significantly different from each other.

Treatment	Oregano seedlings dry weight (g)
Control (H ₂ O)	0.28 $\pm$ 0.066 B
<i>S. violaceoruber</i>	1.18 $\pm$ 0.227 A
<i>K. rhizophila</i>	0.84 $\pm$ 0.280 AB
Consortium	1.36 $\pm$ 0.242 A

day period following the third inoculation and field transplantation (at 90 DAP). Remarkably, an increase in SPAD values was already evident by day 67 (shortly after oregano pot planting) indicating an early onset of the treatment effect (Fig. 5). At 92 DAP, *S. violaceoruber* treated plants had an average SPAD value of 45.94 SPAD units, the highest value of the set if compared to the control plants (control: 38.18 SPAD units; commercial biofertilizer: 39.93 SPAD units) (Fig. 5).

3.7. Oregano plant elongation in response to actinomycetotal inoculations in field

Evaluating the morphological response of oregano to microbial inoculants is essential to determine their potential as biostimulants (Loera-Muro et al., 2021). As a primary indicator of crop growing status, plant height was measured to compare the performance of the actinomycetotal strains. At full bloom (141 DAP), plants inoculated with either *K. rhizophila* or the commercial biofertilizer exhibited higher height than the other administered inocula (Fig. 6; Figure S3). The other two actinomycetotal inoculations (*S. violaceoruber* and the consortium) did not significantly affect plant height in respect to the control.

Oregano plants were harvested at 151 DAP, corresponding to the end of the experiment. To provide a preliminary yield estimate and ensure enough material for subsequent phytochemical analysis, five plants per treatment were harvested at 151 DAP. There were no significant differences among the weight of actinomycetotal treated plants and the control plants; only the commercial biofertilizer stimulated oregano growth in comparison to the uninoculated plants (Table 2).

3.8. Relative variation in flower volatile compounds in response to actinomycetotal strain inoculants

Oregano flowers were sampled directly from the field at the time of full blooming (141 DAP) and immediately analyzed. A total of 35 volatile compounds were detected by GC-MS in all samples, consisting primarily of monoterpenes, aromatic monoterpenoids and sesquiterpenes (Table 3). Despite the different inoculations, the overall qualitative composition of the volatilome profile remained stable across all experimental groups. Monoterpenes (from cis-sabinene hydrate to p-cymene), monoterpene phenols (thymoquinone, o- isothymol methyl ether, methylthymol) and sesquiterpenes (α -guaiene to α -bisabolene) appeared to be different depending on the actinomycetotal inoculation (Fig. 7). Flowers from consortium-inoculated plants showed a relative increase in monoterpene levels, particularly for terpinolene and p-cymene. In contrast, a decreasing trend was observed for the sesquiterpenes (such as α -guaiene and α -bisabolene) in *S. violaceoruber*-inoculated plants compared to control. However, these differences should be interpreted as indicative trends, given the semi-quantitative nature of the analysis.

3.9. Actinomycetotal inoculation can be associated to variation in yield and composition of oregano EOs

EOs extracted from inoculated and non-inoculated plants harvested at 151 DAP were analyzed to explore the potential impact of actinomycetotal treatments on essential oil yield and composition. Preliminary observations indicated an EO yield of 4.02%, 4.43%, 4.47%, 4.43%, 7.99% (v/w), respectively in uninoculated, *S. violaceoruber*, *K. rhizophila*, commercial biofertilized and consortium inoculated plants (Table S4). This trend, if confirmed, would suggest an influence of actinomycetotal inoculum on essential oil production. A qualitative analysis of the essential oil constituents was also performed, identifying a profile of 23 compounds across all treatments, including both inoculated and non-inoculated plants. These compounds (reported in Table 4) remained constant regardless of the treatment, indicating that the actinomycetotal inoculants primarily influenced the semi-quantitative proportions of the oils rather than inducing the presence or absence of specific metabolites. While these preliminary results suggest that microbial consortia may exert a more pronounced influence on secondary

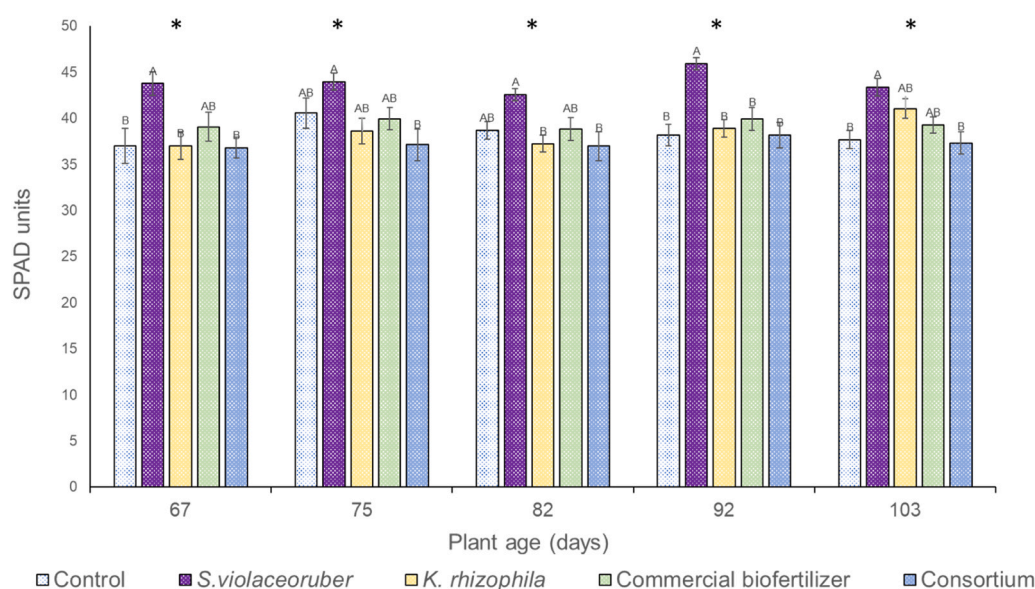


Fig. 5. Mean values of chlorophyll meter readings (SPAD units) measured on healthy leaves ($n = 20$) selected on inoculated and non-inoculated plants located in stems of the same age. Asterisks indicate significant differences among treatments (ANOVA). Letters on the bars indicate post-hoc Tukey's HSD test results at $p < 0.05$. Bars indicate standard errors.

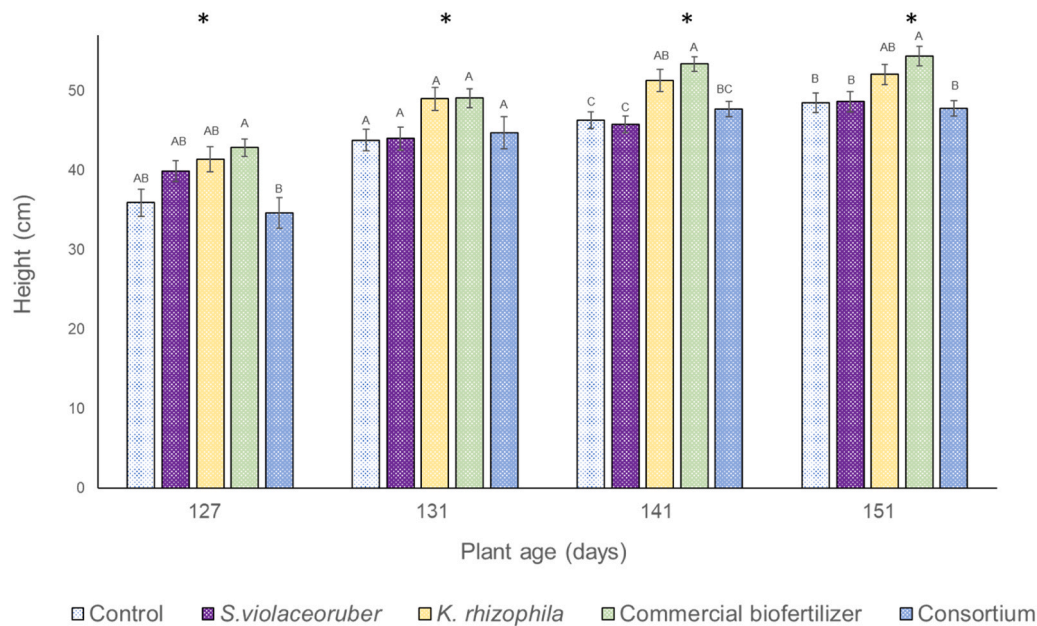


Fig. 6. Oregano plant height during field growth. Each bar represents the mean $\pm$ SE (n = 15). Asterisks indicate significant differences among treatments (ANOVA) at each survey. Letters on the bars indicate post-hoc Tukey's HSD test results at p < 0.05.

Table 2
Dry weight biomass (g) of plants treated with the two actinomycetotal inoculants, their consortium and the commercial biofertilizer at 151 DAP. Data are presented as mean (n = 5) $\pm$ SE. Different letters indicate significant differences (p < 0.05) among treatments after Tukey's HSD test. Treatments that share the same letters are not significantly different from each other.

Treatment	Plants dry weight (g)
Control (H ₂ O)	2.67 $\pm$ 0.90 BC
<i>S. violaceoruber</i>	3.83 $\pm$ 0.93 ABC
<i>K. rhizophila</i>	1.91 $\pm$ 0.78 C
Commercial Biofertilizer	6.07 $\pm$ 0.96 A
Consortium	4.78 $\pm$ 0.88 AB

metabolism than individual strains, further validation is required to confirm this trend.

4. Discussion

Among the PGPB, members of actinomycetota phylum have emerged as key players due to their metabolic versatility and robust colonization strategies (Sousa et al., 2016). PGPB have been extensively studied in major food crops (Wani and Gopalakrishnan, 2019), but their application in high-value aromatic plants, such as oregano, remains largely underexplored.

As one of the most widespread and drought-resistant species in the Mediterranean basin, oregano is a significant source of bioactive compounds, primarily found in its EOs (Leyva-López et al., 2017). Consequently, optimizing both its growth and secondary metabolism represents a primary goal for sustainable cultivation for specialized agriculture. Recent studies show that members of the *Streptomyces* genus promote plant growth and improve tolerance to both biotic and abiotic stresses, and that their enrichment in rhizosphere soils positively impacts plant performance (Pang et al., 2022). Similarly, isolates from *Kocuria* genus have been shown to exert growth-promoting effects on tomato plant under varying irrigation conditions (Almalkawi et al., 2025).

To address this challenge, we investigated the potential of two specific actinomycetotal strains, *K. rhizophila* PA220, known to alter

Table 3
Detected VOCs in Oregano flowers, Retention Time and classification.

Compound	Retention time (min)	Class
1. Methyl 2-methylbutyrate	12.80	Ester
2. 3-Octanol	16.50	Alcohol
3. α -Thujene	20.00	Monoterpene
4. α -Pinene	20.45	Monoterpene
5. Camphene	21.65	Monoterpene
6. Verbenene	23.40	Monoterpene
7. Sabinene	23.70	Monoterpene
8. β -Pinene	24.65	Monoterpene
9. α -Phellandrene	25.95	Monoterpene
10. α -Terpinene	26.60	Monoterpene
11. p-Cymene	27.20	Aromatic Monoterpenoid
12. Limonene	28.05	Monoterpene
13. β -Ocimene	28.80	Monoterpene
14. γ -Terpinene	29.60	Monoterpene
15. cis-Sabinene hydrate	30.55	Oxygenated Monoterpenoid
16. Terpinolene	31.55	Monoterpene
17. Linalool	32.00	Monoterpene
18. trans-Sabinene hydrate	32.75	Oxygenated Monoterpenoid
19. Neo-allo-ocimene	34.60	Monoterpene
20. 4-Terpinenyl acetate	38.25	p-menthane Monoterpenoid
21. O-Methylthymol	41.50	Monoterpene
22. Isothymol methyl ether	42.10	Aromatic Monoterpenoid
23. Thymoquinone	42.90	Ketone Monoterpenoid
24. Thymol	45.89	Aromatic Monoterpenoid
25. Carvacrol	47.99	Aromatic Monoterpenoid
26. α -Ylangene	50.74	Sesquiterpenoid
27. β -Bourbonene	51.19	Sesquiterpene
28. β -Elemene	51.39	Sesquiterpene
29. β -Caryophyllen	53.39	Sesquiterpene
30. β -Copaene	53.99	Sesquiterpene
31. α -Guaiene	54.19	Sesquiterpene
32. α -Bisabolene	55.54	Sesquiterpene
33. Aromadendrene	55.79	Sesquiterpene
34. α -Cubebene	57.04	Sesquiterpene
35. β -Bisabolene	58.64	Sesquiterpene

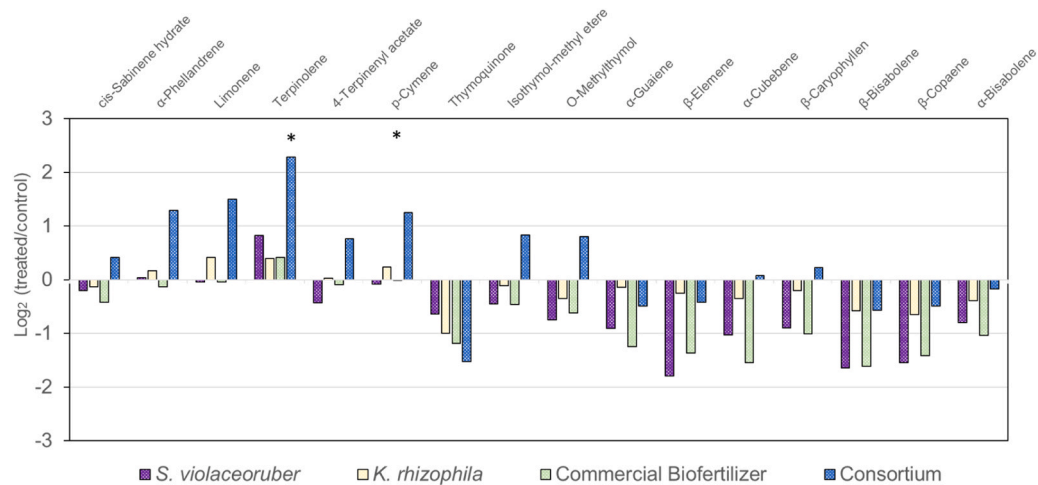


Fig. 7. Log₂ fold change (treated vs. control) of 16 among 35 oregano flowers' VOCs, identified by GC-MS in response to four different inoculations: *S. violaceoruber*, *K. rhizophila*, commercial biofertilizer, and Consortium. Positive values indicate increased abundance compared to control, while negative values indicate decreased abundance. The threshold for biologically relevant changes was set at ± 1 (log₂ scale). Samples, obtained by pooling flowers from three individual plants per condition in order to ensure sufficient biomass for analysis, were analyzed in technical duplicates. Asterisks indicate statistical differences among pooled samples (ANOVA, $p < 0.05$; Tukey's post hoc test).

Table 4
Detected compounds in Oregano EOs, Retention Time and classification.

Compound	Retention Time (min)	Class
α-Thujene	14.65	Monoterpene
α-Pinene	15.00	Monoterpene
Camphene	15.80	Monoterpene
Sabinen	16.85	Monoterpene
β-Pinene	17.10	Monoterpene
β-Myrcene	17.60	Monoterpene
β-Thujene	18.45	Monoterpene
α-Terpinene	18.95	Monoterpene
p-Cymene	19.35	Aromatic Monoterpenoid
3-Methyl-apopinene	19.80	Monoterpene
β-Ocimene	20.35	Monoterpene
γ-Terpinene	21.00	Monoterpene
cis-Sabinenhydrate	21.60	Oxygenated Monoterpenoid
Linalool	22.95	Monoterpenoid alcol
Neo-allo-ocimene	24.25	Monoterpene
4-Terpineol	26.80	Monoterpenoid alcol
Methyl thymyl ether	28.90	Aromatic Monoterpenoid
Isothymol methyl ether	29.35	Aromatic Monoterpenoid
Thymol	31.95	Aromatic Monoterpenoid
Caryophyllene	37.15	Sesquiterpene
Germacrene D	39.60	Sesquiterpene
β-Bisabolene	40.55	Sesquiterpene
δ-Cadinene	40.95	Sesquiterpene

S. lycopersicum L. primary and secondary metabolism (Mauceri et al., 2024) and *S. violaceoruber* DSM40783, previously characterized for multiple PGP traits and tested in vitro assays on *S. lycopersicum* L. seedlings (Faddetta et al., 2023; Ricciardi et al., 2026).

In the present work, genomic analysis confirmed that strain PA220 exhibits high Mash similarity (0.99957) to *K. rhizophila* NCTC 8340, clearly distinguishing it from more distant references such as ASM3599006. This precise taxonomic assignment reinforces the PGP potential of our isolate, consistent with independent research documenting *K. rhizophila* strains as established PGPB (Li et al., 2020; Almalkawi et al., 2025). On the other hand, members of the *Streptomyces* genus are known to be the major producers of bioactive compounds, in particular, the *S. violaceoruber* strain used in this work, also known as *S. coelicolor* A3 (2) in scientific literature, is able to produce secondary

metabolites, including actinorhodins and other polyketide antibiotics lantibiotics, siderophores and terpenes.

The *in-silico* analysis of genes typically involved in PGP capabilities revealed that *S. coelicolor* A3 (2) shows a broader capacity for polymer degradation and iron acquisition, while *K. rhizophila* presents complementary strengths in phosphate regulation and polyamine metabolism, with phosphate solubilization and siderophore-dependent iron uptake representing the most ecologically relevant PGP traits shared between the two strains. The presence of genes involved in IAA biosynthesis, including *trpC*, *iaaM*, and *iaaH* in *S. coelicolor* A3(2), is consistent with the observed effects on plant height and shoot development, in light of the well-established role of microbial auxins in regulating cell division and elongation (Tang et al., 2023). Similarly, the identification of genes associated with phosphate solubilization - such as *gdh/gno* and *gnd*

homologs involved in gluconic acid-mediated phosphate mobilization in both strains- and siderophore biosynthesis (with *K. rhizophila* encoding three siderophore transport genes and *S. coelicolor* harboring a more extensive eight-gene cluster) suggests mechanisms linked to enhanced nutrient acquisition. These traits may explain the increased chlorophyll content observed in treated plants, given the critical roles of phosphorus and iron in photosynthesis (Yang et al., 2024). Finally, the presence of predicted pathways related to polyamine metabolism, uniquely detected in *K. rhizophila* through three putrescine import systems, is consistent with biological processes involved in plant growth regulation and stress-related physiological responses (Pál et al., 2026). The reduced BGC repertoire observed in *K. rhizophila* suggests a more streamlined and specialized secondary metabolism compared to model actinomycetes. Despite this limitation, the presence of terpene-related clusters indicates a potential role in modulating plant-associated metabolite profiles. These differences in BGCs and PGP-associated genetic determinants may be related to their differential association with plant growth-related specific traits.

S. violaceoruber and *K. rhizophila* inoculations were successful in establishing early presence on root surfaces, as observed in SEM micrographs of inoculated oregano seedlings (Fig. 2). *S. violaceoruber* stimulated seed germination, whereas *K. rhizophila* did not show appreciable effects at this stage. Enhanced germination and early seedling development have frequently been associated with microbial auxin production in PGPB *Streptomyces* strains (Zhang et al., 2023), and bacterial priming is known to influence germination rate and uniformity (Hata et al., 2021). Trait-specific responses observed under controlled conditions were also detected under field conditions, confirming their activity beyond controlled environments (Bhatti et al., 2017). *K. rhizophila* promoted shoot elongation at full blooming, indicating a plant growth-promoting effect during a key phenological phase (Meier et al., 2009). No significant differences were observed in dry biomass, which may reflect the uncoupling of height growth and biomass accumulation typical of Mediterranean shrubs (Chatzistathis and Therios, 2013; De Cáceres et al., 2019). *S. violaceoruber* influenced SPAD values after repeated inoculation. Increased chlorophyll content is often associated with improved physiological status and enhanced nutrient assimilation (Lichtenthaler et al., 1981), and similar effects have been reported in plants treated with *Streptomyces* spp. (Manulang and Chuang, 2020). In this context, genomic predictions provide a functional framework for interpreting these observations.

Beyond growth promotion, recent research highlights the role of microorganisms in modulating plant secondary metabolism. Indeed, our study on actinomycetotal strains was not limited to assessing oregano growth but also extended to investigating relative differences in its VOC composition, providing a descriptive perspective on plant physiological responses. A similar regulatory effect on secondary metabolism has been observed in other species, consistent with previous reports describing associations between PGPB and plant metabolic pathways (Zhou et al., 2026).

Flowers' volatiles play a fundamental role in environment-related communication and other organisms' interactions (Bouwmeester et al., 2019). Flower sampling was performed during the full blooming stage (BBCH 65; Meier et al., 2009) at 140 DAP. Due to this narrow phenological window, the biomass available per plant was limited, and pooling flowers from different individuals was necessary to ensure sufficient material for reliable volatilome analyses. While this sampling approach limits the resolution of treatment-level variability, the descriptive relative abundance patterns of selected VOCs highlight interesting trends. Specifically, samples from consortium-inoculated plants exhibited higher relative values for certain monoterpenes compared to control samples. This observation is consistent with previously reported associations between PGPB strains and plant secondary metabolism, providing a contextual framework for interpretation

(Ricciardi et al., 2026). Within this metabolic framework, terpenoids are the most abundant VOCs in plants. Monoterpenes are key components of plant secondary metabolism and are often involved in plant defense mechanisms and signaling processes (La Scala et al., 2024; Huang and Osbourn, 2019). Their biosynthesis relies on the plastidial methyl erythritol-4-phosphate (MEP) pathway yielding mono- and diterpenes (Li et al., 2023). Among them, terpinolene and p-cymene - widely produced by a variety of plant species (Marchese et al., 2017; Pyo and Jung, 2024) - were particularly representative. P-cymene is an alkyl-substituted aromatic monoterpene and a known precursor of several bioactive compounds (Balahbib et al., 2021), while terpinolene exhibits a broad spectrum of biological activities (Aydin et al., 2013) including antimicrobial and antioxidant properties. Interestingly, some PGPB, including *Streptomyces* species, are themselves able to produce terpene compounds, albeit via biosynthetic routes distinct from those operating in plants (Komaki et al., 2018b).

Therefore, these findings raise the possibility that microbial metabolism may be associated with the observed volatilome and EO profiles of *O. vulgare* plants, adding an additional layer of complexity to plant-microbe interactions. The exploratory analysis of EO profiles revealed that the major metabolites detected were thymol, γ -terpinene and p-cymene, which are key compounds underlying the characteristic pharmacological properties of Mediterranean Lamiaceae (Gago et al., 2025). Although the pooling strategy limits the GC-MS dataset to a qualitative profile, it still enables a semi-quantitative assessment and preliminary comparison of the dominant chemical constituents across treatments. The observed trends are nonetheless consistent with the broader hypothesis found in literature that beneficial microorganisms may influence plant secondary metabolism (Ketehouli et al., 2025). Future studies, including proteomic, transcriptomic and metabolomic analyses, will be necessary to support these observations and to clarify whether they involve transcriptional modulation within the MEP pathway (Li et al., 2023; Pérez-Pérez et al., 2024).

These preliminary findings are in line with previous studies on aromatic Lamiaceae. For instance, PGPB inoculation under controlled conditions has been shown to enhance EO accumulation in *Origanum × majoricum*, likely through stimulation of terpene biosynthesis and improved nutrient acquisition (Banchio et al., 2010); Similarly, improved plant nutritional status and secondary metabolite production were reported in *Mentha piperita* upon PGPB application (Chiappero et al., 2019) and a broader modulation of EO content and composition has been described in oregano following inoculation with multiple PGP strains (Çakmakçı et al., 2023).

Overall, the results of the present study highlight relative differences in morphophysiological and metabolic traits under field conditions, while underscoring the need for future studies integrating biological variability and multi-omics approaches. The differences observed in both morphophysiological and metabolic traits by the strains *S. violaceoruber* DSM40783 and *K. rhizophila* PA220, inoculated individually or in combination, suggest their potential role as bioinoculants for the Mediterranean aromatic crop *O. vulgare* L. Microbial-based biofertilizers are emerging as key nature-based tools for building more sustainable and resilient agricultural systems, and these actinomycetota may offer promising opportunities for innovative applications particularly suited to Mediterranean environments.

This pioneering study indicates that novel Actinomycetota-based biofertilizer formulations for aromatic plants can be explored: they are associated with improvements in specific plant traits, including seed germination and shoot elongation under both greenhouse and open-field conditions, and with differences in the metabolic profiles of adult oregano plants under field conditions. These findings pave the way for the development of targeted microbial products capable of supporting crop performance while promoting environmentally friendly agricultural practices.

5. Conclusion

Oregano is a Mediterranean aromatic crop well adapted to arid environments and valued for its EOs with antibacterial and antioxidant properties. In this study, oregano samples inoculated with the strains *S. violaceoruber* DSM40783 and *K. rhizophila* PA220 exhibited higher germination rates, improved shoot development, and increased chlorophyll content compared to controls, thus reflecting genome-based predictions. However, these improvements did not translate into an increase in overall biomass productivity.

Beyond these trait-specific responses, the strains were also associated with relative differences in oregano metabolite profiles, including terpene, with potential implications for EO composition. Given the semi-quantitative and pooled nature of the metabolomic dataset, these observations should be interpreted as descriptive and hypothesis-generating rather than conclusive, and further studies—integrating biological variability and multi-omics approaches—will be essential to expand these findings.

By integrating laboratory experiments and field observations, this work provides evidence supporting the potential of these strains as PGPB inoculants under the tested conditions. At the same time, the indication of possible metabolite-level variations opens new perspectives for future investigation on Actinomycetota-based biofertilizers as tools for optimizing the production of high-value compounds in aromatic crops.

CRedit authorship contribution statement

Silvia La Scala: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sara Amata:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Teresa Faddetta:** Writing – review & editing, Methodology, Investigation. **Loredana Abbate:** Writing – review & editing, Methodology. **Francesco Paolo Bonomo:** Writing – review & editing, Investigation. **Guglielmo Puccio:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation. **Francesco Mercati:** Writing – review & editing, Resources. **Antonio Palumbo Piccionello:** Writing – review & editing, Resources. **Alessandra Carrubba:** Writing – review & editing, Resources, Methodology, Conceptualization. **Giuseppe Gallo:** Writing – review & editing, Writing – original draft, Resources, Project administration, Funding acquisition, Conceptualization. **Paola Quatrini:** Writing – review & editing, Writing – original draft, Resources, Project administration, Funding acquisition, Conceptualization.

Ethical approval

Not applicable.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used Microsoft Copilot (GPT-based tool) for manuscript language editing. The author(s) reviewed and edited the output as needed and take full responsibility for the content of the published article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.micres.2026.128733](https://doi.org/10.1016/j.micres.2026.128733).

Data availability

Data will be made available on request.

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