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PLANT-GROWTH-PROMOTING AND ANTIFUNGAL ASSET OF INDIGENOUS DROUGHT-TOLERANT RHIZOBACTERIA ISOLATED FROM OLIVE (*Olea europaea* L.) RHIZOSPHERE

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ABSTRACT

Faced with global environmental challenges, the quest for sustainable food production has gathered momentum. While abiotic stresses adversely affect plant health and productivity, Verticillium wilt causes considerable yield losses worldwide, particularly in crops such as olive. Recently, drought-tolerant bacteria have been used to alleviate both abiotic stress and pathogen pressure in crops. In this context, our work focuses on the isolation of tolerant indigenous rhizobacteria to mitigate these challenges by investigating their role in biocontrol and abiotic stress tolerance. Thus, a total of 94 rhizobacterial strains were isolated from the rhizospheres of olive trees in southeastern Morocco and characterized to identify tolerant plant growth-promoting rhizobacteria that inhibit *Verticillium dahliae*. 24 strains demonstrated *in vitro* suppression of *Verticillium dahliae* Klebahn, and exhibited tolerance to different abiotic stresses (drought, salinity, and high temperature). In addition, they proved xerotolerant (Aw ≤ 0.91), halotolerant (≥10% NaCl), and thermotolerant (≥ 55°C) capabilities. Beyond, these isolates showcased multifaceted plant growth-promoting traits, such as phosphate solubilization and significant synthesis of essential bioactive compounds like siderophores, indole-3-acetic acid and hydrolytic enzymes. Evaluating outcomes, three standout rhizobacterial isolates emerged due to their exceptional stress tolerance, unique plant growth-promoting qualities, and potent antagonistic potential. Molecular analysis identified them as *Bacillus paranthracis* (OZ-60) and *Bacillus licheniformis* (OZ-48 and OZ-77) through 16S rRNA sequencing. Besides enhancing plant abiotic stress resistance, these isolates hold promise in bolstering the sustainability of olive cultivation and fortifying plant defenses against pathogens.

Keywords: Abiotic stress, Antifungal, *Bacillus*, PGPR, *V. dahliae*, 16S rRNA

INTRODUCTION

In recent years, the pursuit of sustainable food production has emerged as a pivotal challenge amidst the backdrop of global environmental concerns such as climate shifts, plant pathogenic ailments, and degradation of natural resources encompassing soil and biodiversity **(Mahasneh *et al.*, 2024)**. Within this context, the imperative for ecologically benign practices has become pressing. Among cultivated flora, the olive tree (*Olea europaea* L.) holds unique status, being an anciently cultivated species and the sole representative of the *Oleaceae* family with edible fruits **(Eliuz, 2020; Kamran *et al.*, 2023)**. In Morocco, the olive fruit reigns as a paramount produce, with the nation ranking as the world's second-largest producer of canned olives and the fifth-largest producer of olive oil **(Ater *et al.*, 2016; El Qarnifa *et al.*, 2019)**.
Nevertheless, among the Earth's arid regions, those within the western Mediterranean sphere, including Morocco, confront pronounced vulnerability to the dual impact of abiotic and biotic stresses, a consequence of climatic fluctuations, persistent drought, and demographic imbalances **(Harkousse *et al.*, 2021)**. Prominently, globally pervasive stressors such as soil salinity and drought exact significant tolls on productivity and growth, imposing substantial ramifications on the agricultural landscape **(Lahsini *et al.*, 2022; Montes-Osuna *et al.*, 2021; Verner *et al.*, 2018; Yang *et al.*, 2021)**. The olive tree's vitality is further jeopardized by an array of afflictions stemming from microbial agents **(Alshammari *et al.*, 2022; Barguigua *et al.*, 2021; Chliyeh *et al.*, 2014; Mahasneh *et al.*, 2024)**, including *Verticillium dahliae* Klebahn, the causal agent of Verticillium Wilt of Olive (VWO), which classified among the most widespread and damaging olive diseases, inducing a vascular affliction that menacing olive trees worldwide **(Serrano-García *et al.*, 2023)**, with pronounced impact in Morocco **(Barguigua *et al.*, 2020; Boutaj *et al.*, 2020)**. The olive tree is invaded by the fungus through its roots and subsequently spreads to the trunk, branches, and leaves. Within these plant parts, it obstructs the xylem vessels' transportation of water and nutrients, leading to the manifestation of wilting symptoms and ultimately culminating in the mortality of the infected tree **(Antón Domínguez *et al.*, 2023)**.
Within this framework, our study aimed to assess drought, salt, and high temperature tolerance of bacterial isolates from olive tree rhizospheres, as well as *in vitro* antagonistic mechanisms against the olive fungal pathogen *V. dahliae*. Investigate key indicators as plant growth-promoting rhizobacteria (PGPR): phosphate solubilization, siderophore production, indole-3-acetic acid (IAA) production, and protease activity. Furthermore, we evaluate the taxonomic placement of selected isolates through 16S rRNA sequencing.

MATERIALS AND METHODS

Sampling and isolation

The samples were obtained from the rhizosphere soil of olive cultivars from different geographical zones known for olive cultivation in Zagora region in south-east Morocco (30° 15' N, 5° 40' W and 30° 15' N, 5° 41' W). This region has a hot desert climate in the Köppen-Geiger climate classification **(Beck *et al.*, 2018)**. Olive groves were selected from small farms whose soils had never been treated with chemicals. Soil and roots were collected from the base of the olive trees, after removing about 15cm of top-soil, then placed in sterile bags and transported to the laboratory for of viable bacteria isolation. Using a serial dilution method such as described by **Dutta *et al.* (2021)**, viable bacteria were isolated and conserved in 20% (*v/v*) glycerol and Tryptic Soy Broth (TSB) then stored at -80°C for future use.
Rhizospheric bacterial isolates were first characterized based on phenotypic observation under optical microscope to determine their shape using Gram staining observation then biochemical tests for the two bacterial enzymes, oxidase and catalase were performed.

The fungal strain of *V. dahliae* Klebahn, isolated from infested olive trees, was provided by the Moroccan Coordinated Collections of Micro-organisms (CCMM) (https://www.ccmm.ma/).

Screening for antagonistic activity against *V. dahliae*

Bacterial isolates were screened for their *in vitro* antagonistic activity, by dual culture assay, against the fungal pathogen *V. dahliae*. Briefly, 10 µL of fungal spore suspension (10^6 spore. mL^{-1}) was spread onto a Potato Dextrose Agar (PDA) plate and incubated overnight at 28°C, then one drop of each bacterial suspension was placed at the center. Next, plates were incubated at 28°C for 7 days. An inhibition zone of over 20mm was considered as a positive result.

Salinity, high temperature and drought tolerance

In order to evaluate salinity tolerance, isolates were inoculated in Tryptic Soy Agar (TSA) supplemented with 10% NaCl and incubated at 28°C for 48h until colonies appeared (positive result). While, temperature tolerance was examined by culture and incubate isolates at 55°C for 48 h in TSA **(Lahsini *et al.*, 2022)**. A plate without added NaCl was used as control.
The ability of isolates to tolerate drought stress conditions was assessed by influencing the water activity (A*w*) in TSB supplemented with high amounts of D-sorbitol (the higher the concentration of D-sorbitol added, the lower the A*w* obtained in the medium) **(Hallsworth *et al.*, 1998)**. 2 mL of TSB supplemented by 550 $g.L^{-1}$ of D-sorbitol (giving an A*w* of 0.91) was inoculated with 1% bacterial fresh cultures, then incubated at 28°C for 48 h with continuous shaking at 180 rpm. The growth was monitored by measuring optical density (OD) periodically (1-hour intervals) at OD_{600nm} using a microplates reader spectrophotometer (Epoch 2, Agilent BioTek). A sterile TSB was used as a blank.
Drought-tolerant isolates were further categorized using OD values into four categories: highly sensitive (OD < 0.3), sensitive (OD 0.3 < OD < 0.39), tolerant (OD 0.4 to 0.5), and highly tolerant (OD > 0.5) (Ashry *et al.*, 2021). All experiments were performed in duplicate.

Evaluation of PGP traits

All inoculums used in the experiments below were prepared by inoculating each bacterial isolate into 5 mL TSB tubes followed by an incubation at 28°C for 48h in a shaking incubator (180 rpm).

Phosphate solubilization

The approach described by **Ambrosini *et al.* (2017)** was applied for the identification of isolates exhibiting phosphate solubilization from an insoluble source like tricalcium phosphate. The medium composition included 10g glucose, 2g yeast extract, 15g agar, and 1 L distilled water.
A sterile solution of K_2HPO_4 (5 g/50 mL water) and $CaCl_2$ (10g/100 mL water) was then prepared to achieve a 10% (*w*/*v*) concentration and added to the medium before pouring it into Petri dishes. The isolates were inoculated then incubated at 28°C for 3 days. The formation of a transparent halo surrounding bacterial growth indicated successful solubilization of tricalcium phosphate.

Indole acetic acid production

The bacterial isolates were screened for IAA production using the method described by **Chaudhary *et al.* (2021)**. Bacterial isolates were cultured in TSB supplemented with 1 mM L-tryptophan and incubated at 28°C for 3 days in an orbital shaking incubator at 150 rpm. After centrifugation at 9000xg, 2 mL bacterial-free extract was mixed with 2 mL of Salkowski reagent (12 g of $FeCl_3$ per liter in 7.9M H_2SO_4). The mixture was then incubated in the dark for 30 min at room temperature, then OD was measured at 535nm using a spectrophotometer (Epoch 2, Agilent BioTek). IAA concentration was calculated by a standard curve prepared with a range of IAA concentrations. Sterile TSB served as a negative control.

Siderophore production

Two different types of siderophores (hydroxamate and catecholate) were determined using the method described by **Carson *et al.* (1992)**. First, TSB cultures were centrifuged at 9000xg for 15 min to collect bacterial-free extracts from the supernatants. Then,1 mL of the extract was mixed with 3 mL of 2% aqueous $FeCl_3$ solution. This mixture was then measured at two wavelengths in the UV–visible spectrum using the Epoch 2 spectrophotometer (Agilent BioTek). The first measure was done at 430nm which correspond to a brown color generated for hydroxamate nature siderophore, while a wine-colored complex is formed for catecholate nature siderophores at 495nm. A solution of 1 mL of distilled water and 3 mL of 2% aqueous $FeCl_3$, was used as a blank.

Protein hydrolysis

To evaluate protein hydrolysis activity of bacterial isolates, Skim-Milk Agar containing in g/L: Skim-milk powder 100, Peptone 5; Agar 15, was used. Following inoculation and incubation of the agar plate cultures (28°C for 48h), protease-secreting strains displayed a zone of proteolysis representing a positive reaction **(Ambrosini *et al.*, 2017)**.

Lipid hydrolysis

The lipolytic activities of rhizospheric isolates was performed on the nutrient agar supplemented with Tween20 as lipid substrate. The medium contained 10g peptone, 2g yeast extract, 5g NaCl, 20 mL Tween20 and 15g agar in 1 liter distilled water **(Kumar *et al.*, 2012)**. After incubation at 28°C for 48h, lipase-excreting bacteria exhibit a zone of haziness surrounding the bacterial growth.

Mechanisms Associated with the Antagonistic Activity

To further explore the antagonistic potential of rhizospheric isolates *V. dahliae* Klebahn, we employed the direct diffusion and volatile organic compound (VOC) methods detailed below.

Direct diffusion assay

A suspension of each isolate was inoculated, forming a straight line at the center of each culture plate. After 24h of incubation, two 5mm discs of *V. dahliae* fresh culture were placed 2 cm from the middle line of the bacteria culture (Figure 1.A). The plates were then incubated at 28°C for 7 days. The percentage of inhibition was estimated by measuring the radius of *V. dahliae* colonies on the side of the challenged isolate. The radius of colonies from the control grown plate was also assessed. Results are expressed as the mean ± standard error (SE) calculated between replicates. The following formula was used to calculate the percentage of fungal growth inhibition: **(C - T/C) × 100**, where **C** and **T** are the distance across the edges of the fungal growth in the assay and control Petri dish, respectively.

Production of VOC

A compartmented PDA petri-dish was inoculated with the bacterial isolates on one side of the plate; the other side was used for fungal strain cultivation (Figure 1.B). Afterward, plates were incubated at 28°C for 7 days, then the diameters of fungal growth were measured. The results were calculated and expressed as percentages of mycelial growth inhibition, as follows: **(C - T/C) × 100**, where **C** is the value of the control, and **T** is the measurement of the fungus in each antagonist-fungal plate. Three replicates were performed for each isolate.

Molecular identification

Gene primers

Complete 16S rRNA coding gene (~1500pb) was amplified using universal primer pair fD1 (5'-AGAATTTGATCTTGGTTCAG-3') and rP2 (5'-ACGGCTACCTTGTTACGACTT-3') **(Weisburg *et al.*, 1991)**.

DNA extraction and sequencing

Genomic DNA was extracted using the BIOLINE extraction kit (Meridian Bioscience, UK) according to the manufacturer's instructions. PCR was carried out in a 25 µL reaction volume using KAPA kit (KAPA Biosystems) adhering to the manufacturer's instructions. Amplified PCR products were electrophoresed on a 1% agarose gel and stained with ethidium bromide. PCR products were purified using the ExoSAP-IT purification kit (Thermo Fisher Scientific, Waltham, MA). Sequencing was performed using the Big Dye Terminator Cycle Sequencing v3.1 kit (Life Technologies, Inc. Foster City, CA) in both forward and reverse directions with the same primers used for PCR following the manufacturer's instructions. Sequence data were collected on an ABI Prism 3130XL genetic analyzer (Life Technologies, Inc. Foster City, CA).

Sequences analysis

The obtained 16S rRNA sequences were assembled using DNA Baser Sequence Assembler (V5.15) software. The BLAST algorithm incorporated within the EzBioCloud 16S rRNA database was employed for the identification and comparative analysis of sequences with references **(Yoon *et al.*, 2017)**. Following, a phylogenetic trees was constructed using MEGA XI software, employing the maximum likelihood method with 1000 Bootstrap replicates **(Kumar *et al.*, 2018)**, based on both 16S rRNA sequences.

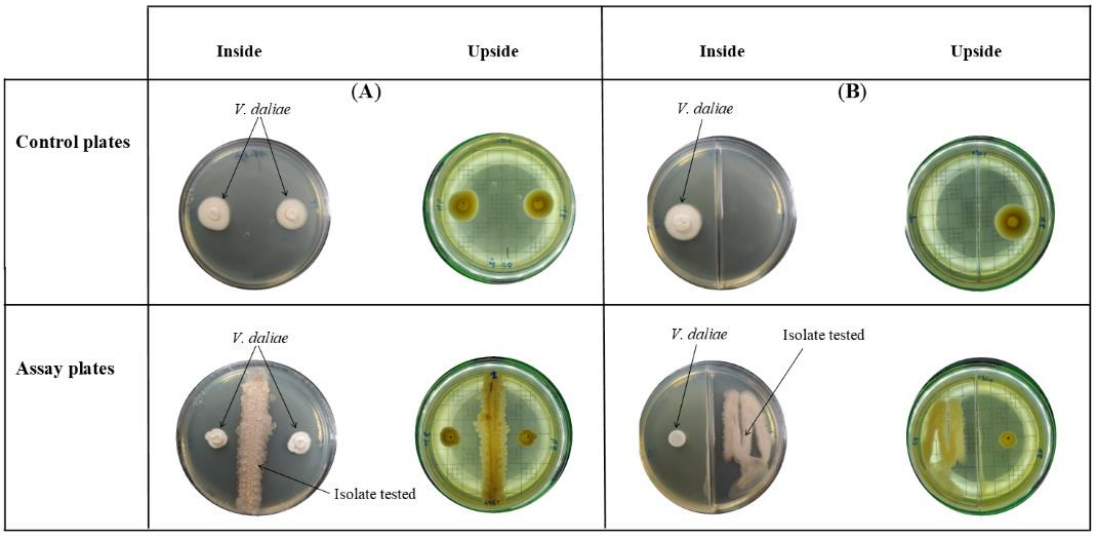


Figure 1 Illustration of antimicrobial activity of rhizospheric bacterial isolates against pathogenic fungi *V. daliae* by dual culture (**A**) and volatile diffusion method (centrally partitioned plates) (**B**).

Statistical analysis

The significance of the difference between replicates, according to the studied parameters, was determined using a one-way ANOVA followed by a Tukey's HSD test. We consider as significantly different only those parameters that had a significance level (*p*-value) <0.05.

RESULTS

Antagonistic activity and abiotic stress tolerance screening

A total number of 94 different bacterial isolates were isolated from several rhizospheric soils of olive trees in Zagora – Morocco and named "OZ" followed by their isolation number. All isolates were gram-positive, rod-shaped cells, and catalase/oxidase positive.

The *in vitro* antagonistic activity against the fungus *V. dahliae*, revealed that 35 (37%) bacterial strains were able to significantly reduce its growth according to their level of inhibitory effects (data not shown). Under osmotic stress concentrations, applied with D-sorbitol, 14 isolates (15%) were categorized as highly sensitive, 6 (7%) as sensitive, 54 (57%) as tolerant, and 21 (22%) as highly tolerant. For salinity tolerance, 73 bacterial isolates (78%) were tolerant and 21 (22%) were sensitive to a 10% concentration of NaCl in the medium. While for temperature screening, 79 isolates (84%) grew at 55°C, the remaining 15 isolates (16%) were considered sensitive. These results revealed that most isolates were both halotolerant and thermotolerant.

Building upon the outcomes of the screening process, a total of 24 bacterial strains were identified, each exhibiting notable resistance to stress encompassing NaCl, high temperature, and drought. Furthermore, these strains exhibited marked efficacy in suppressing fungal growth (Table 1). These 24 strains were designated for subsequent investigations, as elaborated in the following sections.

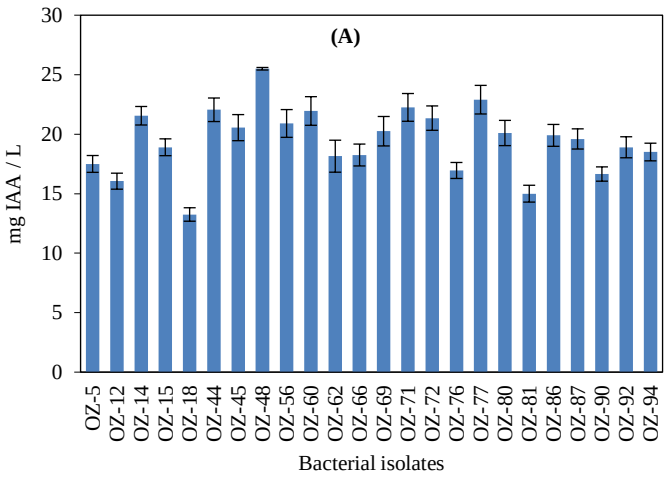
Table 1 Stress tolerance characteristics of 24 selected rhizosphere isolates.

Isolate	Drought stress	Isolate	Drought stress
OZ-5	T	**OZ-69**	HT
OZ-12	HT	**OZ-71**	T
OZ-14	T	**OZ-72**	T
OZ-15	T	**OZ-76**	HT
OZ-18	T	**OZ-77**	T
OZ-44	T	**OZ-80**	HT
OZ-45	HT	**OZ-81**	HT
OZ-48	T	**OZ-86**	HT
OZ-56	T	**OZ-87**	HT
OZ-60	T	**OZ-90**	T
OZ-62	HT	**OZ-92**	HT
	(B)		
OZ-66	T	**OZ-94**	HT

Legend: T – tolerant, HT – highly tolerant

Evaluation of PGP traits

The twenty-four chosen isolates underwent evaluation for growth-promoting attributes, encompassing their capacity to generate phosphate, IAA, and siderophores, alongside their protease and lipase activity. It is noteworthy that all the bacterial strains displayed a discernible halo surrounding their colony growth, indicative of phosphate solubilization. Concerning IAA production, a majority of isolates demonstrated proficiency in IAA synthesis, with values spanning a notable range of 13 to 25 mg/L. The most noteworthy IAA synthesis was recorded by isolate OZ-48, displaying a remarkable production of 25.5 mg/L. Following this, isolate OZ-77 showcased a substantial production of 22.9 mg/L, while the lowest quantity was observed in the case of isolate OZ-18, with a recorded amount of 13.25 mg/L (Figure 2.A). Moving to siderophore production, both catecholate (Figure 2.B) and hydroxamate (Figure 2.C) types were evaluated. Remarkably, all bacterial strains exhibited the capability to generate both categories of siderophores. Among them, isolate OZ-48 emerged as the most proficient in terms of siderophore production, closely followed by isolates OZ-5 and OZ-62. Moreover, the investigation into protease and lipase activities yielded positive outcomes for all isolates, evincing distinct halos and zones of precipitation adjacent to bacterial growth.



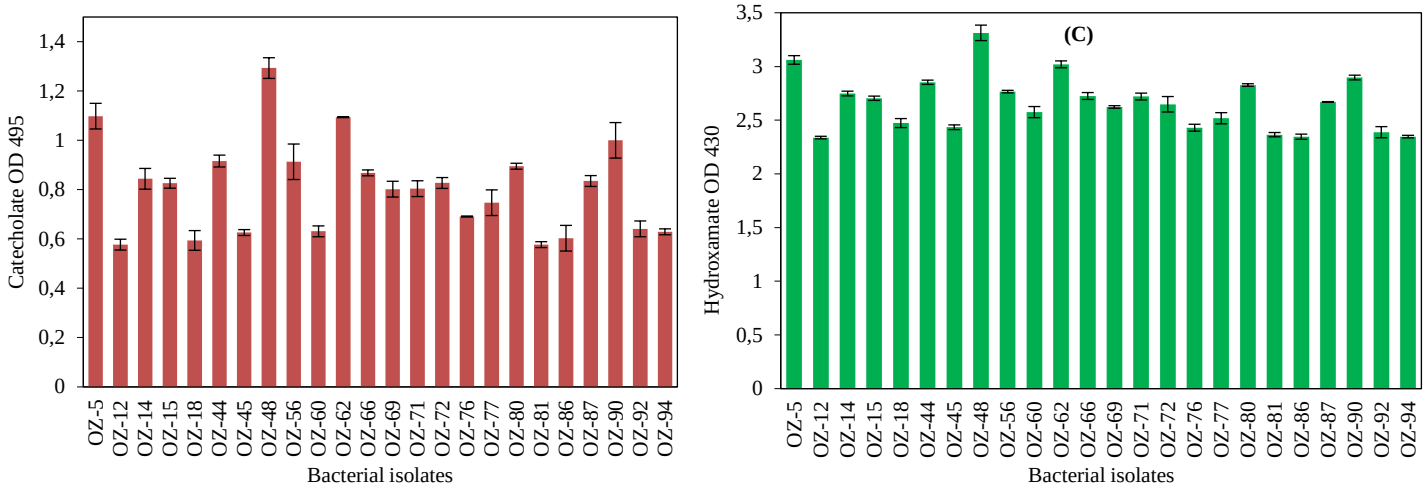


Figure 2 Bacterial isolates from the olive rhizosphere have their PGP characteristics plotted as bars on a graph. (**A**) IAA, (**B**) siderophore type catecholate and (**C**) siderophore type hydroxamate. Data represent mean ± standard deviation.

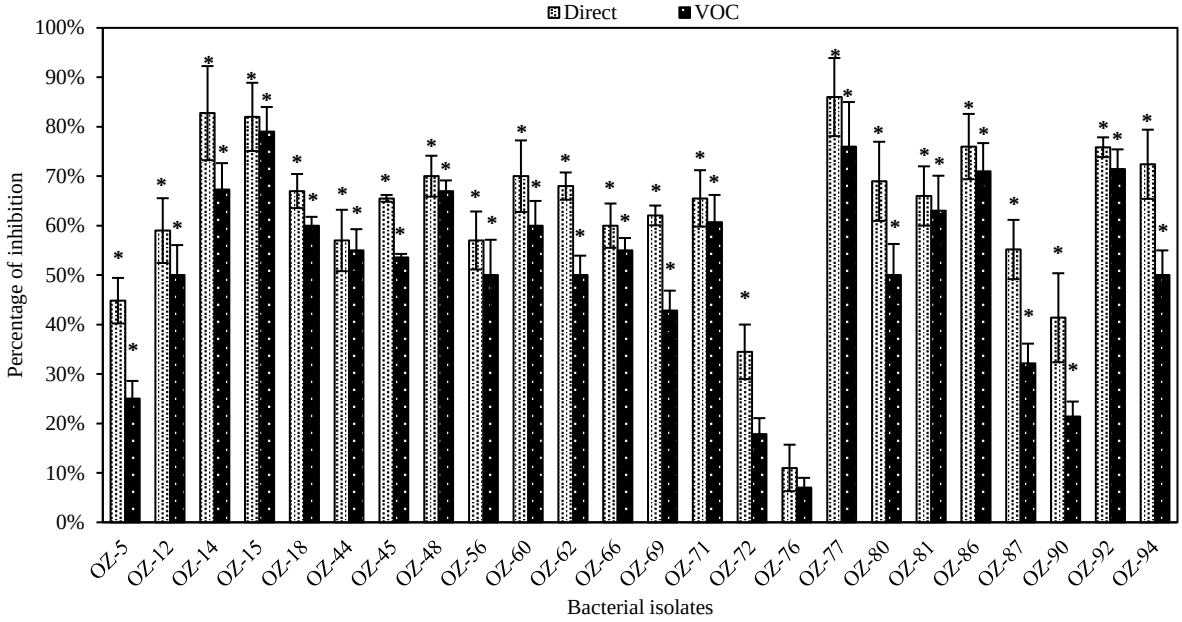


Figure 3 Antagonistic activity of the 24 rhizospheric isolates against *V. dahliae* by direct diffusion assay (dual culture) and volatile organic compounds (centrally partitioned plates). Data represent mean ± standard deviation. Bars followed by (∗) show a statistically significant difference ($p < 0.05$) with control plates (not exposed to bacteria).

Diffusible substance and VOC-mediated antifungal activity

The investigation into the production of antifungal compounds by the 24 selected rhizospheric isolates was conducted using both the diffusible and volatile organic compounds (VOC) assays (Figure 1). In the diffusible substances test, the highest degree of antifungal activity was observed with isolate OZ-77, showcasing an efficacy of 86%. This was closely trailed by OZ-14 with 83%, and OZ-15 at 82%. Conversely, the lowest antifungal activity was evidenced by OZ-76, demonstrating a modest 11% effectiveness. Moving to the VOC production assay, the range of mycelial growth inhibition spanned from 79% to 7% across isolates, with OZ-15, OZ-77, and OZ-76 registering 79%, 76%, and 7%, respectively (Figure 3).

Molecular identification and phylogenetic analysis

DNA Sequencing of the complete 16S rRNA gene was carried out to identify the selected 24 isolates. The 16S rRNA sequences were deposited in GenBank NCBI under the following accession numbers: OP901387 to OP901410.
BLAST results using EzBioCloud are represented in Table 2. Following, a phylogenetic tree was constructed utilizing the derived sequences in conjunction with reference strains sourced from GenBank (Figure 4). Through BLAST results and phylogenetic analysis, isolates were separated into four distinct clusters. Fifteen strains named: OZ-5, OZ-14, OZ-15, OZ-44, OZ-48, OZ-62, OZ-66, OZ-69, OZ-71, OZ-72, OZ-77, OZ-80, OZ-90, OZ-92 and OZ-94, were clustered into *Bacillus licheniformis* clade and three to its closet related strain *Bacillus paralicheniformis* (OZ-56, OZ-76 and OZ-87) with the highest degree of sequence similarity. While the *Bacillus subtilis* clade regrouped 4 strains, OZ-45, OZ-81 and OZ-86 were closely related to *Bacillus cabrialesii*. OZ-12 was mainly related to *Bacillus rugosus* with 100% sequence similarity. The two remaining strains: OZ-18 and OZ-60, were referred to *Priestia endophytica* and *Bacillus paranthracis*, respectively.

DISCUSSION

Climate change as well as plant pathologies are now increasingly leading to undesirable environmental situations with increased abiotic stress in the context of a major droughts in various locations, thereby reducing agricultural productivity. Multiple approaches to minimize such problems have been developed, but they have potentially negative effects besides being costly **(Montes-Osuna *et al.*, 2021; Verner *et al.*, 2018; Yang *et al.*, 2021)**. Thus, it is recommended to explore the utilization of innovative PGP bacteria in agricultural applications, encompassing roles as biofertilizers, biopesticides, and phytostimulators. This strategic approach has the potential to significantly increase agricultural yields, elevate produce quality, counteract drought-induced oxidative stress and offer protection against pathogenic agents **(Oleńska *et al.*, 2020)**. Consequently, contemporary agricultural research primarily centers on the investigation of the rhizosphere, driven by its wealth of microbial diversity.
Within the context of this specific study, indigenous rhizobacterial isolates were meticulously isolated from the rhizosphere soil of olive trees. A total of 94 bacterial isolates were successfully isolated and cultured on nutrient agar employing olive rhizosphere soil as samples. Each isolate underwent comprehensive characterization, encompassing parameters such as drought, salt and high temperature tolerance as well as PGP proprieties and *in vitro* growth inhibition of *V. dahliae* Klebahn.
Through evaluation of screening outcomes, three standout rhizobacterial isolates, named OZ-48, OZ-60 and OZ-77, emerged due to their exceptional stress tolerance, unique PGP qualities and potent antagonistic potential. The 16S rRNA

sequencing of the bacterial strains revealed their closest genetic affinities with *Bacillus* genera. Hence, identifying OZ-60 as *Bacillus paranthracis* and OZ-48 and OZ-77 as *Bacillus licheniformis*. These findings align with prior studies reporting the identification of various *Bacillus* spp. within the olive rhizosphere **(Bennis *et al.*, 2022; de Los Santos Villalobos *et al.*, 2019; Lahsini *et al.*, 2022)**. Notably, these isolates demonstrated substantial drought resistance at 55% D-sorbitol concentration (550 $g.L^{-1}$), indicating their potential for alleviating drought stress in olive trees at A*w* 0.91. Similar investigations have reported the utility of drought-tolerant rhizobacteria thriving at 520 $g.L^{-1}$ D-sorbitol concentration as a strategy to mitigate drought stress in plants **(Moreno-Galván *et al.*, 2020)**. Moreover, it has been observed that co-inoculation with drought-tolerant PGP bacteria can effectively modulate plant stress responses, ultimately enhancing the plant's ability to adapt to drought-induced conditions **(Ashry *et al.*, 2021; Vurukonda *et al.*, 2016)**. Furthermore, these rhizobacteria exhibited tolerance to high salt levels (up to 10% NaCl) and elevated temperatures (up to 55°C), which serves as a crucial element in ameliorating plant resistance to stressors. Moreover, it's crucial to acknowledge that salt stress can hinder plant growth promotion by affecting plant physiology, biochemistry and gene expression **(Upadhyay *et al.*, 2011)**. As well, soil pH can be a limiting factor for soil enzyme activities which is modulated directly or indirectly by salt **(Yu *et al.*, 2019)**. Indeed, researches has documented the enhancement of plant performance in saline environments through the utilization of salt-tolerant PGP bacteria **(A. Kumar *et al.*, 2021; Zhang *et al.*, 2018)**.

Prior scientific investigations have emphasized the superior auxin-producing capabilities of microorganisms originating from the rhizosphere in comparison to those dwelling in non-rhizosphere environments **(Hakim *et al.*, 2021)**. This phenomenon holds significant importance, given the pivotal role of auxins, particularly IAA, in promoting plant growth through root development facilitation and nutrient uptake enhancement **(Hakim *et al.*, 2021; Oleńska *et al.*, 2020)**. The assessment of IAA production within the isolates revealed approximately a level of 25.5 mg/L and 22.9 mg/L and 21.95 mg/L, for OZ-48, OZ-77 and OZ-60, respectively. These isolates were proficient auxin producers, as reported in the literature, reaffirming the prevalence of auxin-producing microorganisms within the rhizosphere. Phosphate-solubilizing bacteria have garnered substantial interest among agricultural microbiologists due to their capacity to enhance phosphorus availability in soil, thereby stimulating plant growth and bolstering yields **(Ahkami *et al.*, 2017)**. Noteworthy, the rhizosphere is recognized for harboring elevated concentrations of such bacteria compared to bulk soil **(Lahsini *et al.*, 2022; Vurukonda *et al.*, 2016)**. Within this study, the three studied isolates demonstrated the capability to solubilize insoluble phosphate. These findings align with previous research identifying olive rhizobacteria as phosphate solubilizers **(Lahsini *et al.*, 2022)**. Rhizobacteria enhance plant growth by sequestering iron (Fe^{3+}) that is available in the environment. They accomplish this by synthesizing iron-chelating compounds known as siderophores. These siderophores assume a crucial role in the biological regulation of soil-borne pathogens, which entails limiting the accessibility of iron to these pathogens, thereby impeding their growth **(Hakim *et al.*, 2021)**. Within this investigation, all isolates were confirmed to produce siderophores both types, hydroxamate and catecholate. In addition, hydrolytic enzymes, encompassing proteases and lipases, play an essential role in preventing plant diseases by disintegrating pathogenic microorganisms in close proximity to the plant **(Barbosa *et al.*, 2020; Rasool *et al.*, 2021)**. In this context all of the studied isolates exhibited positive production for both proteases and lipases.

The OZ-48, OZ-60 and OZ-77 PGPR strains synthesizing these enzymes have displayed the highest biocontrol capabilities against phytopathogenic fungal, *V. dahliae* Klebahn. This soil-borne phytopathogen of *V. dahliae* hinges upon the generation and establishment of robust mycelia capable of infiltrating the host plant's protective cuticle **(Acharya *et al.*, 2020)**, resulting in diminished productivity and economic losses. Various control tactics, including crop rotation, utilization of resistant plant varieties, and soil amendments, have shown restricted effectiveness **(Depotter *et al.*, 2016)**. Thus, the utilization of PGPR offers a natural biocontrol approach to managing these phytopathogen, thereby reducing the reliance on chemical fungicides that may contribute to soil infertility. Importantly, the current isolates exhibited robust inhibitory effects on *V. dahliae*, displaying the highest inhibition rates either in direct diffusion or VOC assays.

Table 2 Locations, geographical coordinates and 16S rRNA sequence analysis for the 24 rhizobacterial isolates.

Isolate	Location			Closest reference strains	Accessions	Ident. %
	(M)	(N)	(W)			
OZ-14	687	30º 15,311'	5º 40,956'	*B. licheniformis ATCC 14580 T*	AE017333	99
OZ-44				*B. licheniformis ATCC 14580 T*	AE017333	99
OZ-45				*B. cabrialesii TE3 T*	MK462260	100
OZ-48				*B. licheniformis ATCC 14580 T*	AE017333	100
OZ-56				*B. paralicheniformis KJ-16 T*	KY694465	99
OZ-60				*B. paranthracis Mn5 T*	MACE01000012	99
OZ-62				*B. licheniformis ATCC 14580 T*	AE017333	99
OZ-66				*B. licheniformis ATCC 14580 T*	AE017333	99
OZ-5	692	30º 15,099'	5º 40,984'	*B. licheniformis ATCC 14580 T*	AE017333	100
OZ-12				*B. rugosus SPB7 T*	JABUXO010000041	100
OZ-76	690	30º 15,441'	5º 41,497'	*B. paralicheniformis KJ-16 T*	KY694465	100
OZ-77				*B. licheniformis ATCC 14580 T*	AE017333	99
OZ-80				*B. licheniformis ATCC 14580 T*	AE017333	99
OZ-81				*B. cabrialesii TE3 T*	MK462260	100
OZ-86				*B. cabrialesii TE3 T*	MK462260	100
OZ-87				*B. paralicheniformis KJ-16 T*	KY694465	99
OZ-90				*B. licheniformis ATCC 14580 T*	AE017333	99
OZ-92				*B. licheniformis ATCC 14580 T*	AE017333	99
OZ-94				*B. licheniformis ATCC 14580 T*	AE017333	99
OZ-15	699	30º 15,385'	5º 41,490'	*B. licheniformis ATCC 14580 T*	AE017333	99
OZ-18				*Priestia endophytica 2DT T*	AF295302	99
OZ-69				*B. licheniformis ATCC 14580 T*	AE017333	99
OZ-71				*B. licheniformis ATCC 14580 T*	AE017333	99
OZ-72				*B. licheniformis ATCC 14580 T*	AE017333	99

Legend: M – Altitude, N – Latitude, W: Longitude

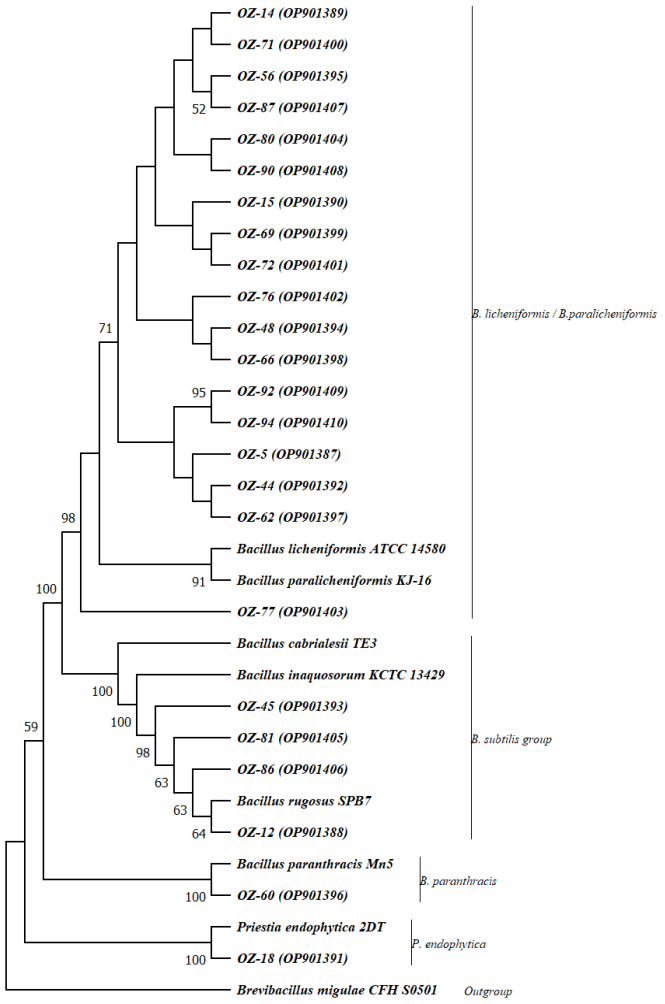


Figure 4 Phylogram representing the phylogenetic tree of the 24 rhizobacterial isolates based on 16S rRNA sequences. Maximum likelihood method was used, with bootstrap at 1000 repetitions. GenBank accession numbers are given in parentheses and *Brevibacillus migulae* was used as outgroup.

CONCLUSION

Our study has illuminated the dynamic presence of highly active rhizobacterial communities within the olive tree rhizosphere. The three selected strains have been emerged as exemplars distinguished by their pronounced repertoire of abiotic stress tolerance, PGP attributes and antagonistic activity. This environmentally friendly approach is aimed at assisting in combating abiotic stress and pathogenic agents. They have potential as novel indigenous rhizobacterial isolates for an eco-friendly approach with prospective applications in improving the sustainability of olive cultivation. However, further studies utilizing the selected strains as inoculants to verify their efficacy *in situ* are worthwhile.

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