IMPACT OF THE RHIZOSPHERE BACTERIUM *STENOTROPHOMONAS PAVANII* ON *CURCUMA LONGA*'S GROWTH, YIELD, AND CURCUMINOID BIOSYNTHESIS EXPRESSION

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Abstract. *Curcuma longa* (Turmeric) is a spice and culinary ingredient that is high in antioxidants and utilized in medicine. It has been demonstrated that applying beneficial plant growth-promoting rhizobacteria (PGPR) may alter the composition of bioactive compounds in a variety of plant species and increase plant growth and production. Using a variety of plant measurements and gene expression studies, this study investigated the impact of *Stenotrophomonas pavanii* on the growth, yield, and chemical composition of *C. longa*. This bacterium was isolated from the rhizosphere of *C. longa* and described by means of 16S rRNA genomic sequencing. Real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to focus on the transcription patterns of curcuminoid-related genes Curcumin synthase 1, -2, and -3 (CURS-1, -2, and -3) and diketide-CoA synthase (DCS). Compared to the control treatment, *S. pavanii* significantly increased the plant's height, the number and dry weight of *C. longa* leaves and roots, and the number of rhizomes and rhizome dry mass. Treatment with *S. pavanii* resulted in a significant rise in carotenoid and chlorophyll b levels, as well as a positive effect on the nitrogen (N) and potassium (K) contents in the leaves of *C. longa*. Moreover, the contents of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in *C. longa* rhizome considerably increased by approximately 1.86-, 1.69-, and 1.77-fold, respectively, in comparison to the control treatment. Differential upregulation of curcuminoid-related genes upon application of *S. pavanii* led to an increase in the concentration of curcuminoid compounds, including bisdemethoxycurcumin, demethoxycurcumin, and curcumin. The aforementioned discoveries illuminate the possibility of *S. pavanii* functioning as a beneficial plant growth-promoting rhizobacterium to augment the development, yield, and bioactive composition of *C. longa*. Moreover, the results offer a substantial understanding of the molecular mechanisms underlying these advancements. This study determined the feasibility and advantages of employing biofertilizers based on microbial compost as a possible alternative to chemical fertilizers.
Keywords: *effect compound, turmeric, microbial, HPLC, biofertilizers, qRT-PCR*

Introduction

Agriculture plays a crucial role in global economies, contributing to poverty eradication, industrialization, diversification of the economy, efficient, sustainable resource utilization, and environmental protection. Since ancient times, agriculture has been essential to human civilizations. However, overuse of chemical pesticides and fertilizers is lowering the soil and groundwater quality, endangering human health and plant growth. Sustainable methods of agriculture and mitigation strategies can help to reduce contamination, boost crop output, and preserve environmental habitats (Srivastav, 2020).

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Microbial bio-fertilisers play a pivotal role in sustainable agriculture as many microorganisms in agricultural soil are helpful to the soil's qualities as well as plants. Food safety and environmental concerns have led to increased interest in biofertilizers, biopesticides, and bioherbicides (Ahirwar et al., 2020). Microbiological achievements have become a powerful tool for improving sustainable agriculture and environmental health. The production of sustainable agriculture necessitates a combined use of microorganisms that promote plant growth while preventing soil degradation. Microorganisms guarantee the availability of essential nutrients and the effectiveness of their utilization (Ahirwar et al., 2020). Biofertilizers are compounds that are applied to the seeds, soil, or surfaces of plants that comprise living organisms. They enhance the availability of primary nutrients, which in turn promotes plant growth. These microorganisms stimulate microbial activity, which raises the availability of nutrients that are easier for plants to absorb. Crop yields are increased by using organic fertilizers, which enhance soil fertility and natural nutrient uptake (Akram, et al., 2020) Biofertilization is a fertilization technique that is used as a substitute for chemical fertilization and helps prevent pollution in the environment (Kurniawati et al., 2023). Although chemical fertilizers help to increase agricultural output and soil fertility, they can be hazardous to the crops as well. The immoderate use of chemical fertilizers such as nitrogenous, phosphate, and potassium can cause soil hardening, diminished fertility, and air, water, and soil pollution. Microbial activity in the cropping system is influenced by the sole reliance on chemical fertilizers (Pahalvi et al., 2021). Continuous application of chemical fertilizer can change the pH of the soil, rendering it vulnerable to pests, promoting acidification, and creating soil crust. These results lead to a drop in beneficial organisms, humus and organic matter levels, inhibition of plant growth, and even greenhouse gas emissions (Pahalvi et al., 2021).

In agriculture production, one crucial aspect of biological fertilization is phytostimulant-associated plant growth-promoting bacteria (PGPB). These bacteria have a variety of functions, including active participation in nutrition availability, biocontrol properties, and biochemical cycle disruption (Ramakrishna et al., 2019; García-Trejo et al., 2021). Specifically, soil bacteria that infiltrate plant roots and participate significantly in plant growth are commonly known as plant growth-promoting rhizobacteria (PGPR). PGPR are bacteria that live freely to connect with the origins of plant and stimulate plant growth (de Andrade et al., 2023). The search for biological sources to utilize for fertilization has lately spread as one of the most essential approaches to identify types of bacteria that grow near the roots of specific plants, characterizing them, and reusing them in biological fertilization (Abedinzadeh et al., 2019; López Restrepo et al., 2020; Saeed et al., 2021). PGPR can directly affect plant metabolism, enhance root development, and suppress pathogens. They also indirectly safeguard plants through nutrient competition, biocontrol of microbes and systemic response promotion. Direct stimulation promotes the development of plants by providing compounds or facilitating nutrient uptake, while indirect promotion prevents harmful effects (Beneduzi et al., 2012). Members of the bacterial genus Stenotrophomonas, belonging to the family Xanthomonadaceae, are recognized as promising PGPR due to their ability to produce spermidine, phytohormones, and siderophores and facilitate phosphate solubilization (Cutiño-Jiménez et al., 2020; Ulrich et al., 2021). Stenotrophomonas bacteria, particularly *Stenotrophomonas pavanii*, are gaining increasing attention in research because of their prospective usage as effective bioinoculants for boosting plant growth and treating many food crop diseases (Kumar et

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al., 2023). *S. pavanii* is a rod-shaped Gram-negative bacterium that does not generate spores. It is a separate genus of PGPR that is thought to enhance crop growth and increase yield by fixing atmospheric nitrogen (Chowdhury and Sengupta, 2020; Feng et al., 2023; Reed and Glick, 2023).

Curcuma longa L. is the scientific name for turmeric, an evergreen herbaceous plant in the Zingiberaceae family (Ahmad et al., 2010). Rhizomes, the part of the plant that is used by humans, contain a variety of substances, including bioactive, non-volatile curcuminoids and volatile oil molecules (Itokawa et al., 2008; Sharifi-Rad et al., 2020). *C. longa* is a plant with many therapeutic uses, such as blood-purifying, hepatoprotective, antioxidant, anti-inflammatory, antitumor, antiprotozoal, stomachic, and carminative effects. It protects the heart and arteries, lowers high plasma cholesterol, and shields lymphocyte DNA from oxidative damage. Curcuminoids, especially curcumin, are the main bioactive ingredients in turmeric. They have anti-inflammatory, anticancer, anti-acidogenic, radioprotective, and neuroprotective qualities (Amalraj et al., 2017; Sharifi-Rad et al., 2020). Curcumin synthase 1, -2, and -3 (CURS-1, -2, and -3) and diketide-CoA synthase (DCS) are enzymes involved in the metabolism of curcuminoid substances in *C. longa*. Diketide-CoA is a precursor molecule formed by DCS and serves as the curcuminoid building block. CURS1, CURS2, and CURS3 convert diketide-CoA into specific curcuminoid compounds, leading to the production of bisdemethoxycurcumin, demethoxycurcumin and curcumin DCS, CURS1, CURS2, and CURS3 genes were discovered to be involved in the process of curcuminoid metabolism in *C. longa* (Katsuyama et al., 2009a; Katsuyama et al., 2009b; Sandeep et al., 2017; Chakraborty et al., 2021) Studying the genes encoding enzymes involved in curcuminoid metabolism has a major impact on agricultural practices, pharmaceutical applications, and the creation of enhanced turmeric cultivars. In order to support the sustainable production of essential phytochemicals, it is helpful to understand the regulation and expression patterns of these genes as they provide an insight into the molecular mechanisms underpinning the synthesis of bioactive compounds in medicinal plants.

This article summarizes our research on the impact of the bacterium *S. pavanii*, which we isolated from the rhizosphere of *C. longa*, on the plant species' development, yield, and production of curcuminoid compounds. To explore the molecular mechanisms behind the influences of *S. pavanii* treatment, real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) was utilized to study the expression patterns of genes involved in curcuminoid synthesis in the turmeric rhizomes. The viability and benefits of using microbial compost-based biofertilizers as a potential substitute for chemical fertilizers were assessed in this study.

Materials and methods

Separation and description of S. pavanii

Collection of soil samples

In June 2022, soil samples were gathered from the uprooted portion of *C. longa* plants grown on the King Faisal University farm, Saudi Arabia, from a depth of 30 cm (three replicates from different places). The samples were then transferred to a sterile petri dish and securely delivered to a microbiology laboratory in the Department of

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Biological Science, Faculty of Science, King Faisal University. For future research, the soil adhering to the root sections was carefully removed and kept at 4°C.

Physiochemical property determination of the soil

To ascertain the physiochemical parameters, soil samples were tested. The method defined in Martin et al. (2013) was used to calculate the pH of the soil. EC of each soil sample was determined using the method described by Richards (1954). In a hot-air oven set to 105°C, the sample's moisture content was measured once it reached a stable weight. A thermometer and a hydrometer were used to measure the temperature and humidity (de Andrade et al., 2023).

Bacterial isolation

A serial dilution approach was used to identify the soil microorganisms on nutrient agar medium (N.A.M.). Separately, one gram of the sample soil was suspended in ten milliliters of distilled water, thoroughly agitated for 15 min, and then vortexed. From 10^{-1} to 10^{-6}, each suspension was serially diluted. The spread plate technique was used to separate the microorganisms from the diluted sample. A glass L-shaped rod was used to spread 0.1 ml of the solution onto nutrient agar plates, which were then incubated for 24 h at 37°C. The most notable colonies were separated and kept at 4°C to conduct more research (Gaete et al., 2020; Kannan et al., 2018).

Bacterial identification using 16S ribosomal RNA gene sequencing

Inoculation of isolates

Every bacterial isolate was cultured in five-milliliter tubes with two milliliters of Luria broth (L.B.). During the overnight incubation period, the isolate-containing tubes were shaken horizontally at 37°C in an incubator shaker (Lab-line Instruments, I.N.C.). Two milliliters of L.B. medium was centrifuged for 5 min at 5000 rpm to precipitate bacterial pellets for DNA extraction. The most prominent colonies were chosen based on distinct observable traits. These criteria included colony morphology, including as size, color, shape, texture, and growth patterns on the agar media. Other factors to examine were opacity or translucence, edge definition, and pigmentation intensity. Colonies with distinguishing or distinctive morphological characteristics were prioritized to ensure a representative spectrum of phenotypic variety. Furthermore, the selected colonies were stained with Gramme stain (Bartholomew and Mittwer, 1952) and then inspected under a microscope to determine if they were Gram-positive or Gram-negative, allowing for further study.

DNA extraction and polymerase chain reaction (PCR)

DNA was extracted from the bacteria using the protocol described by Dellaporta et al. (1983). The universal primers 27F 5′-AGA GTT TGA TCM TGG CTC AG-3′ and 1492R 5′ TACGGYTACCTTGTTACGACTT were used to intensify the 16S rRNA gene from bacterial isolates according to the method described in Weisburg et al. (1991). The purified PCR products were sequenced by Macrogen Inc. (Korea) using 27F and 1492R primers in both directions. The arrangements of the sequences were edited by MEGAX.

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Evolutionary relationships of taxa

Using the Neighbor-Joining approach, the evolutionary history was deduced (Kumar et al., 2018). The ideal tree is showcased. The percentage of duplicate trees, when related taxa clustered together in the bootstrap test (100 recurrences), is displayed above the branches (Saitou and Nei, 1987). Using the same units as the evolutionary distances that were used to build the tree, the branch lengths (next to the branches) in the phylogenetic tree are displayed to scale (Felsenstein, 1985). The evolutionary distances were estimated using the maximym composite likelihood technique with regard to based replacements per site. There were twelve nucleotide sequences in this investigation. Locations that were partially erased allowed under ten percent of alignment gaps, missing data, and uncertain foundations at any position; all places with less than 90% site coverage were removed. The final dataset contained 420 locations in total. In MEGA11, evolutionary analyses were carried out (Tamura et al., 2004).

Resources and growth requirements for plants and microorganisms

Daughter rhizomes of turmeric with one or two buds (40 g) (Narendhiran et al., 2024) were surface sterilized for 5 min using a 5% (v/v) sodium hypochlorite solution to prevent fungal attack. Following that, they were subjected to three rounds of sterile water washing. Turmeric rhizomes were collected from the King Faisal University farm. Next, for 12 h at 25°C, the samples were submerged in an *S. pavanii* solution with an optical density of 600 nm (OD600). Optical density were measurement using a NanoDrop^{TM} 2000c spectrophotometer (Thermo Fisher Scientific) at a wavelength of 600 nm (Tamura et al., 2021). Rhizomes that were soaked in distilled water served as the control treatment. Turmeric rhizomes were planted in sand-filled germination trays in a greenhouse (the temperature ranged from 32 to 36°C, the relative humidity was 47–56%, and the photoperiod was 14 h) on April 1, 2022. After one month, the seedlings were moved into 20 cm diameter plastic containers with a depth of 15 cm and 4.5 kg of sand per pot (*Table A1*) (one plant in each pot) in the same greenhouse as before, and the percentage of germinated rhizomes was measured. The trials used a fully randomized block design with 15 replicas (pots), two treatment groups of *S. pavanii*, and distilled water as the control. Throughout the experimental period, plants were irrigated with groundwater (*Table A2*). Various agricultural activities, such as weeding, were performed as recommended. Hu's (2019) method was used to identify the soil and irrigation water components (*Tables A1* and *A2*). Following 240 days of cultivation, the whole plant was harvested; ten randomly chosen plants from each treatment were used to measure the plant's height (cm); the number of roots, leaves, and rhizomes on each plant (n); the dry mass of these components on each plant (g); and the diameter of the rhizome (mm).

Chemical analysis

Photosynthetic pigment measurement

By measuring the third-top new leaf of four 240-day-old turmeric plants chosen at random, the amounts of photosynthetic pigments were determined. As mentioned above, carotenoids and chlorophyll a and b concentrations were extracted and quantified following the protocol described in (A.O.A.C., 1984).

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Mineral composition

After 48 h of drying at 60°C, the leaves of the plants from different treatments were treated with sulfuric acid to destroy them 240 days after the plants were planted (Piper, 1942). The nitrogen (N) content was determined by utilizing the modified micro-Kjeldahl technique (Jockson, 1967). The phosphorus (p) level (Murphy and Riley, 1962) and potassium (K) content (Mazumdarand Majumder, 2003) were measured by means of atomic absorption flame photometry and calorimetry, respectively.

High-performance liquid chromatography (HPLC) examination of the curcuminoid contents in dried C. longa rhizome ethanolic extracts

The bisdemethoxycurcumin, demethoxycurcumin and curcumin contents in air-dried *C. longa* rhizome powder from three randomly selected plants in each treatment (control and *S. pavanii*) were determined using a Waters 2690 Alliance HPLC system (Waters, Milford, MA, U.S.A.) equipped with a Waters 996 photodiode array detector (Waters, Milford, MA, U.S.A.) and a C18 Inertsil column (4.6 mm x 250 mm, 5 um). The analysis was performed by following the protocols specified by Al Dayel and El Sherif (2022).

Real-time RT-PCR CURS1, -2, -3, and DCS analysis and gene expression

Curcuminoid gene transcript levels (*Table A3*) were measured in *C. longa* rhizomes from four 240-day-old plants chosen at random in each experimental sub-group using real-time RT-PCR, using techniques outlined by Al Dayel and El Sherif (2022). The CURS-1, -2, and -3 as well as DCS (target) gene expressions were normalized with that of the Actin (reference) gene and quantified using the $2^{-\triangle\triangle CT}$ method (Livak and Schmittgen, 2001). Relative expressions of the CURS1, -2, and -3 and DCS genes in SAE (1-, 2-, 3 g/L) treatments were expressed as fold expressions of that of control treatment.

Statistical evaluation

Ten repetitions of a fully randomized block design were employed in the experiment. The data from all measurements were analyzed using the Statistica 6 software Statistica 6 program analysis of variance/multivariate analysis of variance ANOVA/MANOVA (StatSoft, 2001). The mean difference between the treatment groups was evaluated at a probability level of $p = 0.05$. Duncan's test was employed to evaluate the significance of mean differences.

Results

Identifying bacterial strains and physiochemical properties of soil isolates

Strain isolation and molecular characterization

The physiochemical properties of the soil samples were investigated. The pH of the soil was approximately 6.5. This pH level in the soil improves nutrient availability. The soil had a moisture content of 45.8% and a temperature of 32°C. The temperature benefits the plants by increasing nutrient availability, and the moisture content fosters the growth of microorganisms that indirectly support plant growth. The entire 16S rDNA gene was amplified and sequenced in order to taxonomically identify isolated

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bacteria. These sequences were used to create the phylogenetic tree (*Fig. 1*). It was determined that the Gram-positive, rod-shaped, non-spore-forming bacterium originated from the soil. The identification of *S. pavanii* was achieved via molecular characterization. By utilizing the closely related 16S rDNA sequences, a neighbor-joining phylogenetic tree for *S. pavanii* was built (*Fig. 1*).

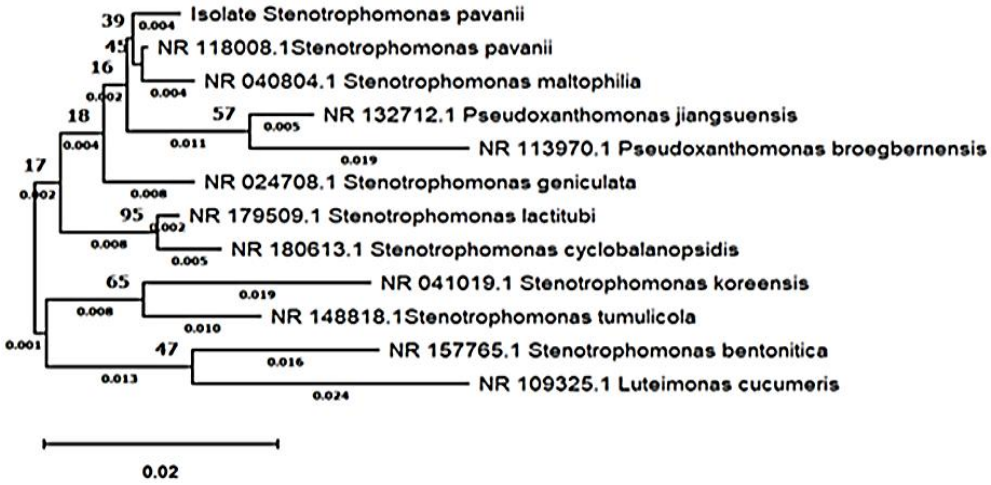

Figure 1. *Depiction of a phylogenetic tree constructed using the neighbor-joining method*

Influence of S. pavanii on the development and productivity of plants

Table 1 displays the corresponding vegetative growth measures. Compared to the control treatment, applying *S. pavanii* significantly enhanced the plants' height, the number of leaves and roots and the dry mass of leaves and roots, and the dry mass of *C. longa* leaves and roots (*Table 1*). The data in *Figure 2* show that the *S. pavanii* treatment considerably increased the number of rhizomes and rhizome dry mass (-1.4- and -1.3-fold, respectively) compared to the control group and these increments were significantly increased according to Duncan's test. However, the rhizome diameter decreased after treatment with *S. pavanii* (*Fig. 2*).

Impact of S. pavanii on the photosynthetic pigment contents of C. longa leaves

As demonstrated in *Figure 3,* compared to the control group, *S. pavanii* treatment significantly increased the amount of carotenoid and chlorophyll b in *C. longa*. In contrast, the highest value of chlorophyll a (chl a) was found in the control treatment (*Fig. 3*).

Table 1. *Impact of S. pavanii treatments on C. longa, including plant height (cm), leaf and root count (n), and dried mass (g) of leaves and roots*

Treatments	Plant height (cm)	No. of leaves (n)	No. of roots (n)	Root length (cm)	Dried weight of roots (g)	Dried weight of leaves (g)
Control	121.67 b	9.67 b	30.67 b	15.67 b	1.73 b	17.77 b
S. pavanii	127.00 a	11.25 a	33.25 a	17.25 a	2.78 a	21.40 a

Values with the same letter within a column are not significantly different at the 0.05 level of probability, as per Duncan's test

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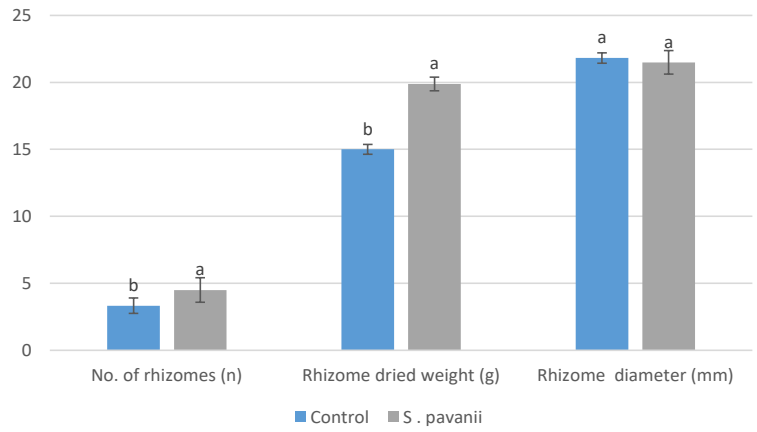

Figure 2. *Impact of S. pavanii treatments on number of rhizomes (n), dried weight of rhizomes (g), and rhizome diameter (mm) of C. longa*

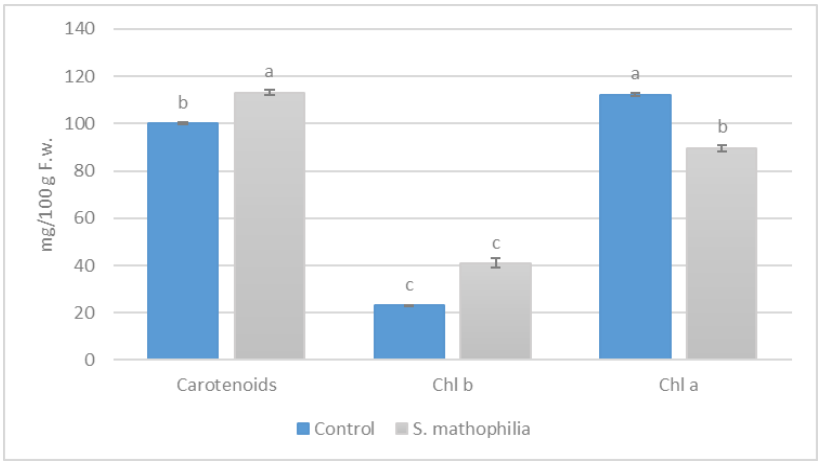

Figure 3. *Influence of S. pavanii treatments on the concentrations of chlorophyll a (chl a), chlorophyll b (chl b), and carotenoids in the leaves of C. longa*

Influence of S. pavanii on the nitrogen, phosphorus, and potassium contents of C. longa leaves

The data in *Table 2* show how the concentrations of nitrogen (N), phosphorus (P), and potassium (K) in *C. longa* leaves were influenced by *S. pavanii*. The treatment with *S. pavanii* positively impacted on the N and K contents, and these improvements were significant according to Duncan's test. The highest contents of P were observed in the control treatments, but this increase was not significant Duncan's test.

Table 2. *Effects of S. pavanii treatments on nitrogen (N), phosphorus (P), and potassium (K) percentages in C. longa leaves*

Treatments	K (ppm)	P (ppm)	N (ppm)
Control	1.9014 b	0.1421 a	11.097 b
S. pavanii	1.9434 a	0.1409 a	11.585 a

*Values with the same letter within a column are not significantly different at the 0.05 probability level, as per Duncan's test

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Influence of S. pavanii on curcuminoid contents of C. longa rhizomes

The *S. pavanii* treatment significantly increased the bioactive components present in the methanolic extracts of *C. longa* rhizome, as assessed by means of HPLC, increasing the contents of curcumin, demethoxycurcumin, and bisdemethoxycurcumin by approximately 1.86-, 1.69-, and 1.77-fold, respectively, in contrast to the control treatment (*Figs. 4* and *5*).

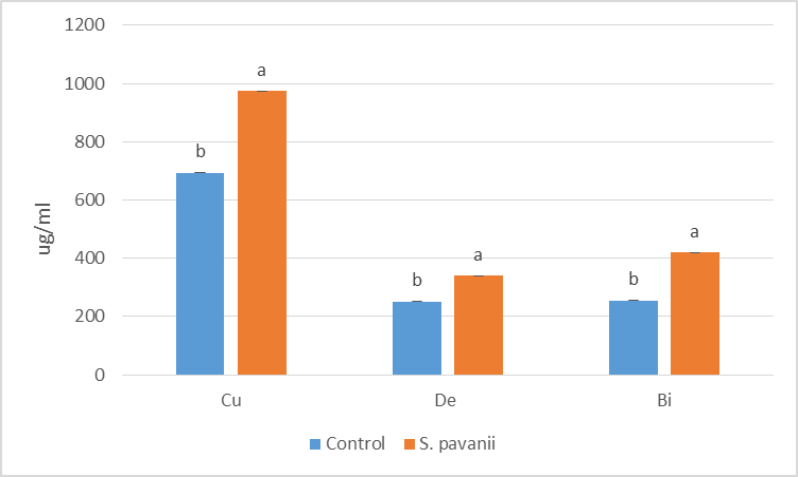

Figure 4. *Effects of S. pavanii treatments on curcumin (Cu), demethoxycurcumin (De), and bisdemethoxycurcumin (Bi) (ug/ml) contents of C. longa*

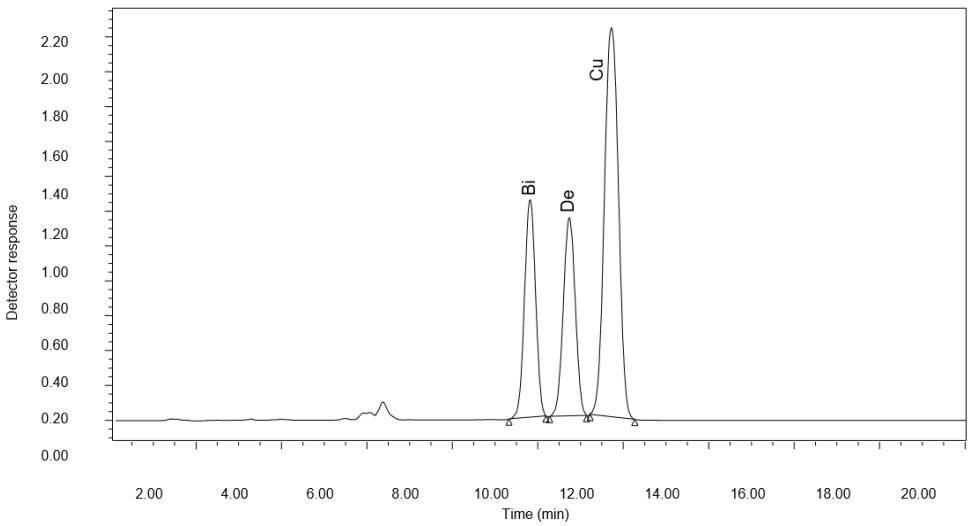

Figure 5. *HPLC chromatogram depicting the organic extracts of C. longa following exposure to S. pavanii. The chromatogram displays peaks corresponding to distinct curcuminoid compounds, namely, bisdemethoxycurcumin (Bi), demethoxycurcumin (De), and curcumin (Cu)*

Influence of S. pavanii on expressions of Curcumin synthase 1, 2, 3, and diketide-CoA synthase genes

Curcuminoid gene expression levels were assessed via real-time PCR in *C. longa* rhizomes treated with *S. pavanii* and control measures. In contrast to the control

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treatment, the *C. longa* rhizome treated with *S. pavanii* had greater levels of gene expression for CURS1, -2, -3, and DCS, according to the data shown in *Figure 6*. The genes expression value were significantly increased confirmed by statistical analysis using Duncan's test.

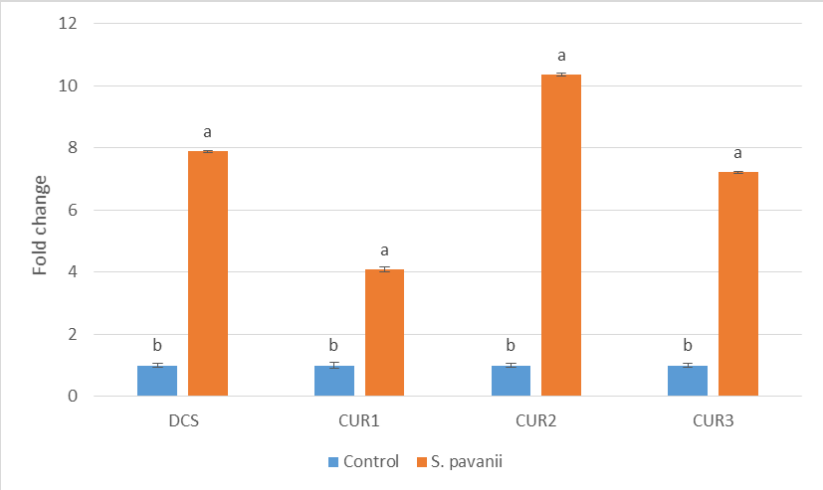

Figure 6. *Profiling the variation in the expression of curcuminoid synthase genes, namely, Curcumin synthase 1, -2, and -3 (CURS-1, -2, and -3) and diketide-CoA synthase (DCS), in the rhizomes of C. longa under both the control and S. pavanii-treated conditions. Data were normalized using actin as an internal reference gene*

Discussion

Organic, natural, and chemical-free foods are becoming increasingly popular. It is critical to screen microbial species for vital plant growth-promoting qualities in order to build viable biofertilizers that benefit agriculture, the environment, and farmers' economies (Abdel-Hamid et al., 2021; Suppakul et al., 2003). In this study, *S. pavanii* was isolated from the uprooted soil of *C. longa* plants and characterized using molecular characterization based on morphology, biochemical properties, and 16S rRNA genome sequence assessment. The effects of *S. pavanii* on turmeric plant growth, production, phytochemical content, and curcuminoid gene expression patterns were studied. The application of *S. pavanii* according to Duncan's test significantly increased plant height, number of roots and leaves, and dry mass of leaves and roots compared to the control treatment. The number of rhizomes and rhizome dry mass also increased with *S. pavanii* treatment, however, the diameter of the rhizome reduced, which was not significant and did not affect the rhizome yield. Significant differences between the control and *S. pavanii* treatments were confirmed by statistical analysis using Duncan's test. In contrast to the control, the *S. pavanii* treatment greatly raised the levels of carotenoid and chlorophyll B (Chl B) in the leaves of *C. longa*, with the control treatment showing the greatest value of Chl A. While the phosphorus content of *C. longa* leaves did not significantly change, the *S. pavanii* treatment had a favorable effect on the nitrogen and potassium contents. When compared to the control treatment, the *S. pavanii* treatment substantially raised the levels of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in the rhizomes of *C. longa*. When *S. pavanii* was applied to *C. longa* rhizomes, real-time PCR analysis showed that the expression levels of CURS1, CURS2, CURS3, and DCS genes were higher than in the control treatment. For instance,

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in various plant species, isolation and bacterial features employing morphology, biochemical properties, and 16S rRNA gene sequence analysis have been described, for example, *Curcuma longa* rhizome (Montesdeoca-Flores et al., 2023), *Thymus vulgaris* (Kumar et al., 2016) *Ipomoea batatas* (Abdel-Hamid et al., 2021) and *Saccharum officinarum* (Dawwam et al., 2013). In comparison to the control treatment, *S. pavanii* treatment uplifted plant developmental metrics, rhizome dry mass, rhizome production and chlorophyll B and carotenoid contents in *C. longa* plants as well as N and K accumulation on *C. longa* leaves. *S. pavanii* is a distinct genus of PGPR (Ramos et al., 2011) which is believed to work on growth enhancement and yield increasing of different crops and can fix atmospheric nitrogen (Chowdhury, 2020; Ghavami et al., 2017; Ramos et al., 2011).

In a study, researchers found that PGPR can improve phytoremediation, a process of using plants to clean up contaminated soil. They tested 50 bacterial isolates from polluted sites, finding that 35 showed positive traits for plant growth promotion, and many could break down pollutants. Seven promising bacteria, like Nocardia sp. and Stenotrophomonas pavanii, significantly improved canola plant growth in contaminated soil (Rahma et al., 2023). Another study investigates the effectiveness of *S. pavanii* on the growth of sunflower plants. The study investigates the ability of a bacterium isolated from sunflower roots, Stenotrophomonas indicatrix BOVIS40, to support plant growth and provide stress resistance. The genetic data of the bacteria indicates genes involved in the breakdown of organic compounds, the creation of biofilms, and the synthesis of plant hormones. According to the research, it could potentially function well as a bioinoculant in agriculture (Alotaibi et al., 2022).

Moreover, PGPR modifies the physiology of plants as well as the nutritional and physical characteristics of rhizospheric soil. It has been observed that rhizobacteria use proton pump ATPase to enhance the uptake of nutritional elements such as Ca, K, Fe, Cu, Mn, and Zn (Adeleke et al., 2021). Since plants release root exudates that contain sugars, growth regulators, amino acids, organic acids, flavonoids, enzymes, fatty acids, and vitamins, PGPR bacteria are extensively distributed throughout the rhizosphere and rhizoplane (Mantelin and Touraine, 2004). Through nitrogen fixation, the production of chemicals such as indole acetic acid, siderophores, organic acids, and exopolysaccharides, as well as the solubilization of phosphorus and other elements to increase micronutrient intake, PGPB support agricultural growth and yield (Gopalakrishnan et al., 2016). The *S. pavanii* treatment greatly raised the amounts of curcuminoid substances, according to HPLC analysis of these compounds. The impact of PGPR bacteria on secondary metabolite synthesis in medicinal plants has been evaluated before (Dawwam et al., 2013; Nguyen et al., 2023; Rat et al., 2021).

RT-PCR amplification and HPLC results revealed a link between curcuminoid gene expression and curcumin production in *C. longa*. The current study's findings, which demonstrated concurrent upregulation of the genes encoding curcuminoid synthase (CURS1, -2, -3, and DCS) and the composition of curcumin, bisdemethoxycurcumin, and demethoxycurcumin, are in line with earlier research that demonstrated the relationship between coordinate genes' activity in response to specific evokers and the accumulation of major secondary metabolic products in plants (Al Dayel and El Sherif, 2022; El Sherif et al., 2022; Khattab et al., 2023).

The regulation of the DCS, CURS1, -2, and -3 genes remain unclear. Different curcuminoid content in turmeric rhizome does not always lead to favorable impacts on DCS and CURS expressions. DCS expression is positively associated with

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curcuminoids, but CURS3 expression is negatively associated with them. CURS1 and CURS2 are also involved in curcumin and demethoxycurcumin production (Ayer et al., 2020; Chakraborty et al., 2021). This process of co-expression of diketide-CoA synthase and numerous curcumin synthases in turmeric rhizome has a major impact on curcuminoid balance across turmeric cultivars and *C. longa* strains may affect the generalizability of the findings.

This study is novel since it focused on the relationship between *S. pavanii* and turmeric plants, investigated the expression of curcuminoid genes, and examined plant growth and the phytochemical content in significant detail. The study investigates *S. pavanii's* capacity to stimulate plant growth, particularly concerning its interactions with *Curcuma longa* plants. The study fills in several research gaps and advances the understanding of how to promote plant growth with the help of microbes. The study explores how *S. pavanii* interacts with turmeric plants, with a focus on turmeric's economic and therapeutic importance. A thorough evaluation of plant growth is also provided in the study, stressing the potential of *S. pavanii* for both environmentally friendly and sustainable agricultural operations, as well as for use as a bioinoculant for turmeric plants. The study focuses on a single strain, *S. pavanii*, from *Curcuma longa*'s rhizosphere, which may not contribute to the complete knowledge of microbial contributions to plant growth. Further research could include looking into field applications of *S. pavanii* to confirm its efficacy outside of controlled settings, investigating its molecular interactions with *C. longa* in the field, and examining its effects across different *C. longa* genotypes to determine broader applicability.

Conclusions

According to our findings, *S. pavanii* is an effective biofertilizer that can boost the production and growth of rhizomes from *C. longa* plants. It was demonstrated that *S. pavanii* changes the chemical composition of bioactive compounds while simultaneously boosting plant growth and yield in *C. longa*. Applying *S. pavanii* to the rhizome of *C. longa* increased the plant's nitrogen content, growth, yield, and concentration of photosynthetic pigments. In this study, it was demonstrated that *S. pavanii* treatments affected the CURS1, -2, -3, and DCS genes differently. The curcuminoid content rose in plants that had been treated with *S. pavanii* in a way that related to the curcuminoid gene's expression level. This study primarily relied on controlled laboratory or greenhouse conditions that incompletely replicate the complexities of field environments. This study identified a positive effect on growth and curcuminoid biosynthesis, but detailed insights are lacking into the effects exerted at the molecular level. Variability in the *C. longa* genotype affects the generalizability of these results to anything other than the specified genotype.

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APPENDIX

Table A1. *Physical and chemical properties of the experimental soil*

Characteristic	Value
Texture	Sandy
Sand %	91.51
Silt %	5.74
Clay %	2.75
Saturation %	23
pH	7.5
Electrical conductivity (EC) (dS/m)	2.2
Organic matter (OM) %	0.05
Total N %	0.014
Available P ppm	3.9
Available K ppm	110

Table A2. *Chemical properties and compositions of the irrigation water*

Salinity level (ppm)	Cations (meq/L)				Anions (meq/L)				Sodium adsorption ratio (SAR)
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	SO_4^{2-}	Cl^{-}	
864	5.72	2.02	7.27	0.38	0.28	2.68	4.03	8.4	3.43

Table A3. *Sequences of forward and reverse primers for real-time RT-PCR*

Gene	Primers sequence	Amplicon length (bp)	GenBank accession number	References
Diketide-CoA synthase (DCS)	5'- GTGCTGTTCATCCTGGACGAG -3' (forward primer)	21	AB495006.1	(Katsuyama et al. 2009a, b)
	5'- CAACAGCACGCCCCAGTCGA-3' (reverse primer)	20		
Curcumin synthase 1 (CURS1)	5'- CATCATTGACGCCATCGAAGC -3' (forward primer)	21	AB495007.1	(Katsuyama et al. 2009a, b)
	5'- TCAGCTCATCCATCACGAAGTACAC -3'(reverse primer)	25		
Curcumin synthase 2 (CURS2)	5'-TCGGGATCAAGGACTGGAACAAC-3' (forward primer)	23	AB506762.1	(Katsuyama et al. 2009a, b)
	5'-TGTTGCCGAACTCGGAGAAGAC-3' (reverse primer)	22		
Curcumin synthase 3 (CURS3)	5'-TGGAGCCCTCCTTCGACGACC-3' (forward primer)	21	AB506763.1	(Katsuyama et al. 2009a, b)
	5'-CCCATTCCTTGATCGCCTTTTCC-3' (reverse primer)	23		
Actin	5'-GGATATGCTCTTCCTCATGCT-3' (forward primer)	21	CP002686.1 AK118354.1 AY087740.1	(Katsuyama et al. 2009a, b)
	5'-TCTGCTGTGGTGGTGAATGA-3' (reverse primer)	20		

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