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Characterisation of *Trichoderma* spp. and rhizobacteria strains isolated from the rhizosphere of strawberry (*Fragaria* × *ananassa* Duch.) in the Menoua Division, Western Cameroon

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ABSTRACT

Grey mould, caused by *Botrytis cinerea* Pers., is the most devastating fungal disease of strawberry (Fragaria × ananassa Duch.), causing yield losses of 50–80% under favourable conditions. Chemical fungicides remain the predominant control strategy, however, they drive the emergence of resistant strains and raise serious environmental, and food safety concerns. Biological alternatives based on plant growth-promoting rhizobacteria (PGPR) and *Trichoderma* spp. represent ecologically sound options with proven efficacy. This study aimed to isolate and biochemically characterizes indigenous microbial strains from the strawberry rhizosphere. To evaluate their plant growth-promoting (PGP) traits, namely indole-3-acetic acid (IAA) production and inorganic phosphate solubilization. Rhizospheric soil samples were collected from four strawberry farms in the Dschang, Western Region of Cameroon, micro orgarnisms were isolated and analysed. A total of 14 fungal and 37 bacterial isolates were obtained. All strains produced IAA and solubilized inorganic phosphate in liquid NBRIP medium. Among fungal isolates, strain D3 (*Trichoderma* spp) was the highest IAA producer (431.6 ± 34.5 µg/mL), while D2 (Trichoderma spp) exhibited the greatest phosphate solubilization capacity (1523.13 ± 34.7 mg/L). Within the bacterial collection, strain A6 (Coccis gram+ purple coloring) produced the highest IAA concentration (172.6 ± 51.3 µg/mL), and A5 (Coccis gram+ purple coloring) displayed the greatest phosphate-solubilizing activity (1612.49 ± 106.2 mg/L). The remarkably high phosphate solubilization values recorded across both collections reflects selective pressure exerted by ferrallitic, iron and aluminium-oxide-rich soils. These findings identify promising biofertilizer candidates, particularly strains of D3, D2, (*Trichoderma* sp) A6, A5, (Coccis gram+) for integration into sustainable strawberry production systems in Cameroon.

Key words: *Botrytis cinerea*, Plant growth-promoting rhizobacteria, *Trichoderma*, Phosphate solubilization, IAA, Biofertilizer, Strawberry

INTRODUCTION

Strawberry (Fragaria × ananassa Duch.) is one of the most economically important fruit crops worldwide, prized for its nutritional quality, sensory attributes, and significant market value.

The crop is a rich source of natural antioxidants, bioactive compounds (flavonoids, carotenoids, and phenolic acids), vitamins, and essential minerals (Giampieri *et al.*, 2012; Gramza-Michałowska *et al.*, 2019). Regular consumption of strawberries has been associated with reduced risk of non- communicable diseases, such as cancer, type 2 diabetes, cardiovascular and neurological disorders (Giampieri *et al.,* 2012). Global production of strawberries was estimated at approximately 10.49 million tonnes in 2023, with China, the United States, and Egypt as the leading producers. African production reached approximately 793,928 t, predominantly contributed by Egypt, Morocco, and South Africa (FAOSTAT, 2025).

In Cameroon, consumers demand for strawberry has increased substantially in recent years, yet local production remains insufficient to satisfy market needs. Cultivation occurs primarily in the Centre, South, and Western regions of the country. The Western and North-Western regions are characterized by temperatures ranging from 12 to 27 °C, offering the most favourable agro-climatic conditions for the developement of strawberries (Ngouana *et al.*, 2023). The crop is currently dominated by older European cultivars (Charlotte, Gariguette, Camarossa, Mara des Bois) introduced decades ago, day-neutral varieties, which extends the production season and are increasingly but unevenly adopted (Chawla and Singh, 2020; Ngouana *et al.*, 2023).

Strawberry production in Cameroon is severely constrained by both abiotic and biotic factors.

Abiotic constraints include progressive soil fertility decline, resulting from erosion and organic matter depletion, which leads to excessive reliance on synthetic fertilizers. The overuse of chemical fertilizers contributes to soil acidification, nutrient imbalances, degradation of the soil microbiome, environmental pollution, and adverse effects on human health (Krasilnikov *et al.*, 2022). A particularly relevant limitation is the strong fixation of phosphorus by iron and aluminium oxides in the ferrallitic soils prevalent in the region, rendering this essential macronutrient largely unavailable to plants despite high total soil Phosphorus concentrations (Mwende, 2019; Osman, 2013).

Among biotic constraints, grey mould, caused by the necrotrophic fungus *Botrytis cinerea* Pers. represents the most devastating disease of strawberry. The pathogen infects flowers and fruits both in the field and during post-harvest storage, causing yield losses of up to 50–80% under conditions of high humidity, moderate temperatures, and limited air circulation (Petrasch *et al.*, 2019; Maghsoodi *et al.*, 2025). Management currently relies predominantly on repeated applications of synthetic fungicides; however, this approach has promoted the emergence of multi-fungicide-resistant *B. cinerea* strains, contributes to soil and water pollution, reduces non-target biodiversity, and leaves pesticide residues on fruits (Islam *et al.*, 2024).

In this context, the development of sustainable biological alternatives is a global research priority. Plant growth-promoting rhizobacteria (PGPR) and *Trichoderma* spp. have gained considerable attention as dual-function microbial agents capable of both enhancing plant growth and suppressing soil-borne and foliar pathogens. PGPR promotes plant growth through direct and indirect mechanisms. Direct mechanisms include biological nitrogen fixation, phosphate and potassium solubilization, phytohormone synthesis (for example, IAA, cytokinins, gibberellins) and production of siderophores. Indirect mechanisms, includes induced systemic resistance (ISR), competitive exclusion, and secretion of cell-wall-degrading enzymes (Bhattacharyya and Jha, 2012; Mohanty *et al.*, 2021). Similarly, *Trichoderma* spp. act as biostimulants and biocontrol agents through mycoparasitism, antibiosis, ISR, and solubilization of soil-bound nutrients (Contreras-Cornejo *et al.*, 2009; Geng *et al.*, 2022).

The integration of indigenous PGPR and *Trichoderma* strains, adapted to local pedoclimatic conditions into

strawberry production systems could substantially reduce dependence on synthetic inputs while maintaining or improving crop yields. However, no study has characterized the plant growth-promoting potential of microbial strains isolated from the strawberry rhizosphere under the agro-ecological conditions of Cameroon.

The present study therefore aimed to: isolate and characterise fungal and bacterial strains from the rhizospheric soil of strawberry farms to identify plant growth-promoting traits, specifically IAA production and inorganic phosphate solubilization, as a basis for the selection of biofertilizer candidates for sustainable strawberry production.

MATERIALS AND METHODS

Study site and rhizospheric soil sampling

Rhizospheric soil samples were collected in June 2025 from four strawberry farms located in the Menoua Division, Western Region of Cameroon. The geographical coordinates of each sampling site were recorded using a GPS within each farm, strawberry plants were selected at random. Plants were carefully uprooted, and the tightly adhering rhizospheric soil fraction was aseptically collected into sterile polyethylene bags. Composite soil samples were prepared by pooling material from five individual plants. All samples were transported to the Research Unit for Phytopathology and Agricultural Zoology (URPHYZA) at the University of Dschang and stored at 4 °C until processing (within 48 h of collection) (Fig. 1).

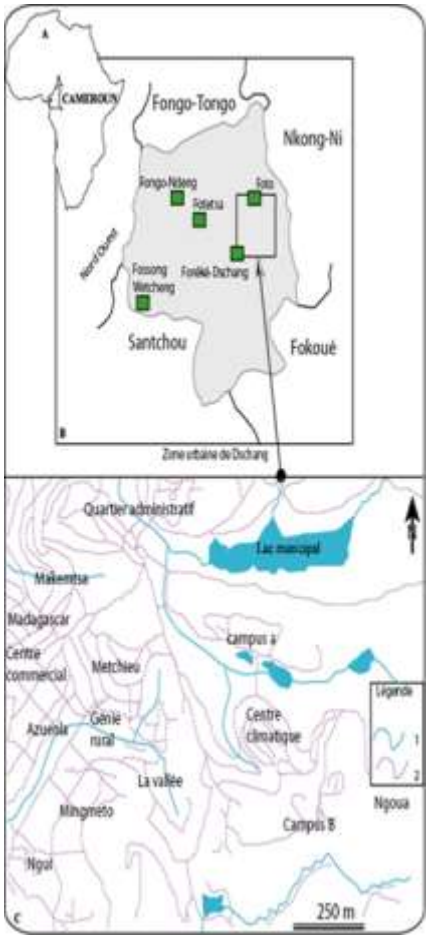

Fig. 1. Location of Dschang

Isolation and purification of microbial strains

Culture media preparation

In the laboratory, two non-selective culture media were used, Nutrient Agar (NA; 8 g/L nutrient broth, 15 g/L agar) for bacterial isolation, and Potato Dextrose Agar (PDA; 200 g/L boiled potato filtrate, 20 g/L dextrose, 15 g/L agar, supplemented with chloramphenicol at 50 mg/L to suppress bacterial growth) for fungal isolation. All these culture media were sterilized by autoclaving at 121 °C for 20 min and dispensed aseptically (~15 mL per Petri dish) after cooling to 45–55 °C.

Serial dilution and pour-plate isolation

Ten grams of rhizospheric soil per site were suspended in 90 mL of sterile distilled water (SDW), vortexed for 5 min to detach microorganisms from soil particles, and subjected to serial tenfold dilution up to 10^{-7}.

For bacterial isolation, 1 mL aliquots from dilutions 10^{-5} to 10^{-7} were mixed with molten NA (45–55 °C) in Petri dishes (pour-plate method) and incubated at 30 °C for 48 h. Morphologically distinct colonies (differing in size, colour, texture, and margin characteristics) were re-streaked on fresh NA plates to obtain pure cultures (Fan *et al.*, 2018).

For fungal isolation, dilutions 10^{-2} to 10^{-4} were used with PDA, following the procedure described by Bedine *et al.* (2020). Plates were incubated at 28 °C and examined daily for five to seven days. Colonies exhibiting morphological characteristics consistent with *Trichoderma* spp. (rapid growth, green to yellow-green

sporulation) were sub-cultured individually on PDA. All pure cultures were stored at 4 °C until further use.

Biochemical characterization of plant growth-promoting traits

Preparation of microbial suspensions

Bacterial suspensions were prepared for 24h in the cultures media by adjusting turbidity to approximately 1.5×10^8 CFU/mL against a 0.5 McFarland standard. Fungal suspensions were prepared from 5-day-old culture media by placing a 5 mm mycelial disc in 5 mL SDW, followed by vortexing to obtain a homogeneous spore/mycelium suspension.

Quantitative phosphate solubilization assay

Inorganic phosphate solubilization was evaluated in the liquid National Botanical Research Institute's Phosphate (NBRIP) medium supplemented with 5 g/L tricalcium phosphate [$Ca_3(PO_4)_2$] as the sole phosphorus source. The medium was autoclaved at 121 °C for 20 min.

After cooling, 100 µL of each microbial suspension was inoculated into 10 mL NBRIP broth in triplicate, uninoculated medium served as a negative control. Cultures were incubated at 30 °C for 5 days with continuous orbital shaking at 120 rpm, then centrifuged at 5.000 × g for 15 min. Soluble phosphorus in the supernatant was quantified colorimetrically by the ammonium molybdate–ascorbic acid method of Murphy and Riley (1962), with absorbance measured at 880 nm. A KH_2PO_4 standard curve (0–40 µg/mL) was used for calibration.

Quantitative IAA production assay

IAA production was assessed following the modified protocol of Islam *et al.* (2016). Briefly, 100 µL of each microbial suspension was inoculated into 10 mL of Nutrient Broth (NB) supplemented with 0.05% (w/v) L-tryptophan (IAA precursor) and incubated at 30 ± 2 °C for 72 h at 120 rpm in darkness. After incubation, cultures were centrifuged at 5,000 × g for 15 min. IAA in the supernatant was detected calorimetrically using Salkowski's reagent [1.5 mL 0.5 M $FeCl_3 \cdot 6H_2O$, 50 mL distilled water, 30 mL concentrated H_2SO_4], added at a 2:1 (reagent: supernatant) ratio. The mixture was incubated in darkness at room temperature for 20 min. The development of a pink to red coloration indicated IAA production. Absorbance was measured at 530 nm, and IAA concentration was calculated from a standard curve (0–60 µg/mL) prepared with pure IAA (Gordon and Weber, 1951).

Statistical analysis

All assays were performed in triplicate. Data are expressed as mean ± standard deviation.

Differences among isolates in IAA production and phosphate solubilization were analyzed by one-way analysis of variance (ANOVA) for fungal isolates and by the Kruskal–Wallis non-parametric test for bacterial isolates, given non-normal distributions in the latter collection. Post-hoc pairwise comparisons (Duncan test) were conducted at a significance level of $\alpha = 0.05$. Statistical analyses were performed using R software (version 4.x).

RESULTS

IAA production and phosphate solubilization by fungal isolates

All fourteen fungal isolates produced detectable IAA and solubilized inorganic phosphate in liquid culture (Table 1). ANOVA structured the collection into two statistically distinct groups for IAA production and into multiple overlapping groups (a–d) for phosphate solubilization.

Strain D3 (*Trichoderma* spp*)* significantly outperformed all other fungal isolates in IAA production, yielding 431.6 ± 43,5µg/mL (group a), a value 2.3 -fold higher than the second ranked isolate B8 (179.03 µg/mL) and approximately 3.8-fold higher than the lowest producer B2 (113.16 µg/mL). The remaining fourteen isolates (including the control, T) formed a single homogeneous group (b), with IAA values ranging from 113.16 (B2) to 184.44 µg/mL (T).

For phosphate solubilization, strain D2 (*Trichoderma* spp) achieved the highest value (1523.13 ± 34.7 mg/L; group a), followed by D3 (*Trichoderma* spp) (1463.29 ± 84.8 mg/L; group ab). The lowest values were recorded for B6 (1163.95 mg/L; group d) and the control T (SDW) (1250.85 mg/L; group cd). The ratio between the highest and lowest

performer was approximately 1.31. Notably, D3 combined the highest IAA production with competitive phosphate solubilization (group ab), identifying it as the most functionally versatile fungal isolate.

Table 1. IAA production (µg/mL) and phosphate solubilization (mg/L) by fungal isolates from strawberry rhizosphere

Strain code	IAA production (µg.mL^{-1})	P solubilization (mg.L^{-1})
D3	431.60±43.50a	1463.29±84.80ab
D2	133.61±64.50b	1523.13±34.70a
B8	179.03± 6.09b	1428.74±79.40abc
A1	146.39±71.60b	1419.94±59.80abc
B9	164.76±44.50b	1341.10±75.60bcd
A2	133.16±8.99b	1336.24±105.50bcd
B7	156.78±28.70b	1308.91±48.40bcd
B5	138.07±2.14b	1317.55±178.60bcd
B4	146.28±7.12b	1258.65±45.20cd
D1	131.79±8.26b	1282.37±87.50cd
A3	129.51±32.80b	1280.80±102.90cd
B1	117.53±11.85b	1302.78±69.70bcd
B2	113.16±17.89b	1300.70±140.70bcd
B6	139.43±14.15b	1163.95±42.90d
Control (T)	184.44±47.20b	1250.85±206.00cd

Values followed by the same letter within a column are not significantly different at α = 0.05 (one-way ANOVA). Data are expressed as mean ± SD of three independent replicates.

Table 2. IAA production (µg/mL) and phosphate solubilization (mg/L) by selected bacterial isolates from strawberry rhizosphere

Strain code	IAA production (µg.mL^{-1})	P solubilization (mg.L^{-1})
A6	172.60±51.30a	1514.70±383.00abc
A5	111.30±5.99cde	1612.49±106.2a
B6	149.02±35.80bcd	1523.60±165.00ab
B14	112.90±8.91cde	1516.37±69.40abc
A10	99.22±10.69de	1472.71±286.00abcd
B5	160.57±19.07b	1405.81±265.00abcdefg
A1	120.20±13.16bcde	1415.70±422.00abcdef
A4	111.40±11.11cde	1436.80±218.00abcde
B7	144.70±15.27bcd	1147.20 ± 220bcdefgh
B8	123.70±8.57bcde	1357.66±85.70abcdefgh
A9	116.80±3.47cde	1333.88±380.00abcdefgh
T9	103.10±10.37cde	1376.91±62.60abcdefg
B15	99.93±8.20de	1375.18±140.20abcdefg
D8	109.10±5.78cde	993.92±87.30fgh
A8	107.20± 6.65cde	935.71±265.00h
Control (T)	105.90±17.65cde	976.75±98.90gh

Values followed by the same letter within a column are not significantly different at α = 0.05 (Kruskal–Wallis test). Data are expressed as mean ± SD of three independent replicates.

IAA production and phosphate solubilization by bacterial isolates

Among the 37 bacterial isolates evaluated, IAA production ranged from 96.82 µg/mL (B1) to 260.4 µg/mL (A6) and was structured into five homogeneous groups (a-e) by the Kruskal-Wallis test. Phosphate solubilization ranged from 935.71 mg/L (A8) to 1612.49 mg/L (A5) and was structured into eight groups (a–h), reflecting greater functional diversity within the bacterial collection than within the fungal collection (Table 2).

Strain A6 (Coccis gram+) was the outstanding IAA producer (260.4 ± 241.0 µg/mL; group a), 1.6-fold higher than the second-ranked strain B5 (160.57 µg/mL; group b). The majority of isolates (groups bcd–cde) produced IAA in the moderate range of 100–150 µg/mL, while strains B13, A10, B15, D6, and B1

(groups de–e, <100 µg/mL) performed least well on this criterion.

Fig. 2. Microscopic and macroscopic view of the bacteria A6 cocci gram+

Fig. 3. Microscopic and macroscopic view of the fungus Trichoderma spp

For phosphate solubilization, strain A5 was the top performer (1612.49 ± 106.2 mg/L; group a), followed by B6 (Coccis gram-) (1523.60 ± 165.0 mg/L; group ab) and B14 (Coccis gram+) (1516.37 ± 69.4 mg/L; group abc). The control strain T (sdw) (976.75 mg/L; group gh) and A8 (935.71 mg/L; group h) showed the lowest phosphate-solubilizing activity (Fig. 2, Table 2). Critically, the vast majority of bacterial isolates outperformed the uninoculated control on this trait, supporting the intrinsic biofertilization potential of the collection. Strain B6 (Coccis gram-) simultaneously displayed competitive IAA production (149.02 µg/mL; group bcd) and high phosphate solubilization (group ab), designating it as a dual-function candidate of particular agronomic interest.

Comparison across the two collections revealed that the best-performing bacterial strains (A5: 1612.49 mg/L) marginally exceeded the best fungal solubilizer (D2: 1523.13 mg/L). However, the fungal isolate D3 (*Trichoderma)* (Fig. 3) was the single highest IAA producer across both collections (432.31 µg/mL), surpassing the best bacterial strain A6 (Coccis gram+) (260.4 µg/mL) by a factor of 1.7 (Table 2).

DISCUSSION

This study provides the first characterization of plant growth-promoting rhizobacteria and *Trichoderma* spp strains from the strawberry rhizosphere in the west region of Cameroon, identifying a collection of functionally diverse indigenous isolates with significant biofertilization potential.

The IAA production levels recorded in this study (96.82–432.31 µg/mL for fungal isolates and 96.82–260.4 µg/mL for bacterial isolates) are broadly consistent with values reported in the international literature. Patten and Glick (2002) documented IAA production of 50–300 µg/mL in rhizospheric *Pseudomonas* and *Bacillus* strains, while Contreras-Cornejo *et al.* (2009) reported values of 100 to > 400 µg/mL in *Trichoderma* spp. The exceptional IAA output of fungal strain *Trichoderma* spp (431.6 µg/mL) falls at the upper boundary of published fungal ranges, comparable to highly productive *Trichoderma* spp isolates reported by Contreras-Cornejo *et al.* (2009). IAA promotes root elongation and lateral root formation, thereby expanding the root surface area available for water and nutrient uptake (Spaepen *et al.*, 2007). Strains with robust IAA-producing capacity are therefore strong candidates for improving crop establishment and stress tolerance.

The phosphate solubilization values recorded across both collections (935–1612 mg/L) are considerably higher than those commonly reported for PGPR in temperate soils (100–800 mg/L; Bargaz *et al.*, 2018). We propose that this elevated activity reflects the selective pressure exerted by the ferrallitic soils of Western Cameroon, which are characteristically rich in iron and aluminium oxides that immobilize phosphate through strong adsorption and co-precipitation reactions (Mwende, 2019). Under these conditions, rhizospheric microorganisms capable of releasing phosphate through acidification (organic acid secretion) or enzymatic mechanisms (phytases, phosphatases) would be subject to strong positive selection, resulting in the enrichment of highly active solubilizers in local soil communities. This interpretation is supported by the finding that the majority of both fungal and bacterial isolates outperformed uninoculated controls, regardless of the collection.

The particularly strong phosphate-solubilizing activity of bacterial strains A5 (Coccis gram+) (1612.49 mg/L) and B6 (Coccis gram-), (1523.60 mg/L), and of fungal strain D2 *(Trichoderma* spp*)* (1523.13 mg/L), suggests these isolates could significantly contribute to phosphorus mobilization in the soil, potentially replacing or reducing mineral phosphate fertilizer inputs. Strain B6 (Coccis gram), which combined high phosphate solubilization (group ab) with competitive IAA production (149.02 µg/mL), represents a particularly attractive single-strain option for bioformulation. Strain D3, which simultaneously exhibited the highest IAA production in the entire study and competitive phosphate solubilization (group ab for fungi), is an equally promising candidate. The use of microbial consortia combining high-IAA producers (D3: *Trichoderma* spp, A6: Coccis gram+) with superior P-solubilizers (A5: Coccis gram+, D2: *Trichoderma* spp) may maximize synergistic effects on plant growth, as previously demonstrated in multi-trait PGPR consortia (Mitter *et al.*, 2021). Such combinations could be particularly impactful in the context of *Botrytis* management, given that *Trichoderma* spp. concurrently act as biocontrol agents against *B. cinerea* through mycoparasitism, antibiosis, and ISR (Geng *et al.*, 2022).

A key methodological strength of this study is the exclusive use of indigenous microbial strains collected from local agroecosystems. Indigenous strains possess inherent adaptations to regional soil chemistry, temperature regimes, and plant genotypes, which substantially increases the probability of stable colonization and consistent performance under field conditions, a critical limitation of commercial bioformulations based on exotic strains (Mitter *et al.*, 2021). This approach aligns with FAO recommendations for the development of locally adapted biological inputs for smallholder farmers.

CONCLUSION

This study isolated and functionally characterized a collection of 14 fungal and 37 bacterial isolates from the rhizosphere of strawberry cultivated in Western Cameroon, demonstrating that all strains possess plant growth-promoting traits, specifically IAA production and phosphate solubilization at levels competitive with or superior to those reported in the international literature. Fungal strain D3 *(Trichoderma* spp*)* emerged as the most potent IAA producer (432.31 µg/mL), while D2 (*Trichoderma* spp*)* was the best fungal phosphate solubilizer (1523.13 mg/L). In the bacterial collection, A6 (Coccis gram-) was the dominant IAA producer (172.6 µg/mL), and A5 (Coccis gram+) the highest phosphate-solubilizing strain (1612.49 mg/L). The exceptionally high phosphate solubilization value across both collections likely reflects positive selection by the iron and aluminium rich ferrallitic soils of the region.

These results constitute a scientifically rigorous basis for the rational selection of biofertilizer candidates. Strains D3, B6, D2 (*Trichoderma* spp), A5, A6 (Coccis gram+), are prioritized for further characterization, including molecular identification, in vivo plant growth promotion bioassays, and biocontrol potential. The development of multi-strain bioinoculants combining the complementary strengths of these isolates could offer an ecologically sound strategy to improve strawberry yield and quality while reducing reliance on synthetic inputs in Cameroonian production systems.

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