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Functional Diversity and Rational Selection of PGPR Rhizobacteria from Durum Wheat: Comparative Performance of a Multi-Strain Consortium (Biostim) and Individual Isolates Against Fusarium culmorum and F. pseudograminearum

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ABSTRACT: Fusarium crown rot, caused by F. culmorum and F. pseudograminearum, seriously constrains durum wheat production in semi-arid regions, and chemical control remains limited. Plant growth-promoting rhizobacteria (PGPR) offer a sustainable alternative, but whether multi-strain consortia outperform single isolates is unclear. Thirty-five fluorescent rhizobacteria from durum wheat rhizosphere were screened for seven growth-promotion and biocontrol traits. Multivariate analysis showed weak trait correlations and identified four functional groups, guiding selection of six multifunctional isolates (BNJ2, BNJ22, BNJ24, BNJ26, BNJ27, BNJ35). Applied alone or as a thirty-five-strain consortium on durum wheat under healthy and pathogen-inoculated conditions, treatments showed significant main and interaction effects. with pathogen-specific responses (BNJ2 vs. F. culmorum; BNJ27/BNJ35 vs. F. pseudograminearum). The Biostim consortium consistently outperformed the control, the isolate average, and the best single isolate, indicating functional complementarity. These results support combining functional screening with multivariate analysis to rationally select robust PGPR consortia for biocontrol of Fusarium crown rot. This work highlights the potential of natural-based biostimulants to promote sustainable wheat production, reduce reliance on chemical inputs, and contribute to human health by supporting the production of safer and healthier food.

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1. INTRODUCTION

Durum wheat (*Triticum turgidum L. subsp. durum*) is a strategically important cereal crop worldwide, especially across Mediterranean regions where it provides a major source of dietary protein. Its productivity, however, is heavily constrained by a range of biotic pressures, foremost among them soil-borne fungal diseases. Fusarium crown and root rot, caused mainly by *Fusarium culmorum* and *Fusarium pseudograminearum*, ranks among the most damaging of these. These pathogens persist in the soil through resistant structures, disrupt root development, cause premature wilting, reduce yield, and compromise grain quality through potential mycotoxin production (Murray and Brennan 2009; Kazan and Gardiner 2018; Miedaner and Juroszek 2021). In semi-arid areas, losses associated with this disease can reach 35–58% in years particularly favorable to the pathogen, representing a substantial economic burden (Murray and Brennan 2009; Kazan and Gardiner 2018).

Disease management still relies heavily on fungicide use, but chemical control remains only partially effective against soil-borne pathogens, largely because of their persistence in the soil and the difficulty of reaching infection sites. Intensive use of plant protection products also carries well-documented costs for ecosystems, human health, and soil microbial communities (Backer et al. 2018; Köhl et al. 2019), a set of concerns that has fueled growing interest in alternative strategies based on beneficial microorganisms.

Plant growth-promoting rhizobacteria (PGPR) stand out as a particularly promising avenue for sustainable agriculture. They act directly, through the production of phytohormones such as indole-3-acetic acid (IAA), phosphate solubilization, and improved mineral nutrition, and indirectly through biocontrol mechanisms including siderophore production, antifungal metabolites, hydrogen cyanide, hydrolytic enzymes, and induced systemic resistance (Haas and Défago 2005; Etesami et al. 2021). Among these, fluorescent Pseudomonas strains have received particular attention for their metabolic versatility and their demonstrated efficacy against several soil-borne pathogens, including Fusarium species (Haas and Défago 2005; Woo and Pepe 2018).

Even so, the field performance of microbial inoculants tends to be inconsistent, a consequence of the intricate interplay between plant, associated microorganisms, and environment. Single-strain formulations in particular often lose effectiveness once soil and climate conditions depart from those under which they were originally selected. Multi-strain consortia, by combining complementary modes of action, could offer greater functional stability (Rajasekar and Elango 2011; Woo and Pepe 2018; Arif et al. 2020), though antagonistic interactions between strains can just as easily undermine this approach, underscoring the need for careful, evidence-based selection (Aleksieienko et al. 2025).

This study set out to characterize a collection of fluorescent rhizobacteria from the durum wheat rhizosphere based on their functional traits, to select promising candidates through multivariate analysis, and to compare, in planta, the performance of a bacterial consortium (Biostim) against individual strains, both under healthy conditions and under pressure from F. culmorum and *F. pseudograminearum*. Ultimately, this research contributes to sustainable agriculture by promoting environmentally friendly biostimulant strategies that reduce chemical inputs, improve crop health, and support human health through safer and more sustainable food production.

2. MATERIALS AND METHODS

2.1. Isolation and Characterization of Bacterial Isolates

Rhizospheric soil samples were randomly collected from durum wheat grown near the Frères Mentouri University agricultural field, following the method of Pepper and Gerba (2004). PGPR bacteria were isolated by serial dilution (up to 10^{-5}) plated on King B (KB) medium, selective for Pseudomonas spp., after 48 h of incubation at 28 °C. Fluorescent colonies were purified through successive subculturing and characterized based on colony morphology (color, shape, margin, elevation, surface texture) and microscopic appearance following Gram staining. Pure isolates were stored at −20 °C in KB medium supplemented with 50% glycerol.

2.2. Plant Growth-Promoting Activities

Siderophore production was assessed on solid Chrome Azurol S (CAS) medium, using a modified version of the method of Pérez-Miranda et al. (2007), based on the original protocol of Schwyn and Neilands (1987).

Inorganic phosphate solubilization was evaluated on solid Pikovskaya medium containing tricalcium phosphate ($Ca_3(PO_4)_2$), after 10 days of incubation at 28 °C, following Pikovskaya's method (1948).

Ammonia (NH_3) production was tested in peptone water according to the method of Cappuccino and Sherman (1992), with isolates incubated for 48 h at 28 °C prior to assessment.

HCN production was determined on King B medium supplemented with glycine, following Bakker and Schippers (1987). Filter paper soaked in picric acid was placed in the Petri dish lids, and cultures were incubated for 96 h at 28 °C.

Indole-3-acetic acid (IAA) production was assessed on King B medium supplemented with L-tryptophan (0.5%), following Bric et al. (1991). Isolates were incubated for 48 h at 30 °C, after which Whatman paper discs soaked in Salkowski reagent were applied to detect IAA.

Extracellular enzymatic activities were assessed on specific media by detecting clear zones of degradation around colonies. Cellulolytic activity was tested on King B medium supplemented with 1% carboxymethylcellulose (CMC), revealed with Congo red after 5 days of incubation at 30 °C, following Cattelan et al. (1999). Amylolytic activity was determined on nutrient agar supplemented with 1% starch, revealed with iodine after 5 days of incubation at 30 °C, following Holt et al. (1994) and Delarras (2014). Protease production was assessed on skim milk agar following Chaiharn et al. (2008). Finally, pectinolytic activity was tested on King B medium containing 0.5% pectin, revealed with iodine after 48 h of incubation, following Delarras (2014).

The antagonistic potential of the bacterial isolates was evaluated through dual-culture confrontation on PDA (Potato Dextrose Agar) against two pathogenic fungal isolates of wheat, Fusarium culmorum (Fus1) and Fusarium pseudograminearum (Fus7), following Vincent et al. (1991) and the protocol described by Sebihi (2016). Confrontation assays were incubated for 10 days at 28 °C, consistent with conditions reported by Hariprasad and Niranjana (2009).

2.3. In Planta Assay: Comparative and Interactive Effects of Biostim and Individual PGPR Isolates on Durum Wheat Inoculated with Fusarium Strains

The effect of bacterial inoculation on durum wheat growth was assessed under controlled conditions, following the protocol described by Sebihi et al. (2016). A subset of isolates, selected based on their PGPR activities and antagonistic potential, was cultured at 28 °C for 24 hours. Fungal suspensions were prepared from 7-day-old cultures.

Durum wheat seeds (GTA variety) were surface-sterilized and germinated on filter paper in Petri dishes. Soil, collected from Aïn El Bey (Constantine), was sterilized by autoclaving (three cycles at 120 °C) and distributed into disinfected plastic pots. Three germinated seeds were sown per pot at a depth of 1 cm and inoculated with 2 ml of bacterial suspension; control pots received sterile distilled water only.

The trial, conducted in pots over a period of two and a half months, included eight treatments: an uninoculated control (T), six bacterial isolates (coded 2, 3, 13, 30, 33, and 35) tested individually, and the Biostim consortium, each replicated three times. These treatments were applied under three health conditions: healthy (no pathogen), inoculation with F. culmorum (+Fus1), and inoculation with F. pseudograminearum (+Fus7). Fungal inoculation was carried out one week after planting.

Biostim is a formulation composed of a consortium of 35 PGPR bacterial isolates, selected for their plant growth-promoting properties and their antagonistic potential against phytopathogenic agents. Each strain was cultured separately and freeze-dried to ensure stable storage while preserving viability and functional properties. The resulting lyophilized powder, reconstituted before application, was blended with maltodextrin and used as a biofertilizer. This setup allowed the performance of a multi-strain consortium to be directly compared with that of individual isolates, across three contrasting health conditions, both in terms of wheat growth and inhibition of the two Fusarium strains, providing a relevant framework for testing this hypothesis under controlled conditions.

2.4. Statistical Analysis

All statistical analyses were performed using OriginPro (version 9.0, OriginLab Corporation, USA), following standard procedures described by Sokal and Rohlf (1995) for parametric and non-parametric tests. The seven functional traits, recorded on an ordinal qualitative scale, were converted into numerical scores (0–3), and their correlations were assessed using Spearman's test (ρ, $p < 0.05$). Functional profiles of the isolates were structured using PCA (on standardized data) and hierarchical clustering (Ward's method, Euclidean distance; $k = 4$ groups), and summarized into a multifunctionality index.

The effects of bacterial treatment (Control, BNJ2, BNJ22, BNJ24, BNJ26, BNJ27, BNJ35, and Biostim), pathogen challenge

(healthy, +Fus1, +Fus7), and their interaction on plant length were tested using a two-way ANOVA (fixed-effects factorial design, with three independent replicate pots per treatment × condition combination), after verifying normality of residuals (Shapiro-Wilk) and homogeneity of variances (Levene). This analysis was complemented by one-way ANOVAs for each health condition, Tukey's HSD and Dunnett's post-hoc comparisons (vs. control), and targeted contrasts comparing Biostim's performance against the control, the average of individual isolates, and the best-performing individual isolate. Finally, principal component analysis (PCA) and hierarchical clustering (Ward's method) were performed on treatment means to distinguish response profiles. The significance threshold was set at $\alpha = 0.05$.

3. RESULTS

3.1. Isolation and Phenotypic Characterization of Isolates

Selection on King B medium yielded a collection of 35 fluorescent bacterial strains. Morphological observation showed colonies ranging from cream to whitish in color, round in shape, with regular margins, a convex elevation, and a smooth surface. Microscopic observation following Gram staining revealed both Gram-negative bacillary forms and Gram-positive coccobacillary forms (Figure 1).



Figure 1*:* Microscopic observation of isolates after Gram staining (×100)

3.2. Plant Growth-Promoting Activities

The thirty-five rhizobacterial strains were characterized in vitro for seven functional traits of agronomic interest, spanning growth-promoting characters (phosphate solubilization, nitrate reduction, hydrogen cyanide and indole-3-acetic acid production) and biocontrol-related characters (siderophore production, direct antagonistic activity against two Fusarium isolates). The intensity of each activity was scored on a four-level ordinal scale (Table 1).

Table 1. Semi-quantitative functional profile of the isolates.

Strain	CAS	HPO4	Antif. Fus1	Antif. Fus7	NO3	HCN	IAA
BNJ1	–	+++	–	–	+	–	++
BNJ2	+	+++	–	+	+	++	+++
BNJ3	–	+	–	–	+	+++	+++
BNJ4	–	+	–	+	–	+	+++
BNJ5	–	+	–	+	++	+	++
BNJ6	–	+	–	–	–	–	++
BNJ7	+	–	–	+	–	–	+++
BNJ8	+	–	–	–	–	+	+++

Strain	CAS	HPO4	Antif. Fus1	Antif. Fus7	NO3	HCN	IAA
BNJ9	+	–	–	–	–	–	++
BNJ10	+	+	–	–	–	–	+++
BNJ11	++	–	–	–	++	+++	+++
BNJ12	++	–	–	+	+	++	++
BNJ13	+	+	–	+	+	–	++
BNJ14	+	+	–	+	++	+++	++
BNJ15	–	++	–	–	–	+++	+
BNJ16	–	++	–	–	+	–	+++
BNJ17	–	–	–	–	+	+++	+
BNJ18	–	–	–	++	++	+++	++
BNJ19	+	+++	+	–	–	+++	+
BNJ20	+	+	–	–	++	+	+
BNJ21	++	++	–	+	+	–	+++
BNJ22	++	+++	–	–	+++	+++	+++
BNJ23	++	++	–	–	+++	+	+
BNJ24	+	++	–	–	+++	–	+++
BNJ25	+++	+	+	++	++	+++	+++
BNJ26	++	++	+	–	++	–	+++
BNJ27	+	+	–	–	++	+++	+++
BNJ28	+	+	–	–	++	+	+++
BNJ29	+	–	+	–	++	+++	++
BNJ30	+	+	–	–	+	+	++
BNJ31	+	+	–	–	++	+++	++
BNJ32	+	+	+	–	–	+++	++
BNJ33	+	–	–	+	++	+++	–

Strain	CAS	HPO4	Antif. Fus1	Antif. Fus7	NO3	HCN	IAA
BNJ34	+	+	+++	–	++	–	++
BNJ35	+	+	–	–	++	++	+++

"–" = no activity (score 0); "+" = weak activity (score 1); "++" = moderate activity (score 2); "+++" = strong activity (score 3). CAS: siderophore production; HPO4: inorganic phosphate solubilization; Antif. Fus1 / Antif. Fus7: in vitro antagonistic activity against Fusarium isolates Fus1 and Fus7; NO3: nitrate reduction; HCN: hydrogen cyanide production; IAA: indole-3-acetic acid production.

The distribution of functional traits across the collection shows a clear predominance of growth-promoting characters. Indole-3-acetic acid (IAA) production was the most frequent trait, detected in 97.1% of isolates, with 45.7% showing high activity. Phosphate solubilization, nitrate reduction, and siderophore production were each expressed by 74.3% of the strains. Hydrolytic enzymatic activities were also widespread, with cellulase and amylase activity detected in 97.14% of isolates, protease activity in 74.29%, and pectinase activity in 54.29%. In contrast, direct antagonistic activity against the fungal isolates remained limited, with only 17.1% of strains active against Fusarium sp. F1 and 31.4% against Fusarium sp. F7. Overall, growth-promotion and enzymatic traits were widely distributed across the collection, whereas direct biocontrol proved to be a rarer, more discriminating character.

3.3. Relationships Among Functional Traits

The Spearman correlation matrix applied to the seven functional traits revealed generally weak associations, pointing to a fair degree of independence among the underlying functional mechanisms. Only one significant positive correlation was observed, between siderophore production (CAS) and nitrate reduction (NO3) (ϱ = 0.38; p = 0.024). No significant relationship was found between antagonistic activity against F1 and F7 (ϱ = −0.11; ns), suggesting that a strain's ability to inhibit one pathogen does not necessarily predict its activity against another (Figure 2).

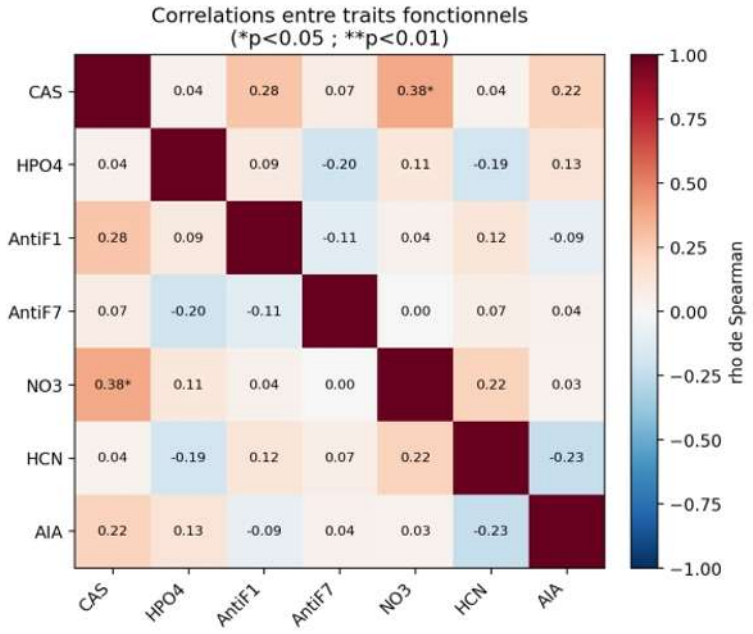


Figure 2. Spearman correlation matrix (ϱ) among the seven functional traits of the 35 strains. * p < 0.05.

Principal component analysis (PCA), performed on the seven standardized traits, explained 42.7% of total variance across the first two axes (PC1 = 22.5%; PC2 = 20.2%) (Figure 3). This moderate level of explained variance is consistent with the low redundancy among traits already suggested by the correlation analysis. The first axis was mainly driven by CAS, NO3, and AntiF1, while the second axis contrasted HCN and AntiF7 activity against HPO4 and IAA.

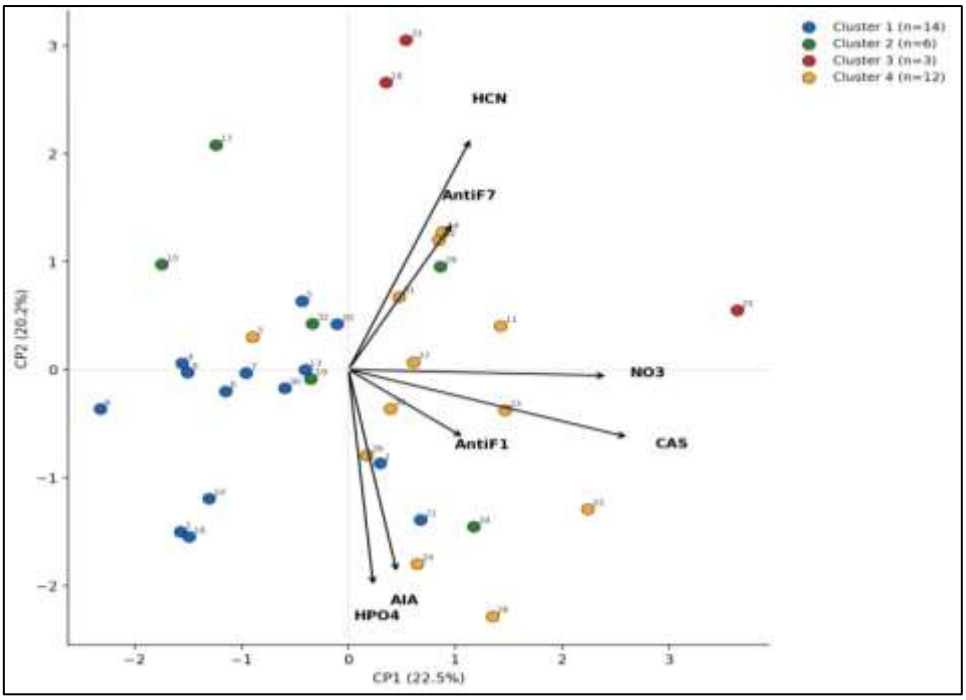


Figure 3. PCA biplot of the functional profiles of the 35 strains (standardized scores). Point color corresponds to the four functional groups identified by hierarchical clustering (Ward's method).

Hierarchical clustering (Ward's method) confirmed this structure, identifying four functional groups consistent with the PCA projection. Cluster 1 (n = 14) corresponded to a profile mainly oriented toward growth promotion, marked by strong IAA expression and weak antifungal activity. Cluster 2 (n = 6) stood out for high HCN production. Cluster 3 (n = 3), which includes strain BNJ25, showed a particularly strong profile, combining high levels of nitrate reduction, HCN production, and antagonism. Cluster 4 (n = 12) displayed an intermediate profile, balanced between NO3 and IAA activity (Figure 4).

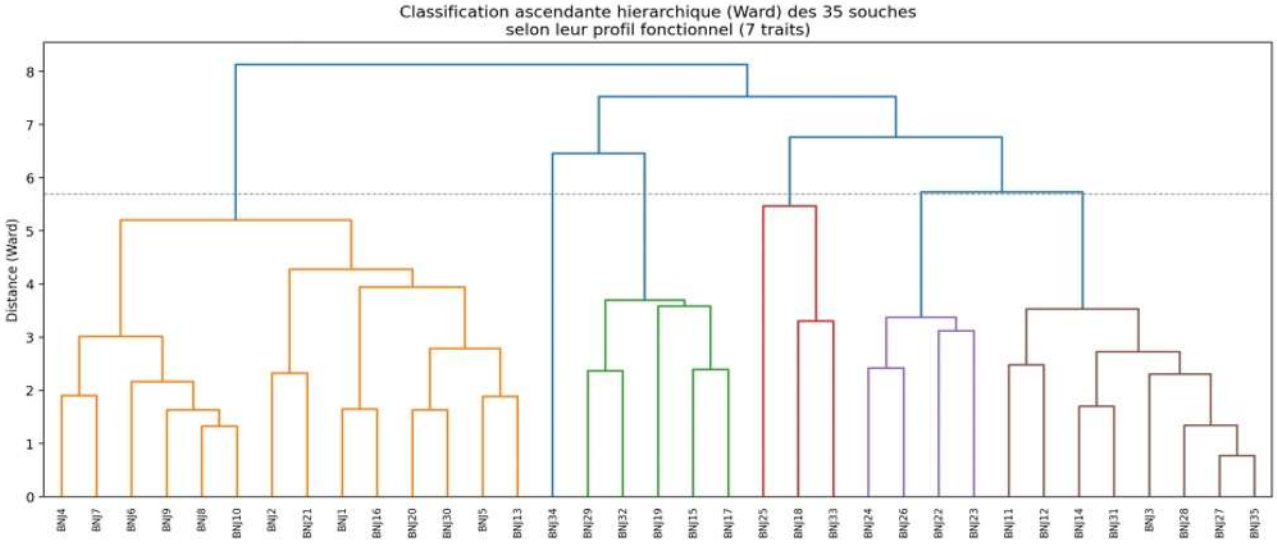


Figure 4. Dendrogram of hierarchical clustering (Ward's method, Euclidean distance) of the 35 strains based on their seven-trait functional profile.

3.4. Selection of Isolates for In Planta Inoculation Trials

Based on the functional profiles characterized above, which combined plant growth-promotion traits and biocontrol-related enzymatic activities, six isolates were selected from the thirty-five strains evaluated: BNJ2, BNJ22, BNJ24, BNJ26, BNJ27,

and BNJ35, for in planta inoculation trials. These strains were tested both individually and combined within a consortium (Biostim), and compared against an uninoculated control, on durum wheat stem length under healthy conditions (Healthy) and in the presence of two Fusarium isolates (+Fus1, +Fus7).

3.4.1. Overall Effect of Treatment and Health Condition on Growth

Analysis of stem length revealed substantial differences among bacterial treatments depending on the health condition applied (Table 2). The Biostim treatment consistently produced the highest mean values, both under healthy conditions and under Fusarium-induced stress. Under healthy conditions, stem length ranged from 38.0 ± 1.0 cm for the control to 64.7 ± 4.5 cm for Biostim, with individual isolates showing intermediate values (39.3–51.0 cm). Under inoculation with Fusarium sp. 1 and Fusarium sp. 7, Biostim maintained its growth-promoting effect, reaching maximum stem lengths of 51.0 ± 2.0 cm and 52.3 ± 2.1 cm, respectively. Some isolates, notably 27 and 35, also performed well depending on the infection condition. Overall, the Biostim consortium significantly enhanced seedling growth, pointing to a stable biostimulant potential under pathogen stress.

Table 2. Mean plant height (cm ± SD) by group and condition (n = 3 per group and condition).

Group	Alone	+ Fus1	+ Fus7
Control	38.0 ± 1.0	28.0 ± 1.0	32.7 ± 0.6
BNJ2	39.3 ± 1.2	39.7 ± 1.2	34.7 ± 1.2
BNJ22	44.7 ± 3.5	38.3 ± 2.1	34.7 ± 0.6
BNJ24	46.0 ± 3.0	36.0 ± 1.7	36.7 ± 3.5
BNJ26	50.0 ± 2.6	38.7 ± 1.5	36.7 ± 1.2
BNJ27	51.0 ± 1.0	34.7 ± 1.2	43.0 ± 8.2
BNJ35	50.7 ± 4.0	34.3 ± 4.2	45.7 ± 6.0
Biostim	64.7 ± 4.5	51.0 ± 2.0	52.3 ± 2.1

A two-way ANOVA (fixed-effects factorial design: Treatment × Health condition, with three independent replicate pots per combination) was performed across the full dataset (Table 3). It revealed a highly significant main effect of treatment ($F(7,48) = 42.54$, $p < 0.0001$) and health condition ($F(2,48) = 78.66$, $p < 0.0001$), together with a significant Treatment × Condition interaction ($F(14,48) = 4.00$, $p = 0.0002$).

These results indicate that plant growth depends jointly on the bacterial isolate applied and the level of pathogen stress. The significant interaction highlights a differential response among isolates depending on the pathogen involved, with some isolates performing better under Fus1 and others under Fus7, confirming the functional variability of the microbial treatments.

Table 3. Two-way ANOVA (fixed-effects factorial design): main effects and interaction.

Source	df1	df2	F	P
Treatment	7	48	42.54	< 0.0001
Health condition	2	48	78.66	< 0.0001
Treatment × Condition interaction	14	48	4.00	0.0002

3.4.2. Simple Effects of Treatment Within Each Health Condition

To further characterize the origin of the Treatment × Health Condition interaction, one-way ANOVAs were run separately for each experimental condition (Table 4). The effect of bacterial treatment was highly significant across all three environments: healthy condition ($F(7,16) = 24.49$; $p < 0.001$), Fusarium sp. 1 infection ($F(7,16) = 29.62$; $p < 0.001$), and Fusarium sp. 7 infection ($F(7,16) = 9.02$; $p = 0.00015$). These results confirm that bacterial treatments elicit differential growth responses both in the absence and in the presence of the pathogen. Discrimination among treatments was strongest under +Fus1 infection and under healthy conditions, and comparatively more limited — though still highly significant — under +Fus7 infection, indicating that isolate efficacy is expressed differently depending on the specific pathogen challenge rather than uniformly increasing under pathogen stress.

Table 4. One-way ANOVA results (treatment effect), by condition (simple effects). Degrees of freedom are given as (df1, df2).

Condition	df1, df2	F	P
Healthy	7, 16	24.49	< 0.001
+ Fus1	7, 16	29.62	< 0.001
+ Fus7	7, 16	9.02	0.00015

3.4.3. Pathogen-Specific Response (Treatment × Condition Interaction)

The Treatment × Condition interaction identified in the two-way ANOVA ($F(14,48) = 4.00$, $p = 0.0002$; Table 3) statistically confirms that the relative efficacy of the isolates was not uniform across pathogens. Examining the relative growth gain of each isolate over the control, condition by condition, helps clarify the nature of this interaction (Figure 5):

BNJ2 showed a near-negligible effect under healthy conditions (+3.4%) and under +Fus7 (+6.1%), but a marked gain under +Fus1 (+41.8%), suggesting a biocontrol activity specific to this Fusarium isolate.

BNJ27 and BNJ35, by contrast, performed best under +Fus7 (+31.5% and +39.8%, respectively), with more modest effects against +Fus1 (+23.9% and +22.5%).

BNJ22, BNJ24, and BNJ26 showed a more generalist profile, with substantial gains both under healthy conditions and in the presence of both pathogens.

This variability in isolate ranking across conditions, statistically validated by the significant interaction in Table 3, distinguishes isolates that act mainly as general growth stimulators (BNJ22, BNJ24, BNJ26) from those that behave more as pathogen-specific biocontrol agents (BNJ2 against Fus1; BNJ27/BNJ35 against Fus7).

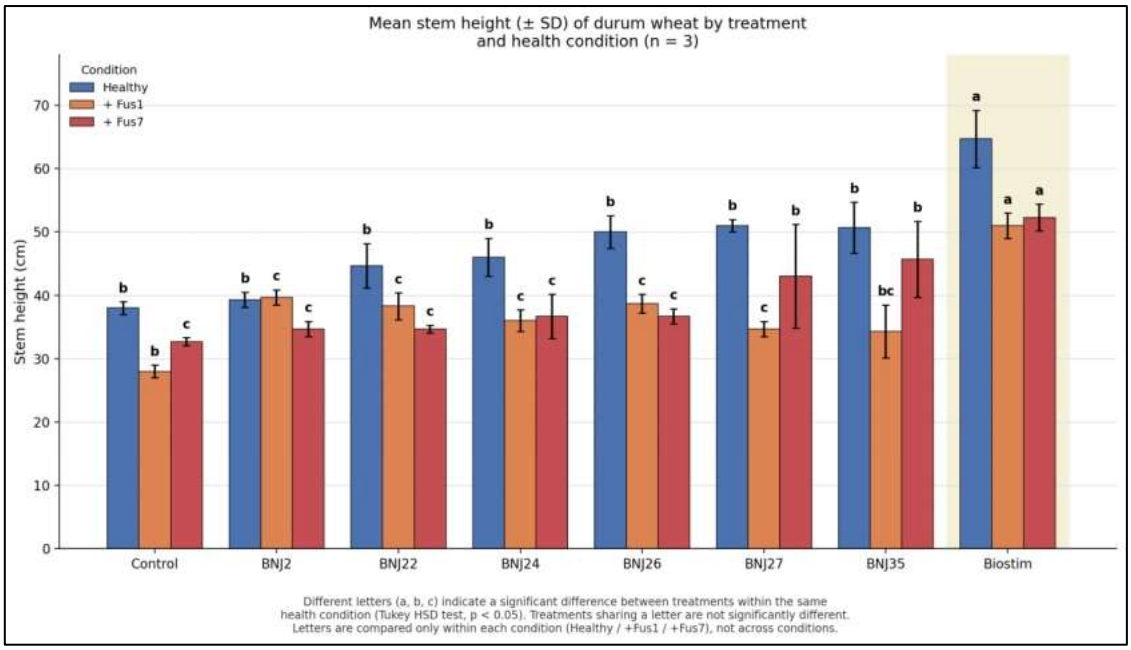


Figure 5. Mean stem height (± SD) of durum wheat by treatment and health condition (n = 3). Different letters indicate a significant difference among treatments within the same condition (Tukey HSD test, $p < 0.05$).

Tukey's HSD test clarified the structure of this interaction, condition by condition. Under healthy conditions, only Biostim differed statistically from the other treatments, which formed a single, undifferentiated group (group b), including BNJ27 and BNJ35. Under +Fus1, Biostim again stood alone at the top (group a); the control differed from the group of isolates most effective under this pathogen (BNJ2, BNJ22, BNJ24, BNJ26, BNJ27; group c), with BNJ35 occupying an intermediate position overlapping both groups (b/c). Under +Fus7, a clear three-tier structure emerged: Biostim (group a), followed by BNJ27 and BNJ35 forming a statistically distinct intermediate group (group b), and finally the control together with the four remaining isolates (group c), statistically confirming the enhanced efficacy of BNJ27 and BNJ35 under this pathogen already noted above.

These results confirm that the bacterial association within the Biostim consortium produces a more robust and stable growth stimulation than the individually applied isolates, both in the absence and presence of Fusarium-induced stress.

3.4.4. Performance of the Biostim Consortium

The Biostim consortium consistently outperformed the uninoculated control, the average of the six individual isolates, and the best-performing individual isolate, across all three tested conditions (Table 5, Figure 5):

Table 5. Growth gain (%) provided by the Biostim consortium relative to the control, the average of individual isolates, and the best individual isolate, by health condition.

Comparison	Healthy	+ Fus1	+ Fus7
Biostim vs. Control	+70.3%	+82.1%	+59.9%
Biostim vs. average of individual isolates	+37.8%	+38.0%	+35.6%
Biostim vs. best individual isolate	+26.9% (BNJ27)	+28.5% (BNJ2)	+14.4% (BNJ35)

The growth gain provided by the consortium exceeded that of the best individual isolate by 26–29% under healthy and +Fus1 conditions, and remained 14.4% higher than that of the best isolate (BNJ35) under +Fus7. This consistently superior

performance relative to the best individual strain suggests a synergistic effect among the combined isolates, rather than a simple additi ve one.

3.4.5. Overall Classification of Treatments by Multivariate Analysis

Principal component analysis (PCA) performed on the mean heights of the eight treatments across the three conditions accounted for 98.0% of total variance across the first two axes (Axis 1: 84.6%; Axis 2: 13.4%) (Figure 6). Axis 1 contrasted the uninoculated control (most negative values) with the Biostim consortium (most positive value, clearly separated from the rest of the cloud), reflecting an overall growth-performance gradient. Axis 2 further separated isolates with a more generalist response (BNJ22, BNJ24, BNJ26, positive values) from isolates with a more pathogen-specific response (BNJ27, BNJ35, negative values). This structure confirms the functional differences observed among isolates and aligns with the specificity profiles identified earlier.

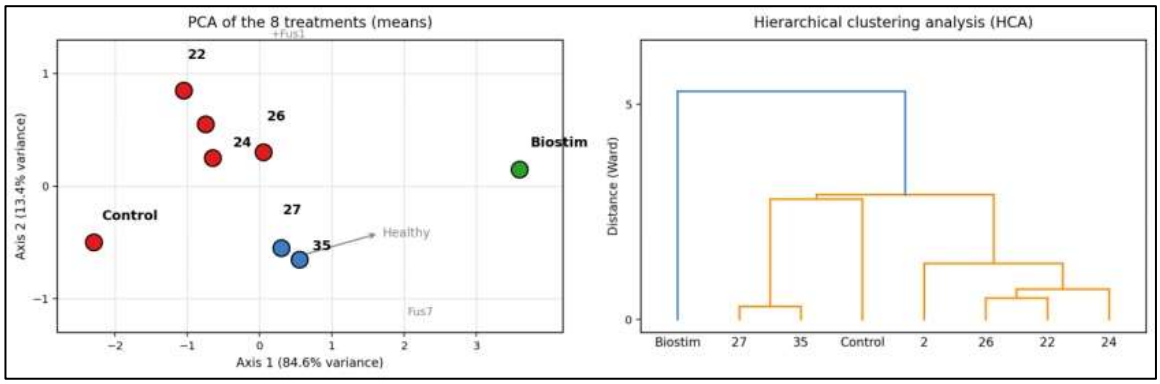


Figure 6. Principal component analysis (left) and hierarchical clustering analysis, Ward's method (right) of the eight treatments.

Hierarchical clustering (Ward's method) applied to the same data clearly singled out the Biostim consortium, which separated from the rest of the treatments at the greatest distance (~5.3), confirming its distinctive growth profile. The remaining seven treatments split into two subgroups: one combining the control with BNJ27 and BNJ35, and another grouping BNJ2, BNJ22, BNJ24, and BNJ26. This structure aligns with the PCA results and reinforces the distinction between a "generalist growth stimulation" pole and a pole with a more context-dependent response profile.

4. DISCUSSION

The functional characterization of the 35 fluorescent isolates revealed a strong prevalence of traits linked to plant growth promotion, particularly IAA production, phosphate solubilization, and extracellular enzymatic activities. This predominance confirms that rhizospheric bacteria represent a valuable source of multifunctional microorganisms capable of improving nutrient availability and stimulating vegetative development. The high frequency of IAA production observed in our collection is consistent with several studies reporting this phytohormone as one of the most widespread mechanisms among PGPR associated with cereals. Bacterial IAA promotes root elongation, increases absorptive surface area, and improves soil exploration, indirectly contributing to greater tolerance of biotic and abiotic stress (Spaepen 2015; Backer et al. 2018).

The weak correlations observed among functional traits in our study, however, suggest that these mechanisms operate largely independently of one another. This lack of strong association indicates that possessing one particular trait does not reliably predict the presence of other beneficial functions. Selecting strains based on a single trait alone could therefore risk excluding isolates with complementary agronomic potential.

Combining PCA and hierarchical clustering allowed us to identify four distinct functional groups, illustrating the considerable metabolic diversity within the collection. This kind of integrative approach is particularly well suited to selecting PGPR candidates, since agronomic efficacy rarely depends on a single mechanism but rather on a combination of complementary functional traits (Mącik et al. 2020).

The *in planta* results show that bacterial application significantly influences durum wheat growth, with clear effects of treatment, health condition, and their interaction. This interaction indicates that isolate efficacy depends on the pathological

context, confirming that PGPR performance is shaped not only by intrinsic potential but also by the capacity to interact with the surrounding biological environment.

Fusarium inoculation led to a significant reduction in control plant growth, confirming the detrimental effect of these pathogens on vegetative development. The losses observed are consistent with the mechanisms described for Fusarium species responsible for root system disruption, water-uptake restriction, and physiological alterations that ultimately reduce plant biomass (Miedaner and Juroszek 2021). Several isolates, however, partially mitigated these negative effects. The specific response of BNJ2 against F. culmorum and of BNJ27/BNJ35 against F. pseudograminearum points to strain–pathogen-dependent interactions. Similar patterns have already been reported for PGPR, where antagonistic efficacy varies depending on the target phytopathogen, the metabolites produced, and the interaction mechanisms involved (Köhl et al. 2019).

The central finding of this study is the superior performance of the Biostim consortium compared with individually applied isolates. The consortium consistently improved plant growth across all three health conditions and maintained its effectiveness under pathogen pressure. This superiority can likely be explained by several complementary mechanisms. First, the diversity of microorganisms within a consortium may enable functional complementarity, with each strain contributing through a different mechanism: hormonal stimulation, nutrient acquisition, antifungal metabolite production, or ecological competition with the pathogen. Second, the coexistence of multiple strains may improve the stability of rhizosphere colonization and reduce dependence on any single set of environmental conditions (Arif et al. 2020).

The clear separation of Biostim in both the PCA and hierarchical clustering analyses confirms its distinct physiological behavior relative to the individual isolates. These results support the idea that a well-designed consortium can produce a response greater than the simple sum of its individual components' performances, through positive interactions among microorganisms. Designing effective consortia, however, requires careful selection to avoid negative interactions between strains. Approaches that combine functional characterization with multivariate analysis, as applied in this study, therefore represent a relevant strategy for developing next-generation biofertilizers.

5. CONCLUSION

This study highlights the functional richness of fluorescent bacteria isolated from the durum wheat rhizosphere and confirms their potential as biological agents supporting plant growth. Characterization of the 35 isolates revealed a strong prevalence of traits associated with vegetative stimulation, particularly IAA production, phosphate solubilization, and extracellular enzymatic activities, while direct antagonistic activity against Fusarium proved rarer and more discriminating.

The integrative approach combining functional analysis with multivariate methods made it possible to identify distinct microbial profiles and to rationally select isolates with complementary properties. The *in planta* trials confirmed that PGPR performance depends on the health context and on the specific interaction between the bacterial strain and the pathogen involved.

The central finding of this study is the consistent superiority of the Biostim consortium over individual inoculations. It significantly stimulated durum wheat growth both under unstressed conditions and in the presence of *F. culmorum* and *F. pseudograminearum*, indicating greater functional stability and a stronger capacity to sustain plant growth under pathogen pressure. This efficacy suggests that combining several complementary strains can yield benefits greater than those achieved with individually selected isolates.

Overall, the Biostim consortium represents a promising approach for developing bioinoculants suited to sustainable cereal production. Further investigation under semi-controlled and field conditions will nonetheless be needed to confirm its ecological stability, agronomic reproducibility, and potential for large-scale application. Moreover, the use of natural biostimulants such as Biostim may contribute to protecting human health by reducing reliance on chemical inputs, promoting more environmentally friendly agricultural practices, and supporting the production of safer, healthier, and more sustainable food.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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