



Assessment of strain-dependent variation of phytostimulatory activity in *Streptomyces ambofaciens*-based bioformulations for agricultural applications

O. V. Andriushchenko, I. V. Strashnova, T. V. Ivanytsia, V. O. Ivanytsia, M. B. Galkin

Odesa National I. I. Mechnikov University, Odesa, Ukraine

Article info

Received 09.09.2025

Received in revised form

19.10.2025

Accepted 14.11.2025

Odesa National

I. I. Mechnikov University,

Vsevoloda Zmiienka st., 2,

Odesa, 65082, Ukraine.

Tel.: +38-097-938-22-91.

E-mail:

kgalkin@onu.edu.ua

Andriushchenko, O. V., Strashnova, I. V., Ivanytsia, T. V., Ivanytsia, V. O., & Galkin, M. B. (2025). Assessment of strain-dependent variation of phytostimulatory activity in *Streptomyces ambofaciens*-based bioformulations for agricultural applications. *Regulatory Mechanisms in Biosystems*, 16(4), e25206. doi:10.15421/0225206

The biotechnological potential of *Streptomyces ambofaciens* is highly strain-dependent, yet practical evaluations of how different strains influence the effects of microbial complexes used for agricultural bioformulations remain unstudied. In this investigation, we evaluated two microbial complexes differing only in their *Streptomyces* component: complex A1 containing *S. ambofaciens* ONU 1016, and complex A3 containing *S. ambofaciens* ONU 561, both combined with *Bacillus subtilis* ONU 1125 and *Trichoderma harzianum* LBX-181. Barley grains were inoculated with microbial suspensions across a concentration range to reveal how *Streptomyces* strain selection affects complex-mediated plant growth responses. Our findings showed that *S. ambofaciens* strains acted differently when applied alone versus in microbial complexes, reshaping the plant growth responses. *S. ambofaciens* ONU 1016 and ONU 561, as single inoculants, increased the fresh weight of the barley seedlings by 9.0–51.4% when applied as 75% suspensions. This gain in weight was the result of ability of the studied strains to increase leaf area and height of the barley seedlings at this concentration. PCA showed that the effects of *S. ambofaciens* ONU 1016 and ONU 561 on plant growth, similar at 10–50% concentrations (distance in PCA space 0.1–0.5), began to diverge when 75% suspensions were applied: ONU 1016 acted evenly across height, leaf area, and roots, whereas ONU 561 shifted away from root promotion, with the distances between them in PCA space 1.7 at 75% and 2.1 at 100%. When included into microbial complexes with *B. subtilis* ONU 1125 and *T. harzianum* LBX-181, differences in phytostimulatory effects became more pronounced. The A1 complex consistently promoted both aboveground biomass and roots at all concentrations, although increases in fresh weights were significant only at 10% and 25%. The A3 complex increased fresh weight only at 10% due to improved leaf growth, but at higher concentrations it only promoted root development while suppressing aboveground biomass growth. PCA confirmed that the A1 and A3 were far apart, with distances of 4.0–5.4 across concentrations, reflecting their fundamentally different orientations. Thus, the inclusion of either strain determines whether multi-microbial mixtures support balanced plant growth (A1) or only root-focused responses (A3). The selection of an appropriate *S. ambofaciens* strain is crucial for enhancing the performance of microbial fertilizers.

Keywords: plant growth promotion; barley; mono-strain inoculants; multi-species inoculants; biofertilizers.

Introduction

Among plant growth promoting bacteria (PGPB) strain-specific differences are known (Matselyukh et al., 2020; Ramírez-Pool et al., 2024; Kulik et al., 2025). Multi-strain or multi-species inoculants for plant growth promotion are carefully investigated with regard to the character of interactions between their participants (Prigigallo et al., 2023). Consortia demonstrating microbial synergy are selected for greenhouse and field experiments. Such complexes can exert much stronger beneficial influences than their single components. Thus, global meta-analysis of 51 live-soil studies showed that microbial complexes improved plant growth by 48% and pollution remediation by 80%, whereas the effects of single-strain inoculants did not reach such a high extent, although they were also very favorable: 29% and 48%, respectively (Liu et al., 2023). Microbial consortia do not behave like the average effects of their participants – complex, subtle relations between microorganisms make the actions of multi-strain complexes unique (Sharma et al., 2025).

Being known as PGPB, *Streptomyces* act as effective stimulators and protectors by producing beneficial compounds (Kirubakaran et al., 2025), promoting plant- and soil-beneficial microbiota (Gates et al., 2023; Sun et al., 2025), and by activating plant defense systems (Ankati et al., 2021) alone and in consortia (Dev et al., 2024). Thus, *Streptomyces* sp. STD57 showed antagonistic activity against *Ralstonia solanacearum* and enhanced growth of tomato, wheat and rice via the synthesis of indoleacetic acid (IAA) (He et al., 2024). Improvement of tobacco seedling development by almost 100% was explained by the action of antifungal volatile compounds 2,4-bis(1,1-dimethylethyl)-phenol and hexanedioic acid dibutyl ester produced by

Streptomyces sp. TOR3209. Presence of these compounds regulated the expression of genes in plant genomes (He et al., 2022). Different strains of *Streptomyces* were able to solubilize phosphorus and produce siderophores (Orouji et al., 2023).

Strain-specific differences of *Streptomyces* determine the possibility of using certain inoculants in agriculture. For instance, strain *S. microflavus* AtB-42 could exhibit beneficial synergistic activity (Staropoli et al., 2021) or antagonistic activity with different strains of *T. harzianum* (Prigigallo et al., 2023). Among 17 *Streptomyces* strains, four strains increased the dry weight of cucumber roots and shoots. Three of these strains were able to solubilize phosphorus and produce siderophores, and all synthesized IAA. Use of the strain SS12 with the best activity made it possible to reduce the dosage of NPK fertilizers and increase the yield of fruits by 40% (Orouji et al., 2023).

Despite vast genome analyses conducted for several *S. ambofaciens* strains (Nguyen et al., 2010; Fondi et al., 2017), information about the diversity and biotechnological potential of this species is still scarce in the literature. It is known that strains of *S. ambofaciens* differ in antagonistic activity (Potapenko et al., 2025). *S. ambofaciens* S-2 was effective against *Colletotrichum gloeosporioides* in red chilli fruits (Heng et al., 2015). *S. ambofaciens* HCIG17 synthesized IAA but did not show the ability for phosphate mobilization (Özdemir Kocak, 2019). In contrast, *S. ambofaciens* 63 was characterized by phyate-degrading activity (Ghorbani-Nasrabadi et al., 2012). Thus, investigations into the biotechnological potential of *S. ambofaciens* strains remain highly relevant today.

Representatives of the *Bacillus* genus tend to enhance plant growth and soil health when mixed in consortia, including the mono-

genus complexes (Ali et al., 2021; Liu et al., 2025). Treatment with *Streptomyces pactum* Act12 and consortium of bacilli, including *B. subtilis* and *B. licheniformis* in a ratio 1:1, improved uptake of metals in polluted soil, increased content of enzymes, and promoted plant-based bioremediation (Ali et al., 2021).

Moreover, consortia including *Bacillus*, *Streptomyces*, and other PGPB are known to influence the plant microbiome by changing the number of certain bacterial and fungal groups, shifting toward the thriving of beneficial microorganisms (Schmidt et al., 2014; Vuolo et al., 2022). A consortium based on eight strains of *Streptomyces* and *Bacillus* promoted plant growth, increased availability of nutrients in soil, inhibited phytopathogens, and enhanced the amount of beneficial microorganisms in experiments with apple seedlings (Qiao et al., 2024).

Fungi of the *Trichoderma* genus are part of many commercial formulations – both mono-species and poly-species consortia (Jin & Alberti, 2025), and new strains with effective properties are currently being discovered (Kuang et al., 2024). In consortia and in soil *Trichoderma* species interact with numerous microbiota representatives, participating in bacterial-bacterial or fungal symbioses (Reid & Gifford, 2024).

Trichoderma and *Streptomyces* enhanced plant biomass accumulation and increased the content of nutraceutical metabolites (Staropoli et al., 2021; Kabir et al., 2024). Complexes of *Trichoderma* and *Bacillus* spp. improved plant health, growth, and resilience to stress, as shown, for instance, in the remediation of saline soils (Santoyo et al., 2024). Treatment of seeds with a complex of *B. subtilis* and *T. harzianum* clearly enhanced the biomass and height of soy bean plants, and positively affected the Rhizobiaceae (Rigobelo et al., 2024).

The aim of our research was to investigate the strain-dependent differences in *Streptomyces ambofaciens* included in two microbial complexes with bacilli and trichodermas.

Materials and methods

Streptomyces ambofaciens ONU 1016 and *S. ambofaciens* ONU 561 were initially isolated from mussels *Mytilus galloprovincialis* gathered in the Black Sea, Odesa Bay, and *B. subtilis* ONU 1125 – from the deep bottom sediments of the northern-western part of the Black Sea (Shtenikov et al., 2018). *Trichoderma harzianum* LBX-181 was isolated at the Scientific and Production Association 'AgroBioInnovation'. These strains were chosen as microbial fertilizers due to their safety and significant antagonistic activity (Shtenikov et al., 2018; Andriushchenko et al., 2024).

The complexes named "A1" and "A3" included *B. subtilis* ONU 1125 and *T. harzianum* LBX-181, and differed in *Streptomyces* strains: A1 included *S. ambofaciens* ONU 1016, while A3 included *S. ambofaciens* ONU 561.

Bacilli were cultivated for three days, and *Streptomyces* – for 7 days, in a liquid nutritional medium containing (g/L): corn extract – 25.0; beetroot molasses – 25.0; malt extract – 20.0; yeast extract – 15.0; demineralized whey – 12.0; KH_2PO_4 – 2.0; K_2HPO_4 – 1.5; KNO_3 – 5.0; MgSO_4 – 0.5; FeSO_4 – 0.01; rapeseed oil-based antifoaming agent – 0.001; pH 6.5–7.0. Conditions of cultivation: 28 ± 1 °C, 210 rpm.

Trichoderma were cultivated on wheat bran for four days at $+28 \pm 1$ °C, and subsequently washed with sterile distilled water (SDW).

Bacterial cells and conidia were adjusted to 10^9 CFU/mL using SDW. Such suspensions, containing cultural liquid, cells and spores, were used for treatment of the grains alone and in complexes (1:1:1 ratio) at different percentages.

Spring barley (*Hordeum vulgare* L.), cultivar "Irina", grains were treated for 15 min with mono-strain or poly-species suspensions, and then sown in soil-filled trays (Chernozem soil) in ThermoStable GC-1000 plant growth chambers.

Each variant of treatment included 1000 grains. The next variants were simultaneously investigated:

- water control (grains were soaked in SDW);

- *S. ambofaciens* ONU 1016 (10%, 25%, 50%, 75%, 100%);
- *S. ambofaciens* ONU 561 (10%, 25%, 50%, 75%, 100%);
- *B. subtilis* ONU 1125 (10%, 25%, 50%, 75%, 100%);
- *T. harzianum* LBX-181 (10%, 25%, 50%, 75%, 100%);
- A1 complex (10%, 25%, 50%, 75%, 100%);
- A3 complex (10%, 25%, 50%, 75%, 100%).

Temperatures in the cultivation chambers ranged from $+18 \pm 1$ °C at night to $+25 \pm 1$ °C during the day, and relative air humidity was maintained at 50%. Lighting conditions corresponded to the photoperiod typical for April and were controlled automatically.

Three independent experiments were carried out with all variants mentioned above.

Seedlings were randomly selected (100 from each variant) on the 5th, 7th, 10th, 13th, 16th, 19th, 22nd, and 25th days of the experiment. Fresh weight of the whole plant (g), height (mm), root length (mm) and leaf area (mm^2) were estimated. Leaf area was evaluated as:

$$\text{leaf area} = \text{leaf length} \times \text{leaf width} \times 0.65 \text{ (Yuvchuk, 2023)}.$$

Mean values (\bar{x}) \pm standard errors (SE) were calculated across three independent experiments ($n = 300$). A one-way analysis of variance (ANOVA) with Tukey's Honest Significant Difference (HSD) test ($P < 0.05$) was performed separately for each experimental day to evaluate treatment effects.

Principal component analysis (PCA) was applied to the standardized mean values of the measured traits in order to reduce the dataset into two main components (PC1 and PC2). The proportion of total variance explained by each component was determined. A PCA biplot was then constructed to illustrate both the treatment centroids and the corresponding feature vectors (arrows). To improve interpretability, the feature vectors were scaled in relation to the PCA scores.

Scipy.stats, Math, Pandas, Sklearn.decomposition, Numpy, Matplotlib, and Seaborn libraries in Python 3.9 (Python Software Foundation, USA, 2024) were used for analysis and graphical outputs.

Results

The effect of microbial suspensions – both mono- and polyspecies – clearly depended on the concentration of the inoculum used. When comparing the average root length of seedlings on the final, 25th day of the experiment, treatment with the A3 complex resulted in 30.0–48.0% improvement in average root length at suspension concentrations of 25–75% (Fig. 1).

The A1 complex was efficient at all applied concentrations, increasing root length by 55.1–102.0%. The best result was obtained with the suspension of 25% concentration.

When investigating the dynamics of root growth, at all concentrations except 25%, the influence of the A1 complex occurred earlier than that of the A3 complex, starting already on the 5th day – the first time point of the experiment. At 25% concentration, by contrast, the effect of the A3 complex appeared earlier and was equal to or higher than that of the A1 until the 19th day of the experiment.

As for *S. ambofaciens* alone, in contrast to the complexes, both strains were not effective in enhancing root length. *T. harzianum* LBX-181 alone showed a root growth-promoting effect at 25–50% (16.0–27.6% increase, respectively). Higher concentrations were not effective. Inoculation of seeds with *B. subtilis* ONU 1125 enhanced root growth by 32.4–58.0% when suspensions of 25–75% concentrations were applied. A 100% concentration decreased the average root length of barley seedlings.

Average height of barley seedlings increased after treatment of seeds with all tested concentrations of the A1 complex. In contrast, application of the A3 complex retarded plant height (Fig. 2).

Surprisingly, the same decreasing effect was observed in seedlings after the treatment with low (10%, 25%) concentrations of mono-strain suspensions (*S. ambofaciens* ONU 1016 and ONU 561), while treatment with 50–75% concentrations had a clear positive effect. In the case of *S. ambofaciens* ONU 1016, the increase reached 19.3–23.5%, in the case of *S. ambofaciens* ONU 561 – 26.0–34.0%. *S. ambofaciens* ONU 1016 alone also improved seedling height by 34.5% at 100% of suspension.

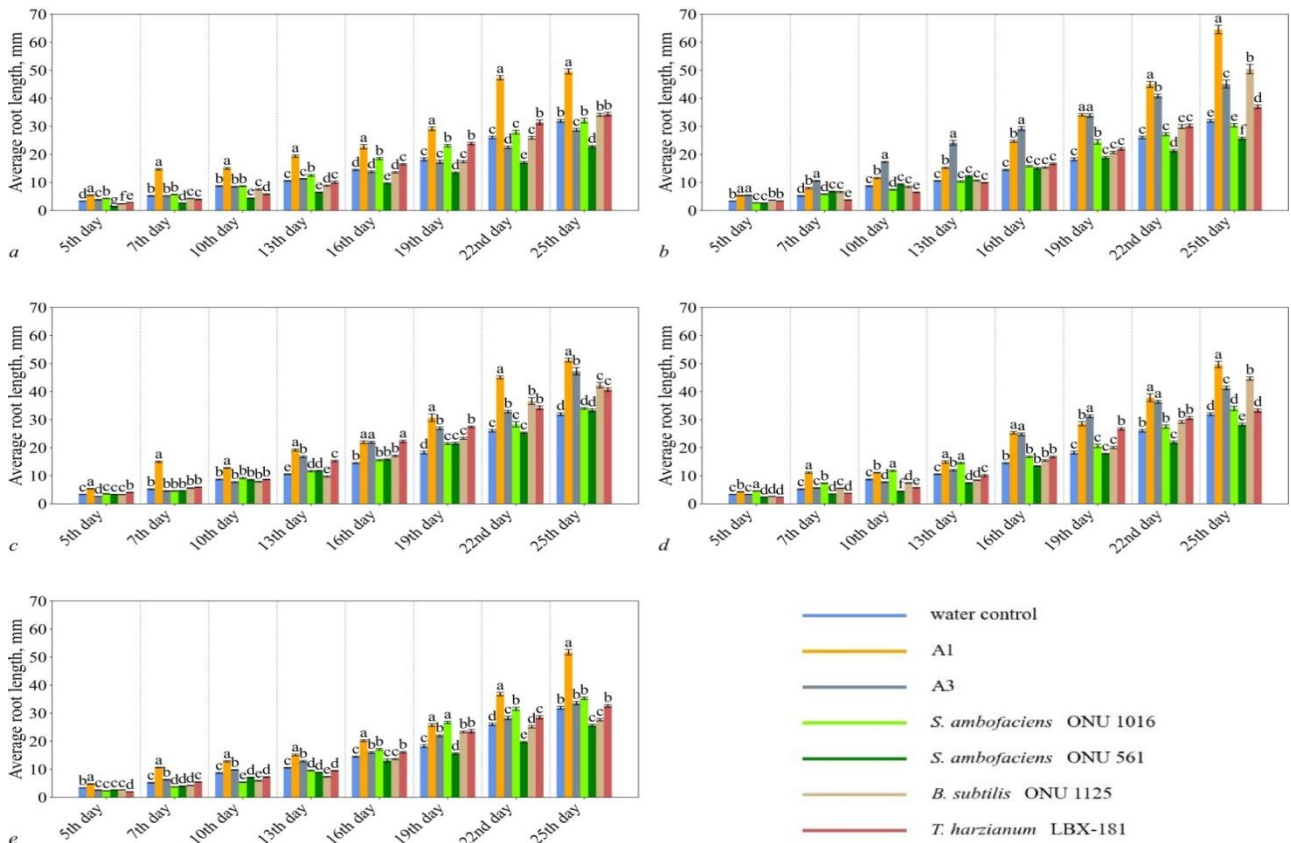


Fig. 1. Effect of barley grain inoculation with microbial suspensions at different concentrations (*a* – 10%, *b* – 25%, *c* – 50%, *d* – 75%, *e* – 100%) on the average root length of seedlings ($\bar{x} \pm SE$; $n = 300$): different letters above the bars indicate statistically significant differences between treatment variants within each day ($P < 0.05$, one-way ANOVA with Tukey’s HSD test)

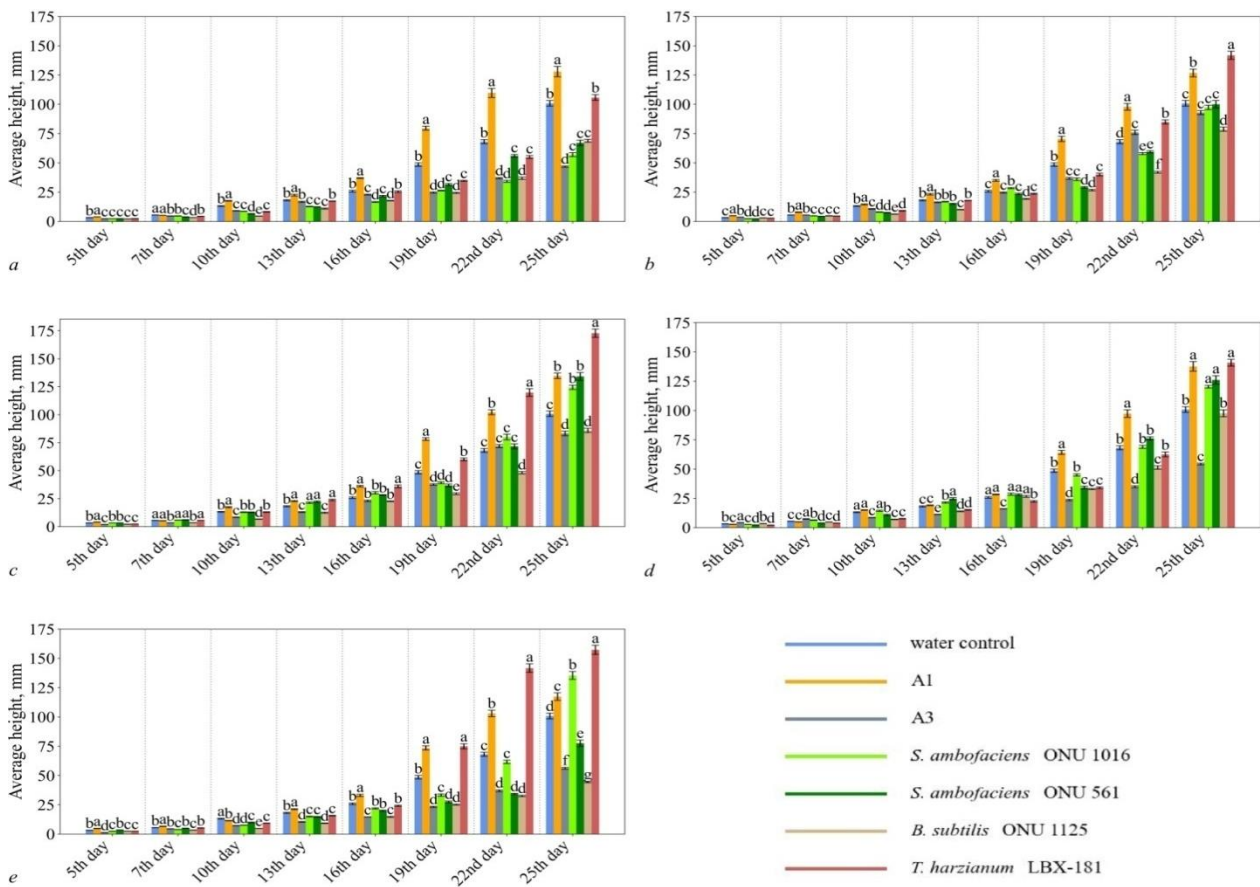


Fig. 2. Effect of barley grain inoculation with microbial suspensions at different concentrations (*a* – 10%, *b* – 25%, *c* – 50%, *d* – 75%, *e* – 100%) on the average height of seedlings ($\bar{x} \pm SE$; $n = 300$): different letters above the bars indicate statistically significant differences between treatment variants within each day ($P < 0.05$, one-way ANOVA with Tukey’s HSD test)

The mono-species suspension of *B. subtilis* ONU 1125 inhibited barley height at all concentrations, whereas *T. harzianum* LBX-181 had a positive impact on this morphological parameter at 25–100% concentrations of the fungal suspension.

Treatment with the A1 complex at concentrations of 10–75% increased the average leaf area by 18.4–45.0% (Fig. 3).

The A3 complex positively influenced this morphological trait only at 10% of suspension, resulting in a 26.1% increase, whereas higher concentrations of the A3 reduced average leaf area.

Mono-strain treatment with *S. ambofaciens* ONU 1016 was effective at 75% suspension, enhancing leaf growth by 34.1%. Improvement after the treatment with *S. ambofaciens* ONU 561 reached 12.6–14.0% at 50–75% concentrations of bacterial suspensions (Fig. 3).

Inoculation of grains with 10–75% suspension of *B. subtilis* ONU 1125 led to 10.0–23.1% increase in average leaf area per plant. Treatment with 25–75% suspension of *T. harzianum* LBX-181 resulted in 11.1–45.0% enhancement of leaf area growth.

On the final day of the experiment, the gain in fresh weight after the treatment with 10% suspension was nearly the same – 11.2% and 12.2% for the A1 and A3 complexes, respectively (Fig. 4).

However, investigation of growth dynamics showed that the sharp increase in weight caused by the A1 complex at 10% occurred as early as the 5th day, whereas the phytostimulating activity of the A3 complex was first detected on the 13th day. Starting from this time point, the effects of the two complexes on seedling fresh mass followed similar patterns, with stable growth enhancement from the 22nd day (Fig. 4).

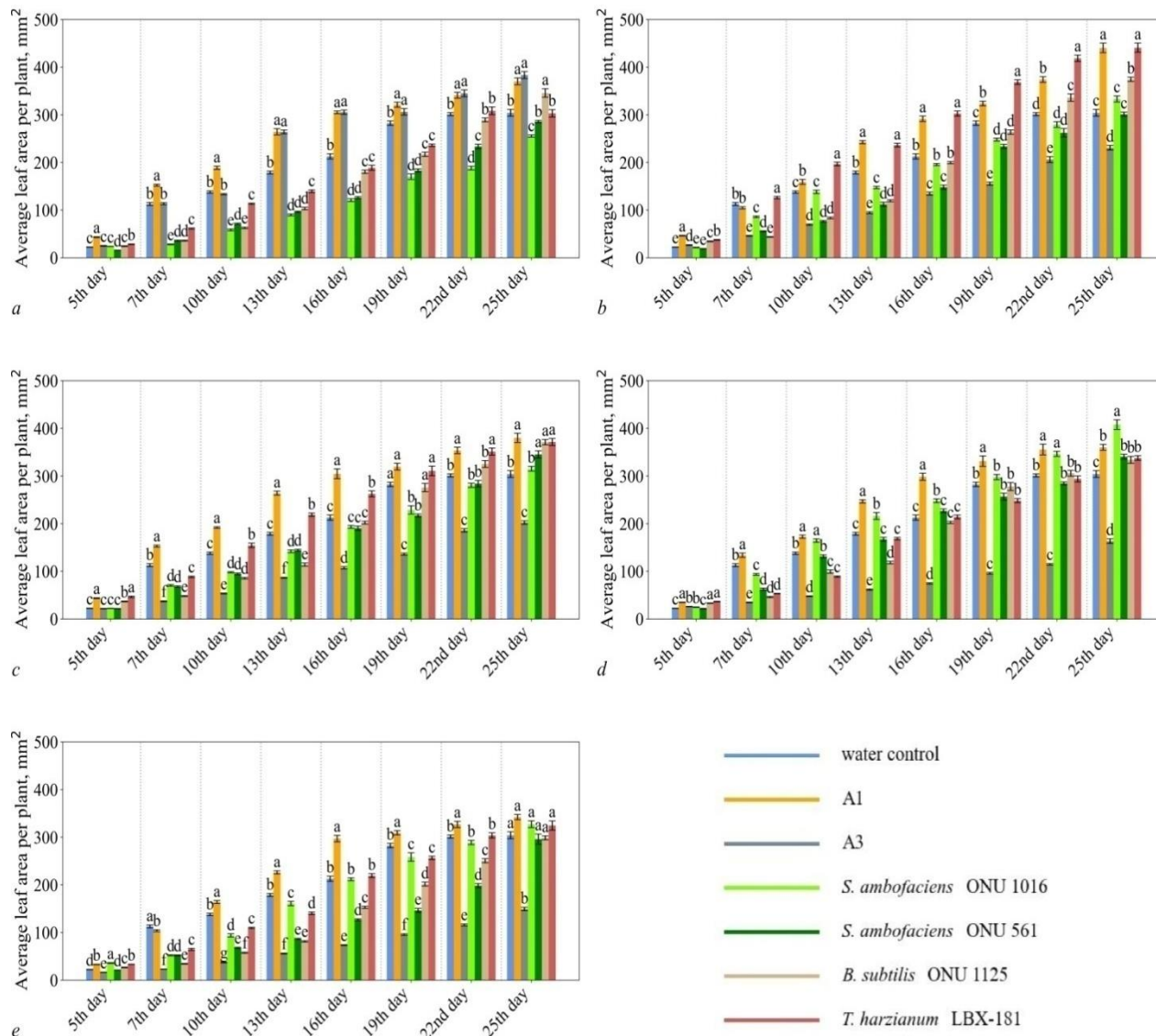


Fig. 3. Effect of barley grain inoculation with microbial suspensions at different concentrations (*a* – 10%, *b* – 25%, *c* – 50%, *d* – 75%, *e* – 100%) on the average leaf area of seedlings ($\bar{x} \pm SE$; $n = 300$): different letters above the bars indicate statistically significant differences between treatment variants within each day ($P < 0.05$, one-way ANOVA with Tukey’s HSD test)

Increasing concentrations of the inoculate revealed different phytostimulating potentials of the studied complexes. Treatment of seedlings with 25% suspension retarded the promoting effect of the A1, which appeared only on the 13th day, whereas the enhancement due to the A3 complex occurred earlier – starting from the 7th day – but disappeared by the 16th day of the experiment. Further increases in concentration (50%, 75%, 100%) had a negative effect in the case of the A3 complex, preventing the manifestation of its stimulating activity on any day of the experiment. In the case of the A1 complex, phytostimulatory activity with a fresh weight gain of 26.2% was detected at

25% concentration. Higher concentrations did not produce a clear promoting effect, as the average weight was not significantly different from that of the water control.

Mono-strain treatments had different impacts clearly depending on concentration. At 10% and 100%, a decrease in weight was detected in case of all inoculates. Mono-strain treatments with *S. ambofaciens* ONU 1016 and *S. ambofaciens* ONU 561 showed similar dynamics at 10%, 25%, 50%, whereas at 75% and 100% the results of the two treatments belonged to statistically different groups. *S. ambofaciens* ONU 1016 had the strongest stimulating effect on fresh weight at

75%, while at this concentration *S. ambofaciens* ONU 561 did not differ significantly from the water control. *T. harzianum* LBX-181 was effective only at 25%, and *B. subtilis* ONU 1125 promoted fresh weight of the plants at concentrations of 25–75% (Fig. 4).

PCA revealed that *S. ambofaciens* ONU 561 and ONU 1016 clustered closely at low doses but diverged strongly at higher ones (Fig. 5). At 10% both strains occupied close positions in PCA space (ONU 1016: PC1 = -1.74, PC2 = -0.98; ONU 561: PC1 = -1.85,

PC2 = 0.03) indicating similar effects on seedlings. At 25% and 50%, they remained nearly indistinguishable (PC1 separation < 0.5; PC2 separation < 0.2), which allowed us to suggest that at these concentrations their contributions to plant growth were very close.

However, at 75%, their profiles began to diverge sharply with ONU 561 (PC1 = -0.50, PC2 = -1.39) shifted in the opposite direction from root promotion as compared to ONU 1016 (PC1 = -1.78, PC2 = -0.20). At 100% this divergence was the greatest.

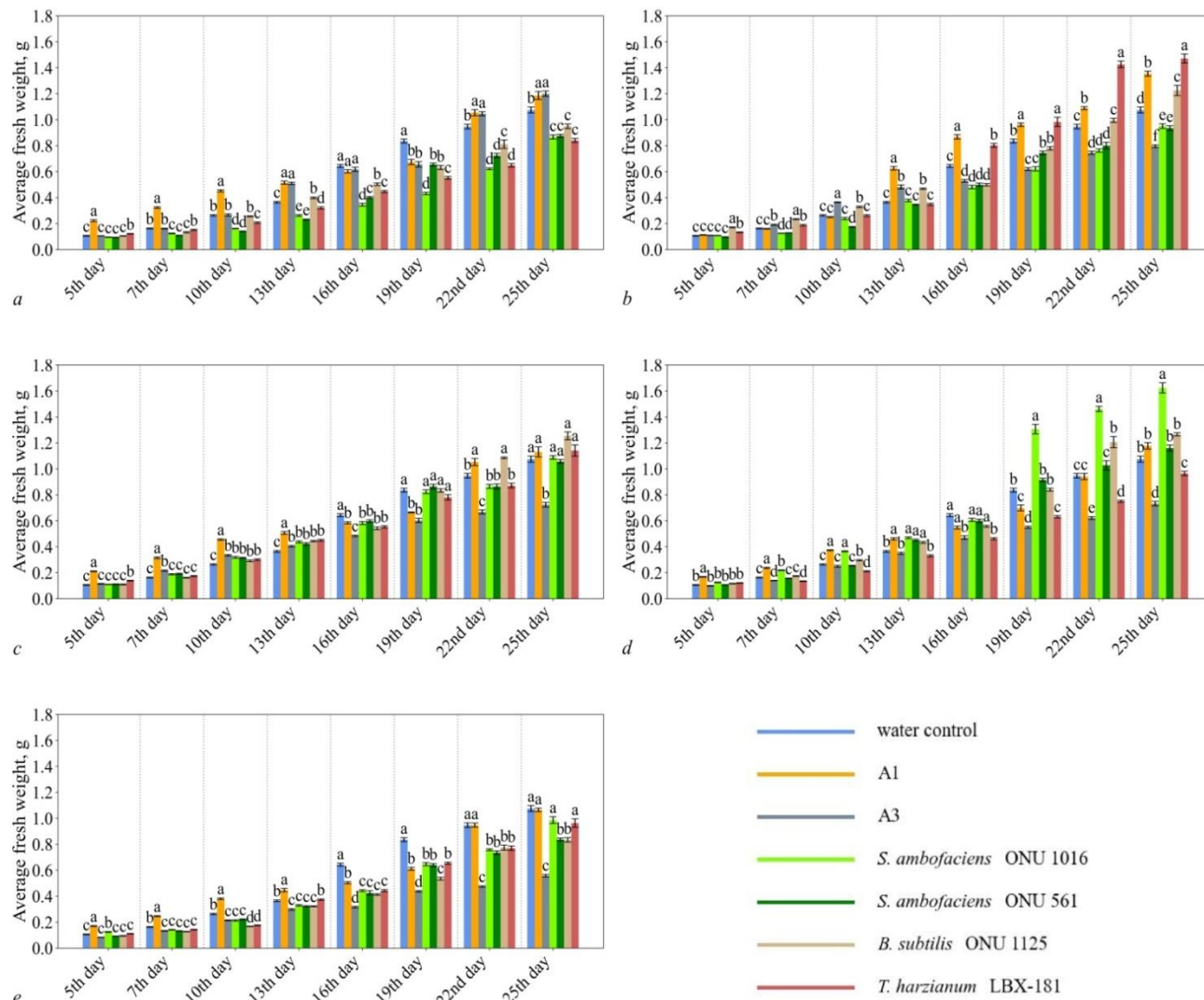


Fig. 4. Effect of barley grain inoculation with microbial suspensions at different concentrations (*a* – 10%, *b* – 25%, *c* – 50%, *d* – 75%, *e* – 100%) on the average fresh weight of seedlings ($\bar{x} \pm \text{SE}$; $n = 300$): different letters above the bars indicate statistically significant differences between treatment variants within each day ($P < 0.05$, one-way ANOVA with Tukey’s HSD test)

The A1 complex consistently aligned with both aboveground plant biomass and root vectors (for instance, PC1 = 2.75, PC2 = 1.08 at 25%; PC1 = -1.56, PC2 = 2.06 at 75%), showing improvements across all traits at all concentrations except 100% (Fig. 5).

In contrast, the A3 complex separated along the root axis at the majority of concentrations (for instance, PC1 = -1.11, PC2 = 2.02 at 25%; PC1 = -2.45, PC2 = 1.34 at 100%), showing strong root promotion (Fig. 5). Other traits (leaf area and fresh weight) were clearly promoted only after the treatment with 10% of suspension. Surprisingly, at this concentration root growth was not stimulated.

Discussion

Strain-specific differences of *S. ambofaciens* are known (Ghorbani-Nasrabadi et al., 2012; Özdemir Koçak, 2019; Potapenko et al., 2025), still the information about this species remains scarce. In our study, concentration was a key in barley seed treatment. Both *S. ambofaciens* strains improved fresh weight (increase of 9.0–51.4%)

when applied as 75% mono-strain inoculates. This reflected their ability to increase leaf area (12.6–34.1%) and height (19.3–26.0%) of the seedlings at 75% concentration. When lower or higher amounts of cells were applied for seed treatment, no improvement of fresh weight was observed.

S. ambofaciens ONU 1016 was more effective in increasing the average height of the seedlings, reaching a 34.5% gain at 100% of inoculate concentration. Despite the similarity in weight gain at 75%, PCA revealed that while the effects of *S. ambofaciens* strains on plant growth were very close at lower concentrations, they began to diverge at 75%. *S. ambofaciens* ONU 1016 influenced all the studied morphological traits – average height, leaf area, and root length of the seedlings – in a more balanced way (PC1 = -1.78, PC2 = -0.20), whereas *S. ambofaciens* ONU 561 diverged in the opposite direction from root growth enhancement (PC1 = -0.50, PC2 = -1.39). Thus, with increasing concentrations, the difference between the effects of the studied strains on plant growth became more obvious.

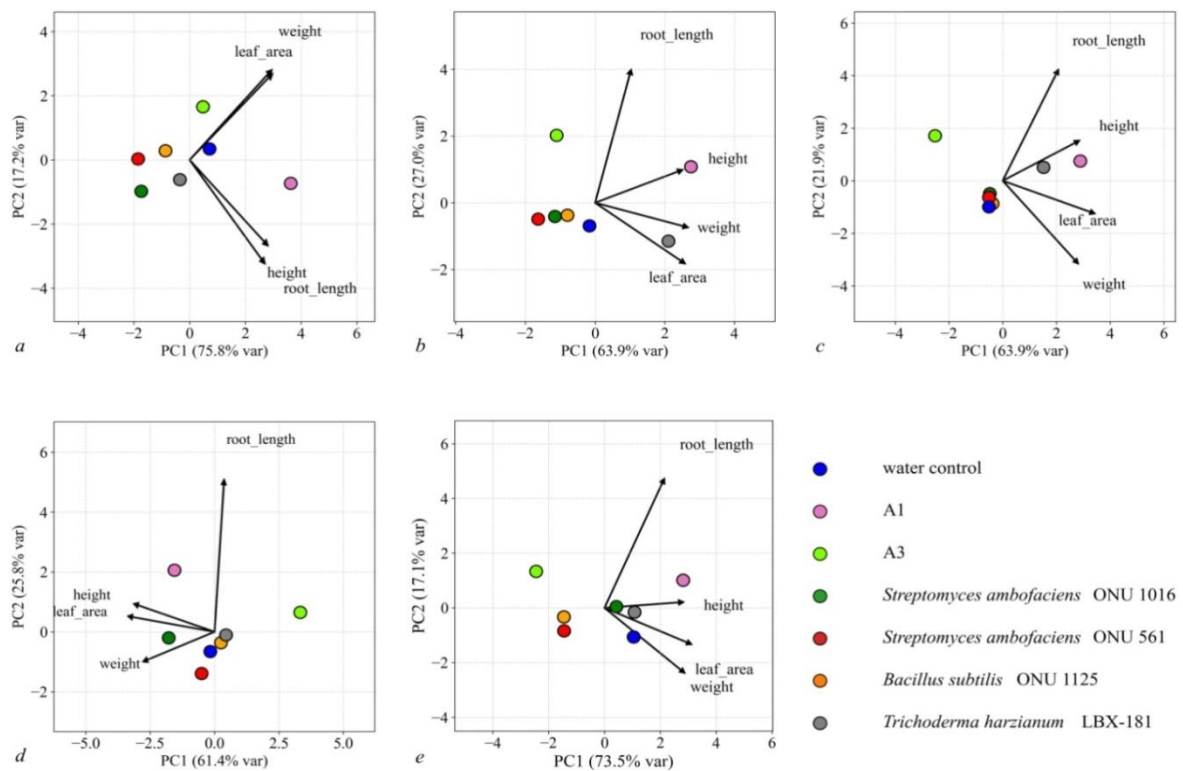


Fig. 5. PCA biplot of morphological traits of barley seedlings ($n = 300$) inoculated with microbial suspensions at different concentrations ($a - 10\%$, $b - 25\%$, $c - 50\%$, $d - 75\%$, $e - 100\%$): treatment centroids are shown as colored points, while arrows represent feature loadings (root length, shoot height, leaf area, fresh weight)

Interactions between microorganisms in complex inoculates change the initial phytostimulating activities of the strains (Prigigallo et al., 2023). Indeed, more differences in strain effects could be revealed when *S. ambofaciens* ONU 1016 and *S. ambofaciens* ONU 561 were included in the multimicrobial mixtures. Being parts of the inoculates together with *B. subtilis* ONU 1125 and *T. harzianum* LBX-181, *Streptomyces* strongly shaped the effect of the microbial mixtures on the seedling growth. Thus, addition of *S. ambofaciens* ONU 561 into the A3 mixture improved the fresh weight of the barley by 12.2% only when a low 10% concentration of the complex was applied. Most probably, such positive effect occurred due to the increase in leaf area (26.1%). PCA supported this, showing that the A3 at 10% ($PC1 = 0.48$, $PC2 = 1.66$) clustered closer to leaf area. At higher concentrations, the A3 was not effective in improving aboveground growth ($PC1$ consistently negative at 25–100%) while shifting toward root orientation ($PC2 = 1.34$ at 100%). Higher concentrations decreased the total fresh weight of the seedlings by inhibiting leaf area and height of the seedlings.

Thus, it can be seen that being a leaf growth stimulator at 50–75% when applied alone, *S. ambofaciens* ONU 561 lost this activity when used in the complex with *B. subtilis* ONU 1125 and *T. harzianum* LBX-181. Moreover, bacilli and trichodermas were also leaf stimulators when applied as mono-strain suspensions, but the resulting mixture – the A3 complex, did not show a positive effect on the aboveground plant growth except for 10% suspensions. It was the gain in leaf biomass (by 26.1%) that resulted in fresh weight improvement after seed treatment with 10% concentration. Strong root development (41.1–48.0% increase) was achieved after the treatment with 25–75% concentrations of the A3, as reflected by the positive $PC2$ loadings for root length at these levels.

The same improvement in root development was also found for the A1 complex with *S. ambofaciens* ONU 1016 included – even to a greater extent (55.1–102.0%) – together with remarkable improvements in height (16.5–36.6%) and leaf area (18.4–45.0%). Thus, inclusion of *S. ambofaciens* ONU 1016 in the bacilli-trichodermas complex resulted in strong aboveground and root growth at all concentrations of the suspensions. PCA confirmed this, placing A1 consistently on the positive side of $PC1$ (for instance, $PC1 = 2.81$ at 100%) indica-

ting its combined promotion of aboveground plant biomass and roots. Our findings are consistent with the study of Haring et al. (2019) describing that while *Streptomyces* sp. strain by itself had no significant impact on growth of shallots, treatment with both *Streptomyces* sp. and *Trichoderma* sp. resulted in higher positive effect that treatment with *Trichoderma* alone. Changes in transcriptomes of *Streptomyces* and *Trichoderma* in consortia were described proving their clear mutual influence (Prigigallo et al., 2023).

The gain in fresh weight of the seedlings in the case of the A1 complex occurred at 10% and 25% concentrations, reaching 11.2–26.2%. The present results are in accordance with the literature data about strain-dependent beneficial characteristics of PGPB. Thus, the positive effect of *T. harzianum* T22 on sorghum growth was promoted by *S. griseus* (Kabir et al., 2024). Consortium of *T. harzianum* T22 and *Streptomyces microflavus* AtB-42 enhanced the growth of shoot biomass of parsley and increased the content of metabolites with nutraceutical value (Staropoli et al., 2021). The same strain of *Streptomyces* and another *T. harzianum* strain (M10) already exhibited reciprocal antagonism on a solid nutrient medium. *Trichoderma* decreased the growth of *Streptomyces* in a liquid medium. No effect on tomato seedling height was observed after the treatment with *S. microflavus* AtB-42 + *T. harzianum* M10 consortium, still the root growth of tomatoes was hindered. Alone, *T. harzianum* M10 increased shoot fresh weight while *S. microflavus* AtB-42 could reduce root weight and height depending on the scheme of treatment. Still, resistance response in tomato seedlings were stronger in case of treatment with consortia compared to the treatment with single strains (Prigigallo et al., 2023). *Streptomyces* could promote growth of beneficial plant bacteria as it was shown for *Bacillus* spp. (Nonthakaew et al., 2022). In other studies this effect was not detected, as in the case of *Bradyrhizobium japonicum* in soy bean roots (de Andrade et al., 2025).

Future investigations of molecular bases of strain-specificity of *S. ambofaciens* intended for agricultural biotechnology are needed.

Conclusion

The present study demonstrated that *S. ambofaciens* exerts highly strain-dependent modulation of phytostimulatory effects in microbial complexes. Inclusion of *S. ambofaciens* ONU 1016 or *S. ambofaciens* ONU 561 distinguished their single-strain activity by redefining the phytostimulatory effect of the microbial mixtures, determining whether plants respond to seed inoculation with balanced growth improvement (the A1 complex) or mainly root development (the A3 complex). The concentration-dependent effects observed in this study further highlight that the efficacy of bioformulations containing *S. ambofaciens* is determined not solely by strain identity but also by amount of cells, indicating that optimization of inoculum concentration must be strain-specific. To our knowledge, this is the first study to examine how inoculation of grains with *S. ambofaciens* suspensions affects barley growth. The results highlight the importance of rational selection of *S. ambofaciens* strains and optimization of inoculum dosage in developing microbial fertilizers. Future investigations should focus on identifying the molecular mechanisms that enable individual *S. ambofaciens* strains to regulate the effects of microbial complexes on plant growth.

This work was supported by the Ministry of Education and Science of Ukraine (2024–2025, project No. 0124U001029 “Development of a biotechnology for the ecologization of cereal crop production using marine biological resources to restore the fertility of soils affected by military actions”).

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Ali, A., Li, Y., Jeyasundar, P.G.S.A., Azeem, M., Su, J., Wahid, F., Mahar, A., Shah, M.Z., Li, R., & Zhang, Z. (2021). *Streptomyces pactum* and *Bacillus* consortium influenced the bioavailability of toxic metals, soil health, and growth attributes of *Symphylum officinale* in smelter/mining polluted soil. *Environmental Pollution*, 291, 118237.
- Andriuschenko, O. V., Strashnova, I. V., Ivanytsia, T. V., Rakytska, S. I., & Galkin, M. B. (2024). Antagonistic activity of Black Sea actinobacteria against phytopathogenic microorganisms. *Microbiology and Biotechnology*, 62(3), 59–81.
- Ankati, S., Srinivas, V., Pratyusha, S., & Gopalakrishnan, S. (2021). *Streptomyces* consortia-mediated plant defense against *Fusarium* wilt and plant growth-promotion in chickpea. *Microbial Pathogens*, 157, 104961.
- de Andrade, da Silva, M. S. R., de Carvalho, L. A. L., Santos, C. H. B., Frezarin, E. T., da Silva, C. G. N., Pinheiro, D. G., Zonta, E., Baba-lola, O. O., & Rogobelo, E. C. (2025). Effect of co-inoculation with plant growth-promoting bacteria on the microbiome of soybean roots. *Frontiers Sustainable Food Systems*, 9, 1505001.
- de Souza, L. F., Oliveira, H. G., Pellegrinetti, T. A., Mendes, L. W., Bonatelli, M. L., Dumaresq, A. S. R., Sinatti, V. V., Pinheiro, J. B., Azevedo, J. L., & Quecine, M. C. (2025). Co-inoculation with *Bacillus thuringiensis* RZ2MS9 and rhizobia improves the soybean development and modulates soil functional diversity. *FEMS Microbiology Ecology*, 101(2), fiaf013.
- Dev, A. S. R., Harish, S., Karthikeyan, G., Nivedha, M., & Sangeetha, C. (2024). Consortia of *Streptomyces* spp. triggers defense/PAMP genes during the interaction of Groundnut bud necrosis orthospovirus in tomato. *3 Biotech*, 9, 196.
- Fondi, M., Pinatel, E., Talà, A., Damiano, F., Consolandi, C., Mattorre, B., Fico, D., Testini, M., De Benedetto, G.E., Siculella, L., De Bellis, G., Alfano, P., & Peano, C. (2017). Time-resolved transcriptomics and constraint-based modeling identify system-level metabolic features and over-expression targets to increase spiramycin production in *Streptomyces ambofaciens*. *Frontiers in Microbiology*, 8, 835.
- Gates, A. D., French, A. M., Demetros, A. A., Kelley, B. R., & Lebeis, S. L. (2023). A *Streptomyces* consortium contributes distinct microbial interactions during *Arabidopsis thaliana* microbiome assembly. *Phytophormones*, 7(4), 515–525.
- Ghorbani-Nasrabadi, R., Greiner, R., Alikhani, H.A., Hamed, J. (2012). Identification and determination of extracellular phytate-degrading activity in actinomycetes. *World Journal of Microbiology and Biotechnology*, 28(7), 2601–2608.
- Haring, F., Rostia, E., Syam'un, E., & Ginting, N.M. (2019). Effect of *Trichoderma* sp. and *Streptomyces* sp. on the growth and production of true seed shallots (TSS). *Earth and Environmental Science*, 343, 012020.
- He, D., Gao, C., Zhao, S., Chen, H., Li, P., Yang, X., Li, D., Zhao, T., Jiang, H., & Liu, C. (2024). Antibacterial, herbicidal, and plant growth-promoting properties of *Streptomyces* sp. STD57 from the rhizosphere of *Adenophora stricta*. *Microorganisms*, 12(11), 2245.
- He, Y., Guo, W., Peng, J., Guo, J., Ma, J., Wang, X., Zhang, C., Jia, N., Wang, E., Hu, D., & Wang, Z. (2022). Volatile organic compounds of *Streptomyces* sp. TOR3209 stimulated tobacco growth by up-regulating the expression of genes related to plant growth and development. *Frontiers in Microbiology*, 13, 891245.
- Heng, J. L. S., Shah, U., Rahman, N. A., Shaari, K., & Hamzah, H. (2015). *Streptomyces ambofaciens* S2 – a potential biological control agent for *Colletotrichum gleosporioides* the causal agent for anthracnose in red chilli fruits. *Journal of Plant Pathology and Microbiology*, 6, S1-006.
- Jin, S., & Alberti, F. (2025). Advances in the discovery and study of *Trichoderma* natural products for biological control applications. *Natural Products Reports*, 42, 1367–1386.
- Kabir, A. H., Thapa, A., Hasan, M. R., & Parvej, M. R. (2024). Local signal from *Trichoderma afroharzianum* T22 induces host transcriptome and endophytic microbiome leading to growth promotion in sorghum. *Journal of Experimental Botany*, 75(22), 7107–7126.
- Kirubakaran, R., Shameem, N., Saranya, E., Meenambigai, K., Dhanasekar, R., Parraj, J. A., Yadav, N., Singh, S., Rustagi, S., Puri, P., Sharma, B., Negi, R., & Yadav, A. N. (2025). *Streptomyces* as endomicrobiome: Potential bioinoculants for agricultural sustainability. *Journal of Applied Biology and Biotechnology*, 13(4), 115.
- Kuang, A., Fu, X., Liu, Z., Chen, Q., Jin, R., & Mao, H. (2024). Biocontrol effect of the complex inoculants of *Trichoderma* and *Bacillus amyloliquefaciens* on chrysanthemum white rust. *Biocatalysis and Agricultural Biotechnology*, 56, 103010.
- Kulik, T., Staniszewska, P., Wisniewski, P., Treder, Z., Przybylski, M., Wronska, E., Mazdziarz, M., Krawczyk, K., Bilska, K., Paukszt, L., & Olszewski, J. (2025). In-depth comparison of commercial *Trichoderma*-based products: Integrative approaches to quantitative analysis, taxonomy and efficacy. *Frontiers in Microbiology*, 16, 1646394.
- Liu, X., Mei, S., & Salles, J. F. (2023). Inoculated microbial consortia perform better than single strains in living soil: A meta-analysis. *Applied Soil Ecology*, 190, 105011.
- Liu, Y., Jia, B., Ren, Y., Xun, W., Stefanic, P., Yang, T., Miao, Y., Zhang, N., Yao, Y., Zhang, R., Xu, Z., Shen, Q., & Mandic-Mulec, I. (2025). Bacterial social interactions in synthetic *Bacillus* consortia enhance plant growth. *IMeta*, 4(4), e70053.
- Matselyukh, B. P., Golembiowska, S. L., & Bambura, O. I. (2020). Screening of soil *Streptomyces* – producers of antibiotics against phytopathogenic bacteria. *Mikrobiologichnyi Zhurnal*, 82(5), 36–40.
- Nguyen, H. C., Karray, F., Lautru, S., Gagnat, J., Lebrilhi, A., Ho Huynh, T. D., & Pernodet, J. (2010). Glycosylation steps during spiramycin biosynthesis in *Streptomyces ambofaciens*: Involvement of three glycosyltransferases and their interplay with two auxiliary proteins. *Antimicrobial Agents and Chemotherapy*, 54(7), 1602–1609.
- Nonthakaew, N., Panbangred, W., & Intra, B. (2022). Plant growth-promoting properties of *Streptomyces* spp. isolates and their impact on mung bean plantlets' rhizosphere microbiome. *Frontiers in Microbiology*, 13, 967415.
- Orouji, E., Fathi Ghare Baba, M., Sadeghi, A., Gharanjik, S., & Koobaz, P. (2023). Specific *Streptomyces* strain enhances the growth, defensive mechanism, and fruit quality of cucumber by minimizing its fertilizer consumption. *BMC Plant Biology*, 23, 246.
- Özdemir Koçak, F. (2019). Identification of *Streptomyces* strains isolated from *Humulus lupulus* rhizosphere and determination of plant growth promotion potential of selected strains. *Turkish Journal of Biology*, 43(6), 391–403.
- Potapenko, K., Lisiutin, G., Vasylieva, N., Strashnova, I., Franke, R., Petriv, N., Duduyemi, O. P., Baklan, K., Korotaieva, N., Gudzenko, T., Mamns, M. P., Broenstrup, M., Lenzen, H., Vital, M., Ivanytsia, V., & Yevsa, T. (2025). Antimicrobial and anticancer activity of *Streptomyces ambofaciens* (Myt 8) and *S. globisporus* ONU 1019 (Myt 11) secondary metabolites isolated from the Odesa Bay, the Black Sea: An *in vitro* study. *Biomedicine and Pharmacotherapy*, 186, 117981.
- Prigigallo, M. I., Staropoli, A., Vinale, F., & Bubici, G. (2023). Interactions between plant-beneficial microorganisms in a consortium: *Streptomyces microflavus* and *Trichoderma harzianum*. *Microbial Biotechnology*, 16, 2292–2312.
- Qiao, R., Xu, M., Jiang, J., Song, Z., Wang, M., Yang, L., Guo, H., & Mao, Z. (2024). Plant growth promotion and biocontrol properties of a synthetic community in the control of apple disease. *BMC Plant Biology*, 24, 546.
- Ramírez-Pool, J. A., Calderón-Pérez, B., Ruiz-Medrano, R., Ortiz-Castro, R., & Xocostle-Cazares, B. (2024). *Bacillus* strains as effective biocontrol agents against phytopathogenic bacteria and promoters of plant growth. *Microbial Ecology*, 87(1), 76.
- Reid, T. E., & Gifford, M. L. (2024). *Trichoderma* gets by with a little help from *Streptomyces*: Fungal-bacterial symbiosis in plant growth promotion. *Journal of Experimental Botany*, 75(22), 6893–6897.

- Rigobelo, E.C., de Andrade, L. A., Santos, C. H. B., Frezarin, E. T., Sales, L. R., de Carvalho, L. A. L., Guariz Pinheiro, D., Nicodemo, D., Babalola, O. O., Verdi, M. C. Q., Mondin, M., & Desoignies, N. (2024). Effects of *Trichoderma harzianum* and *Bacillus subtilis* on the root and soil microbiomes of the soybean plant INTACTA RR2 PROT.M. *Frontiers in Plant Sciences*, 15, 1403160.
- Santoyo, G., Orozco-Mosqueda, M. C., Afridi, M. S., Mitra, D., Valencia-Cantero, E., & Macias-Rodriguez, L. (2024). *Trichoderma* and *Bacillus* multifactorial allies for plant growth and health in saline soils: Recent advances and future challenges. *Frontiers in Microbiology*, 15, 1423980.
- Schmidt, R., Koberl, M., Mostafa, A., Ramadan, E. M., Monschein, M., Jensen, K. B., Rudolf, B., & Berg, G. (2014). Effects of bacterial inoculants on the indigenous microbiome and secondary metabolites of chamomile plants. *Frontiers in Microbiology*, 5, 64.
- Sharma, N., Mahawar, L., Mishra, A., & Albrechtsen, B. R. (2025). Microbial contributions to plant growth and stress tolerance: Mechanisms for sustainable plant production. *Plant Stress*, 17, 100966.
- Shtenikov, M. D., Ostapchuk, A. M., & Ivanytsia, V. O. (2018). Antagonistic activity of endospore forming bacteria of deep water the Black Sea sediments. *Microbiology and Biotechnology*, 43(3), 82–89.
- Staropoli, A., Vassetti, A., Salvatore, M. M., Andolfi, A., Prigigallo, M. I., Bubbici, G., Scagliola, M., Salerno, P., & Vinale, F. (2021). Improvement of nutraceutical value of parsley leaves (*Petroselinum crispum*) upon field applications of beneficial microorganisms. *Horticulturae*, 7(9), 281.
- Sun, T., Liu, H., Wang, N., Huang, M., Banerjee, S., Jousset, A., Xu, Y., Shen, Q., Wang, S., Wang, X., & Wei, Z. (2025). Interactions with native microbial keystone taxa enhance the biocontrol efficiency of *Streptomyces*. *Microbiome*, 13, 126.
- Vuolo, F., Novello, G., Bona, E., Gorrasi, S., & Gamalero, E. (2022). Impact of plant-beneficial bacterial inocula on the resident bacteriome: Current knowledge and future perspectives. *Microorganisms*, 10(12), 2462.