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Article

Dual Role of *Bacillus velezensis* EM-A8 in Maize: Biocontrol of *Exserohilum turcicum* and Enhancement of Plant Growth

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Abstract

Northern corn leaf blight (NCLB), caused by the hemibiotrophic fungus *Exserohilum turcicum*, is a major foliar disease of maize worldwide. Reducing dependence on chemical fungicides requires the development of sustainable strategies to manage foliar diseases in economically important crops. As an eco-friendly alternative, we evaluated a biocontrol agent previously isolated in our laboratory (*Bacillus velezensis* EM-A8; GenBank accession number OL704805) and investigated its effects on maize under both greenhouse and field conditions. The aims of this study were: (i) characterize phytohormone production in two different formulations containing the biocontrol agent; (ii) assess the influence of the bacterium on plant biomass and yield; (iii) compare the efficacy of the two formulations in controlling NCLB under field conditions; and (iv) determine whether the formulations affected salicylic acid and phenolic compound levels in maize tissues. Our results showed that *B. velezensis* EM-A8 synthesized a broad spectrum of phytohormones, including salicylic acid, indoleacetic acid, indolebutyric acid, jasmonic acid, abscisic acid and gibberellic acid, as well as cytokinins such as kinetin, zeatin, and 6-benzylaminopurine. Foliar application of the bacterium increased maize dry biomass by 30%. In field trials, both formulations effectively suppressed NCLB, reducing the number of symptomatic leaves by 25–50% relative to untreated controls. Furthermore, treated plants exhibited yield increases exceeding 1,000 kg/ha. In conclusion, formulations containing *B. velezensis* EM-A8 provided effective biocontrol of *E. turcicum* while simultaneously enhancing maize grain yield under field conditions.

Keywords: maize; biocontrol agents; *Bacillus velezensis*; phytohormones; maize growth stimulation

1. Introduction

Northern Corn Leaf Blight (NCLB) is one of the most widespread foliar diseases of maize. Global yield losses caused by NCLB are estimated at approximately 2.5% [1], corresponding to nearly 30 million tons of maize in 2023 [2]. In Argentina, where maize is one of the most important crops, NCLB causes significant yield reductions. For the 2024/2025 growing season, national maize production was around 50 million tons, and the planted area for the following season is estimated at 9.7 million hectares [3]. Over recent decades, the prevalence of NCLB in Argentinian maize has risen markedly,

reaching nearly 90% annually [4]. In cases involving susceptible hybrids and favourable environmental conditions, yield losses can reach up to 40% [5].

The causal agent of NCLB is *Exserohilum turcicum*, a hemibiotrophic fungus that survives between growing seasons in crop residues and secondary hosts such as *Sorghum halepense* [6], [7]. Its conidia are dispersed by wind and rain, typically reaching the lower leaves of maize plants. Infection can occur through stomatal openings or by direct penetration of the epidermis. The pathogen initially establishes a biotrophic phase with limited tissue damage, but subsequently shifts to a necrotrophic phase characterized by host cell plasmolysis, xylem occlusion, and conidiophore formation [8], [9]. Typical symptoms include gray, elliptical lesions ranging from 2 to 15 cm in length, which may coalesce into extensive necrotic areas [8]. This necrosis, together with premature leaf senescence, reduce the plant's ability to capture solar energy, thereby limiting the translocation of photosynthates required for grain filling [10]. Consequently, yield losses result not only from the reduction in photosynthetically active leaf area, but also indirectly from stem degradation triggered by the remobilization of carbohydrates [10], [11].

Management of this foliar disease primarily aims to avoid environmental conditions favorable for pathogen infection during the crop's critical growth stages. In Argentina, however, late sowing dates are often preferred because they coincide with more stable rainfall patterns [10]. Another widely adopted strategy is chemical control through fungicide applications [12]. Although effective, this approach relies on moderately hazardous compounds and must be implemented before the disease exceeds a certain damage threshold [13], [14]. In this context, biological control represents a promising alternative that can be integrated into pest management strategies [15]. Foliar pathogens interact with other phyllosphere microorganism, and the nature of these interactions – ranging from competition to antagonism- can influence crop health by inducing resistance to pathogens [16]. Previous studies conducted in our laboratory screened native maize phyllosphere microorganisms from their potential antagonistic activity [17], [18], [19]. In these studies, selected bacterial strains were evaluated for their antagonistic effects against *E. turcicum* both *in vitro* and in the field, including competition for nutrients, antibiosis, and effects on pathogen growth. Among the candidates, the biocontrol agent (BCA) *Bacillus velezensis* EM-A8 (GenBank accession number OL704805) was identified as the most promising, owing to its strong antagonistic properties and its tolerance to phyllosphere environmental conditions, as demonstrated by our research group [17], [18], [19].

In addition to suppressing pathogen, biocontrol agents (BCAs) can also promote plant growth. Several studies have demonstrated that certain *Bacillus* species not only control pathogens but also enhance maize growth under field conditions [20], [21]. Both pathogen colonization and BCA activity are known to influence salicylic acid (SA) and phenolic compound levels - molecules central to physiological processes such as germination, flowering, and senescence, as well as plant responses to biotic and abiotic stresses [22], [23], [24], [25]. Salicylic acid is a phenolic compound that promotes the expression of pathogenesis-related proteins that reinforce cell walls, induce lysis of infected cells, and trigger the hypersensitive response, ultimately leading to localized cell death [15], [22]. Furthermore, it is a key molecule in the establishment of systemic acquired resistance (SAR) [26]. The response elicited by enhances plant defenses against subsequent infections, operating both locally and systemically [27]. While SA is strongly associated with resistance to biotrophic pathogens, its role in defence against necrotrophic and hemibiotrophic organisms remains less clearly defined [25].

To better understand the effects of *B. velezensis* EM-A8 on maize and its interaction with *E. turcicum*, the present study pursued the following objectives: (i) to evaluate the biostimulant activity of *B. velezensis* EM-A8 using two formulations under greenhouse and field conditions; (ii) to analyze its capacity for phytohormone synthesis in both formulations; (iii) to assess its biocontrol efficacy against NCLB under natural disease pressure; and (iv) to quantify total phenolic compounds in maize plants following foliar application of the BCA under field conditions with natural *E. turcicum* incidence.

2. Materials and methods

2.1. Inoculum preparation

Bacillus velezensis EM-A8 was originally isolated from the maize phyllosphere in corn fields located in the southern region of Córdoba Province, Argentina. Two inoculum formulations were prepared: Formulation 1 (F1) consisted of cultures grown in nutrient broth, while formulation 2 (F2) was also supplemented with 5 g L⁻¹ molasses and 10 g L⁻¹ yeast extract (Sartori et al., 2025). Both formulations were adjusted with glycerol to reach 0.97 *a_w* and incubated for 24 h at 140 rpm and 25 °C until reaching final level of 10⁸ CFU mL⁻¹.

2.2. Biostimulant activity of *B. velezensis* EM-A8 under greenhouse conditions

To evaluate the biostimulant effect of *B. velezensis* EM-A8 on maize, a greenhouse experiment was conducted using a randomized complete block design with three blocks. Each block consisted of ten replicates of maize plants cultivated in pots containing 0.5 L of natural soil. Treatments included foliar application of F1 and F2 at the V2 stage, with evaluations performed at the V4 stage. Control plants (C) received no formulation. The variables measured were plant height, fresh and dry biomass. For dry biomass determination, plant material was placed in paper envelopes and oven-dried at 60 °C in a forced-air stove for 12 h. Fresh and dry biomass were quantified using a gravimetric scale. Data were subjected to analysis of variance (ANOVA), and means were compared using the DGC test [28] at a significance level of *p* < 0.05. Statistical analyses were performed using Infostat software [29].

2.3. Phytohormone determination and quantification in bacterial formulations

To explore the potential mechanism underlying the biostimulant activity of *B. velezensis* EM-A8, the presence and concentration of phytohormone were determined in both F1 and F2. For extraction, 10 mL of each formulation was centrifuged, and the resulting supernatant was mixed with 2.5 mL of ethyl acetate. This mixture was centrifuged again, and the organic phase was recovered and evaporated to dryness. Samples were then resuspended in 100 µL of methanol, and 10 µL aliquots were injected into an Alliance 2965 chromatograph (Waters Inc., California, EE.UU.) Chromatographic separation was performed under a methanol:0.2% acetic acid (40:60) gradient at a flow rate of 0.2 mL min⁻¹. Data acquired and analysed were conducted using MassLynx™ 2.1 software with a Quattro Ultima™ Pt mass spectrometer (Micromass, Manchester, UK). Data acquisition and analysis were conducted using MassLynx™ 2.1 software with a Quattro Ultima™ Pt mass spectrometer (Micromass, Manchester, UK). Phytohormone quantification was based on calibration curves generated with known concentrations of each hormone and their corresponding deuterated internal standards. The HPLC-MS analysis was performed by the Plant Physiology Group (Grupo de Fisiología Vegetal, FCEFQyN, Universidad Nacional de Río Cuarto, Argentina). The phytohormones quantified included salicylic acid, indoleacetic acid, indolebutyric acid, jasmonic acid, kinetin, zeatin, benzylaminopurine, abscisic acid, and gibberellins.

2.4. Field Trials

The field trials to evaluated the biocontrol efficacy of *B. velezensis* EM-A8 formulations (F1 and F2) against *E. turcicum* were conducted in three maize fields located in the southern region of Córdoba, Argentina (coordinates: -33.013683, -64.132552; -33.009594, -64.111725; -33.006628, -64.488574). The trials were carried out during two consecutive maize growing seasons (2023-2025). The experimental design consisted of eleven randomized complete blocks, each comprising three treatments: foliar application of F1, foliar application of F2, and plants without inoculum control (C). Each experimental unit consisted of two rows, 20 m in length. Treatments were applied at the V8 phenological stage by foliar spraying. The following parameters were evaluated under field conditions after *B. velezensis* EM-A8 application:

2.4.1. NCLB severity and number of affected leaves

Total number of affected leaves per plant was recorded, and disease severity was assessed every ten days on the ear leaf and the leaves immediately above and below. Disease severity was measured according to the Bleicher scale (1988). Leaf tissue samples were also collected to evaluate phenolic compounds concentration. For this, samples were frozen at -80 °C until lyophilisation.

2.4.2. Leaf phenolic compound level

Total phenolic compounds were quantified from 0.1 g of lyophilized leaf tissue incubated for 2 h with 3 mL of methanol:water:hydrochloric acid (80:19:1). Samples were then centrifuged for 5 minutes at 3000 rpm, and 0.1 mL of supernatant was mixed with 0.75 mL of Folin-Ciocalteu reagent (10%). After 5 min, 0.75 mL of Na₂CO₃ was added. Absorbance was read at 725 nm after 90 min of reaction. The standard curve was prepared with different concentrations of gallic acid, ranging from 0.5 to 500 mg . L⁻¹. Results were expressed as mg gallic acid equivalent per g of dry weight.

2.4.3. Yield components

Yield components were estimated by determining i) number of rows per ear; ii) number of kernels per row, and iii) weight of 1000 kernels. Measurements were taken from physiologically mature ears collected from 15 randomly picked plants per treatment and control. Kernel moisture content was measured with a hygrometer, and yield values were adjusted to 14.5% moisture.

2.5. Statistical analysis

Analysis of variance (ANOVA) was performed for NCLB severity, number of affected leaves and phenolic compound concentrations, and yield components using Infostat software [29]. Means were compared using the DGC test at a significance level of $p < 0.05$.

3. . Results

3.1. Biostimulant activity of *B. velezensis* EM-A8 in the greenhouse trial

As described in materials and methods, plant height and biomass (fresh and dry) were evaluated to determine the biostimulant activity of *B. velezensis* EM-A8. Analysis of variance revealed that treatments had no significant effect on plant height (F: 1.55; d.f.: 2; $p=0.2185$). Control plants reached an average height of 47.23 cm, while plants treated with F1 and F2 had 46 and 49.51 cm, respectively. Fresh biomass was higher in treatment F2 (3.77 g.plant⁻¹) compared to F1 (3.15 g.plant⁻¹) and the control (3.13 g.plant⁻¹), although these differences were not statistically significant (F: 2.13; d.f.: 2; $p=0.1255$). In contrast, dry biomass production showed significant differences among treatments (F: 3.68; d.f.: 2; $p=0.0296$). Plants treated with F2 produced significantly more dry biomass (0.44 g plant⁻¹) than those treated with F1 (0.37 g plant⁻¹) or the control (0.34 g plant⁻¹) (Figure 1).

3.2. Phytohormones detection and quantification in formulations

Phytohormone profiling of the bacterial formulations demonstrated that *B. velezensis* EM-A8 is capable of synthesizing growth-promoting compounds. As shown in Table 1, five out of the nine phytohormones assessed were detected in F1, including salicylic acid, indoleacetic acid, kinetin, zeatin, and 6-benzylaminopurine. In contrast, F2 contained all nine phytohormones evaluated and at higher concentrations than those found in F1.

3.3. Field trial

3.3.1. NCLB severity and number of affected leaves

NCLB severity and number of affected leaves were evaluated across three fields at different sampling times. In Field 1, which was evaluated in the same campaign year as Field 2, no significant differences among treatments were detected for severity (F = 1.17; d.f. = 2; $p = 0.3167$) or number of

affected leaves ($F = 1.58$; d.f. = 2; $p = 0.2117$). Mean values were $5.59 \pm 3.46\%$ for severity and 2.62 ± 0.70 for the number of affected leaves (Figure 2).

In Field 2, ANOVA revealed significant differences in NCLB severity ($F=4.14$; d.f.: 2; $p=0.0187$). Control plants showed significantly higher severity (3.78%) compared to F1 (3.06%) and F2 (3.19%). However, the number of affected leaves was not significantly different among treatments ($F= 2.96$; d.f.= 2; $p=0.0561$) with an overall mean of 2.59 ± 0.76 affected leaves.

In Field 3, conducted two years later, no significant differences were observed among treatments for NCLB severity ($F= 1.36$; gl= 2; $p=0.2615$), with mean values of $1.67 \pm 1.42 \%$. Similarly, the number of affected leaves was not significantly different ($F= 2.56$; gl= 2; $p=0.0819$) averaging 1.34 ± 0.95 leaves. However, when analysing individual sampling times, significant differences were detected 47 days after treatment application (sampling time 4) ($F= 9.50$; d.f. = 2; $p = 0.0034$). At this point, control plants had more affected leaves (3.67) compared to F1 (2.50) and F2 (2.33).

3.3.2. Leaf phenolic compound concentration

ANOVA revealed significant differences in phenolic compound concentration between fields ($F= 6.3$; gl= 2; $p=0.0025$). Field 3 presented the highest levels (18.96 mg GAeq per g of dry weight) followed by fields 2 (16.06 mg GAeq per g of dry weight) and Field 1 (15.61 mg GAeq per g of dry weight) (Figure 3). However, within each field, treatments did not significantly affect phenolic compound concentrations (Field 1: $F=0.05$; d.f.=2; $p=0.9473$; Field 2: $F=1.52$; d.f.=2; $p=0.2465$; Field 3: $F=0.39$; d.f.=2; $p=0.6801$).

3.3.3. Yield components

Yield component data are shown in Table 2. Respect to the number of rows per ear, was not affected by the application of the treatments for fields 1 and 3. In Field 2, however, control showed significantly higher values ($p=0.0304$) than F1 and F2.

Regarding the number of kernels per row, in field 1, no significant differences were found, with mean values ranging from 27.67 to 28.27. In Field 2, F2 significantly increased kernel number compared to F1 and C ($p=0.0031$). In Field 3, both formulations (F1 and F2) resulted in significantly higher values compared to the control ($p < 0.0001$).

Thousand kernel weight (g) was significantly affected by treatments in all fields ($p<0.0001$). In Field 1, the highest weight was obtained for F2 treatment, followed by F1 and C. In Field 2, F1 showed the highest value followed by F2 and C. In contrast, in Field 3 this parameter was significantly higher in control ($p<0.0001$), followed by F1 and F2.

The estimated yield was significantly higher in C and F2 in Field 1 ($p<0.0001$), but C yielded significantly less in Field 2 ($p<0.0001$) obtaining significantly higher values for the treatments. There were no statistical differences between treatments for grain yield in Field 3 ($p=0.4628$).

4. Discussion

In this study, we evaluated a biological control strategy for northern corn leaf blight (NCLB). This disease is typically managed through early sowing or chemical fungicides applications; however, these measures are often insufficient to provide reliable protection. Moreover, synthetic fungicides can be hazardous to human and animal health, leaving toxic residues in the environment. In this sense, biological control is an alternative strategy to the use of chemical compounds, involving the use of beneficial microorganisms for disease control. Biological control represents a sustainable alternative, relying on beneficial microorganisms to suppress plant diseases. In this context, we assessed both the biocontrol and biostimulant activities of *Bacillus velezensis* EM-A8 in maize. As a first step, a greenhouse trial was carried out to determine whether the laboratory-prepared formulations (F1 and F2) exerted a biostimulant effect on maize growth. The results of this assay demonstrate that the bacteria promote plant growth, as evidenced by a 30% increase in dry biomass production. One of the mechanisms by which growth-promoting bacteria exert their biostimulant

effect is by through the production of phytohormones that regulate plant development [30], [31]. Therefore, we analysed whether *B. velezensis* EM-A8 releases phytohormones related to growth and defence. The results revealed the presence of five phytohormones in F1 and nine in F2. Moreover, the phytohormones common to both formulations were detected at higher levels in F2. This difference in the type and concentration may be attributable to the composition of the liquid media used for formulation production. In the case of F2, the medium was enriched with molasses and yeast extract, which may have stimulated metabolite production. In both formulations, salicylic acid was the predominant phytohormone. This compound plays a central role in vital processes such as stress tolerance, activation of defence mechanisms, photosynthesis and growth and development [23], [24], [25]. In maize, [32] and Ali et al. (2023) [33] reported that salicylic acid alleviates the deleterious effects of salinity and enhances grain yield, while Li et al. (2023) [34] demonstrated that exogenous salicylic acid maintains photosynthetic rates under heat stress. With respect to defence, salicylic acid enhances antioxidant enzyme activity, regulates cell wall strengthening, and induces systemic acquired resistance [35]. Consistent with this, Li et al. (2022) [36] observed that a maize mutant with enhanced resistance to *Curvularia lunata* accumulated higher levels of salicylic acid and jasmonic acid.

Indoleacetic acid and indole butyric acid are auxins naturally present in plants. These hormones regulate key aspects of plant growth and development, including root elongation, shoot formation, leaf morphogenesis, and kernel development. With respect to grain yield, indoleacetic acid stimulates sugar and protein metabolism during kernel differentiation and promotes nutrient allocation to the endosperm [37], [38]. In this context, IAA production by plant growth-promoting Bacilli promotes root and coleoptiles elongation [39]. Moreover, auxins are involved in plant defence. Our phytohormone analysis in F1 and F2 confirmed that *B. velezensis* EM-A8 produces both indole acetic and indole butyric acid, which may account, at least in part, for the increased dry biomass and yield observed in the greenhouse and field trials.

Jasmonic acid (JA) is known to enhance plant tolerance to abiotic stresses, including high temperature [40] and salinity [41]. In maize, Tayyab et al. (2020) [42] demonstrated that JA positively influences plant performance under drought stress. However, the capacity of *Bacillus* spp. to produce JA has not yet been clearly correlated with their ability to induce resistance in plants [43]. In our study, *B. velezensis* EM-A8 produced JA in formulation F2, whereas no detectable levels were observed in F1.

Besides, both F1 and F2 showed the presence of cytokinins kinetin, zeatin and 6-benzylaminopurine which may help explain the higher biomass observed in the greenhouse trial and the increased grain yield recorded in the field trial. Cytokinins are phytohormones able to induce the cell division and regulate multiple developmental processes, such as nutrient mobilisation, seed germination, root growth, stress response and apical dominance [44]. Absciscic acid is involved in plant development as well as in abiotic stress resistance. This phytohormone mitigates the harmful effects of drought, chilling and soil salinity, thereby contributing to yield improvement [38], [45]. In this research, absciscic acid was detected in F2 but not in F1, which could partially account for the differences in dry biomass production observed between the two formulations of the same bacterium.

Gibberellins are ubiquitous phytohormones that regulate plant growth by promoting cell division, stem elongation and grain development and mitigate abiotic stresses [46], [47], [48]. In maize, Cui et al. (2020) [49] reported that gibberellin application at two phenological stages increased grain yield through different yield components, such as grain weight or kernel number, depending on the timing of application. In this work, gibberellins were detected in F2 but not in F1. Their presence in F2 may help explain the greater biomass accumulation observed in plants treated with this formulation in the greenhouse trial.

Many studies have demonstrated that certain strains of fungi and bacteria can stimulate plant growth through different mechanisms [50], [51], [52], [53], [54], [55]. For example, Adesemoye et al. (2008) [50], evaluated *Bacillus subtilis* and *Pseudomonas aeruginosa* strains in *Solanum lycopersicum* L. (tomato), *Abelmoschus esculentus* (okra), and *Amaranthus* sp. reported up to 30% increase in dry biomass after 60 days, for both bacterial strains. While many studies have documented plant growth

stimulation by applying microorganisms to the rhizosphere or seeds, to our knowledge, no studies have evaluated microbial application directly to the foliar area, as proposed in this research.

Beneficial bacteria provide a wide range of plant growth-promoting abilities for agricultural use, such as biocontrol activity, phytohormone production and nutrient solubilisation. However, the effectiveness of these bacteria under field conditions may differ from greenhouse or laboratory results due to environmental variations [56]. Therefore, effective integration of such tools into production systems requires scaling the experiments to field conditions to confirm bacterial functionality. Since our greenhouse trial demonstrated the biostimulating activity of *B. velezensis* EM-A8 and that Sartori et al. (2017b) [19] confirmed its ability to control NCLB, we conducted a field trial to assess its biocontrol capacity, its effect on foliar concentration of phenolic compounds, and its impact on grain yield in maize under field conditions.

Phenolic compounds constitute a broad group of metabolites that play an important role in resistance to pathogens including resistance to fungal disease [57]. In plant-beneficial microbe interaction, several *Bacillus* spp. have been shown to induce systemic resistance by enhancing phenolic compounds accumulation [58]. However, in our study, no significant differences in leaf phenolic compound concentration were observed between treatments and the control. Thus, we could not demonstrate that *B. velezensis* EM-A8 enhances maize resistance to NCLB through phenolic compounds accumulation under field conditions. In contrast, Wallis & Galarneau (2020) [59] reported that phenolic compounds generally increased in plants following interactions with beneficial microbes, pathogens and insects. Similarly, Li et al. (2022) [36] found elevated levels of phenylpropanoids in a maize mutant line resistant to *Curvularia lunata*.

On the other hand, the three field trials revealed a decrease in NCLB severity and in the number of affected leaves following the application of F1 and F2. These findings are in line with Sartori et al. (2017b) [19], who reported lower incidence of NCLB and common rust in maize after treatment with two antagonists, one of which was *B. velezensis* EM-A8. Similar results have been described in the same pathosystem by Zhang et al. (2021) [60], who observed that *Klebsiella jilinsis* 2N3 reduced NCLB severity, and by Ding et al. (2017) [61], who showed inoculation with *B. subtilis* DZSY21 decreased NCLB intensity in maize. Likewise, Chen et al. (2022) [62] found that treatment with *Paenibacillus polymyxa* SF05 significantly reduced the disease index of banded leaf and sheath blight.

Grain yield in the field trials was estimated based on its components: number of rows per ear, number of kernels per row and thousand-kernel weight. The number of rows per ear did not differ significantly among treatments, averaging around 16 rows. In contrast, application of F1 and F2 increased the number of kernels per row by 4.11% and 8.33%, respectively, in. This is consistent with Satorre et al. (2003) [63], who reported that the number of rows per ear is more strongly determined by genetics while the environment exerts less influence on this trait in the selected hybrid. Thousand-kernel weight was also significantly higher in F1 (9.22%) and F2 (11.29%) compared to the control. Because grain yield is directly affected by biomass production [11], the greenhouse trial results support the expectation of yield improvements not only due to reduced NCLB severity but also through the biostimulant activity of the bacteria. The superior performance of F2 relative to F1 may be explained by the presence of gibberellins, which are known to stimulate plant growth. In this sense, Balderas-Ruiz et al. (2020) [30] showed that *B. velezensis* promoted biomass accumulation in mango plants in addition to its biocontrol effect against anthracnose. Etesami et al. (2023) reviewed multiple applications of *Bacillus* spp. and concluded that *B. velezensis* can enhance crop yields by producing phytohormone and other growth regulators that alleviate stress and promote plant growth.

5. Conclusions

In conclusion, both formulations of *B. velezensis* EM-A8 demonstrated efficacy in controlling NCLB under field conditions and exhibited biostimulant activity in maize. In greenhouse trials, increased biomass production was observed, while in field trials, yield improvements were recorded.

These findings indicate that *B. velezensis* EM-A8 formulations have a dual potential: serving both as a preventive strategy against *E. turcicum* and as a biostimulant to enhance maize productivity.

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References

1. S. Savary, L. Willocquet, S. J. Pethybridge, P. Esker, N. McRoberts, and A. Nelson, 'The global burden of pathogens and pests on major food crops', *Nat Ecol Evol*, vol. 3, no. 3, pp. 430–439, Mar. 2019, doi: 10.1038/s41559-018-0793-y.
2. FAO (Food and Agriculture Organization of the United Nations). 2024. FAOSTAT, Statistical Databases. Agriculture, Fisheries, Forestry, Nutrition. Rome. <http://faostat.fao.org/default>. Consulted on July, 2024.
3. Bolsa de Comercio de Rosario (2025). In: <https://www.bcr.com.ar/es/mercados/gea/estimaciones-nacionales-de-produccion/estimaciones>. Consulted on September, 2025.
4. M. A. Carmona and F. J. Sautua, '¿Cuándo Es Realmente Necesario Aplicar Fungicidas En Maíz ? When To Consider Foliar Fungicide Applications on Corn?', *Agronomía y Ambiente*, vol. 41, no. 2, pp. 149–161, 2021.
5. R. L. De Rossi, E. M. Reis, and R. Brustolin, 'Morfología de conídios e patogenicidade de isolados de *Exserohilum turcicum* da Argentina e do Brasil em milho', *Summa Phytopathol*, vol. 41, no. 1, pp. 58–63, 2015, doi: 10.1590/0100-5405/1948.
6. R. C. Pratt and S. G. Gordon, 'Breeding for Resistance to Maize Foliar Pathogens', in *Plant Breeding Reviews*, vol. 27, John Wiley & Sons, Ltd, 2010, pp. 119–173. doi: 10.1002/9780470650349.ch3.
7. J. Sucher et al., 'The durable wheat disease resistance gene Lr34 confers common rust and northern corn leaf blight resistance in maize', *Plant Biotechnol J*, vol. 15, no. 4, pp. 489–496, Apr. 2017, doi: 10.1111/pbi.12647.
8. S. Hurni et al., 'The maize disease resistance gene Htn1 against northern corn leaf blight encodes a wall-associated receptor-like kinase', *Proc Natl Acad Sci U S A*, vol. 112, no. 28, pp. 8780–8785, Jul. 2015, doi: 10.1073/pnas.1502522112.
9. J. Sibiya, P. Tongona, and J. Derera, 'Combining ability and GGE biplot analyses for resistance to northern leaf blight in tropical and subtropical elite maize inbred lines', *Euphytica*, vol. 191, no. 2, pp. 245–257, May 2013, doi: 10.1007/s10681-012-0806-x.
10. N. A. Formento, 'Enfermedades foliares reemergentes del cultivo de maíz: (*Puccinia sorghi* y *Puccinia polysora*), tizón foliar (*Exserohilum turcicum*) y mancha ocular (*Kabatiella zeae*)', *Estacion Experimental Agropecuaria Paraná*, pp. 89–100, 2010, [Online]. Available: https://inta.gob.ar/sites/default/files/script-tmp-act-tecnica-n2_16_enfermedades-foliares-reemergentes-.pdf.
11. F. H. Andrade, C. Vega, S. Uhart, A. Cirilo, M. Cantarero, and O. Valentinuz, 'Kernel number determination in maize', *Crop Sci*, vol. 39, no. 2, pp. 453–459, 1999, doi: 10.2135/cropsci1999.0011183x0039000200026x.
12. L. Couretot, L. Parisi, G. Ferraris, and G. Magnone, 'Efecto de fungicidas foliares y momento de aplicación sobre la severidad de tizón foliar y enfermedades de raíz y tallo en maíz', *XIV Jornadas Fitosanitarias Argentinas*, vol. 8, pp. 4–11, 2012.
13. 'gráficos FAO'.
14. R. L. De Rossi et al., 'Crop damage, economic losses, and the economic damage threshold for northern corn leaf blight', *Crop Protection*, vol. 154, Apr. 2022, doi: 10.1016/j.cropro.2021.105901.
15. K. K. Pal and B. M. Gardener, 'Biological control of plant pathogens', *Curr Opin Biotechnol*, vol. 7, no. 3, pp. 343–347, 2006, doi: 10.1016/S0958-1669(96)80042-5.
16. M. Legein et al., 'Modes of Action of Microbial Biocontrol in the Phyllosphere', Jul. 14, 2020, *Frontiers Media S.A.* doi: 10.3389/fmicb.2020.01619.

17. M. Sartori, A. Nescia, Á. Formento, and M. Etcheverry, 'Selección de microorganismos epifíticos de maíz como potenciales agentes de biocontrol de *Exserohilum turcicum*', *Rev Argent Microbiol*, vol. 47, no. 1, pp. 62–71, 2015, doi: 10.1016/j.ram.2015.01.002.
18. M. Sartori, A. Nesci, A. Montemarani, G. Barros, J. García, and M. Etcheverry, 'Preliminary Evaluation of Biocontrol Agents against Maize Pathogens *Exserohilum turcicum* and *Puccinia sorghi* in Field Assays', *Agricultural Sciences*, vol. 08, no. 09, pp. 1003–1013, 2017, doi: 10.4236/as.2017.89073.
19. M. Sartori, A. Nesci, J. García, M. A. Passone, A. Montemarani, and M. Etcheverry, 'Efficacy of epiphytic bacteria to prevent northern leaf blight caused by *Exserohilum turcicum* in maize', *Rev Argent Microbiol*, vol. 49, no. 1, pp. 75–82, Jan. 2017, doi: 10.1016/j.ram.2016.09.008.
20. T. Cheng, X. Z. Yao, C. Y. Wu, W. Zhang, W. He, and C. C. Dai, 'Endophytic *Bacillus megaterium* triggers salicylic acid-dependent resistance and improves the rhizosphere bacterial community to mitigate rice spikelet rot disease', *Applied Soil Ecology*, vol. 156, Dec. 2020, doi: 10.1016/j.apsoil.2020.103710.
21. P. Z. Kulimushi, G. C. Basime, G. M. Nachigera, P. Thonart, and M. Ongena, 'Efficacy of *Bacillus amyloliquefaciens* as biocontrol agent to fight fungal diseases of maize under tropical climates: from lab to field assays in south Kivu', *Environmental Science and Pollution Research*, vol. 25, no. 30, pp. 29808–29821, Oct. 2018, doi: 10.1007/s11356-017-9314-9.
22. A. Corina Vlot, D. A. Dempsey, and D. F. Klessig, 'Salicylic acid, a multifaceted hormone to combat disease', *Annu Rev Phytopathol*, vol. 47, pp. 177–206, Sep. 2009, doi: 10.1146/annurev.phyto.050908.135202.
23. D. A. Dempsey and D. F. Klessig, 'How does the multifaceted plant hormone salicylic acid combat disease in plants and are similar mechanisms utilized in humans?', *BMC Biol*, vol. 15, no. 1, Mar. 2017, doi: 10.1186/s12915-017-0364-8.
24. G. Forchetti, O. Masciarelli, M. J. Izaguirre, S. Alemano, D. Alvarez, and G. Abdala, 'Endophytic bacteria improve seedling growth of sunflower under water stress, produce salicylic acid, and inhibit growth of pathogenic fungi', *Curr Microbiol*, vol. 61, no. 6, pp. 485–493, Dec. 2010, doi: 10.1007/s00284-010-9642-1.
25. Y. M. Koo, A. Y. Heo, and H. W. Choi, 'Salicylic acid as a safe plant protector and growth regulator', *Plant Pathol J (Faisalabad)*, vol. 36, no. 1, pp. 1–10, 2020, doi: 10.5423/PPJ.RW.12.2019.0295.
26. Y. Zhang and X. Li, 'Salicylic acid: biosynthesis, perception, and contributions to plant immunity', *Curr Opin Plant Biol*, vol. 50, pp. 29–36, 2019, doi: 10.1016/j.pbi.2019.02.004.
27. D. F. Klessig, H. W. Choi, and D. A. Dempsey, 'Systemic acquired resistance and salicylic acid: Past, present, and future', *Molecular Plant-Microbe Interactions*, vol. 31, no. 9, pp. 871–888, 2018, doi: 10.1094/MPMI-03-18-0067-CR.
28. Balzarini M.G., Gonzalez L., Tablada M., Casanoves F., Di Rienzo J.A., Robledo C.W. (2008). *Manual del Usuario*, Editorial Brujas, Córdoba, Argentina.
29. Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. *InfoStat versión 2020*. Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL <http://www.infostat.com.ar>
30. K. A. Balderas-Ruiz et al., '*Bacillus velezensis* 83 a bacterial strain from mango phyllosphere, useful for biological control and plant growth promotion', *AMB Express*, vol. 10, no. 1, 2020, doi: 10.1186/s13568-020-01101-8.
31. H. Etesami, B. R. Jeong, and B. R. Glick, 'Potential use of *Bacillus* spp. as an effective biostimulant against abiotic stresses in crops—A review', Jan. 01, 2023, Elsevier B.V. doi: 10.1016/j.crbiot.2023.100128.
32. A. H. Elhakem, 'Salicylic acid ameliorates salinity tolerance in maize by regulation of phytohormones and osmolytes', *Plant Soil Environ*, vol. 66, no. 10, pp. 533–541, 2020, doi: 10.17221/441/2020-PSE.
33. Q. Ali et al., 'Synergistic Effects of Rhizobacteria and Salicylic Acid on Maize Salt-Stress Tolerance', *Plants*, vol. 12, no. 13, Jul. 2023, doi: 10.3390/plants12132519.
34. Y. Li et al., 'Exogenous SA or 6-BA maintains photosynthetic activity in maize leaves under high temperature stress', *Crop Journal*, vol. 11, no. 2, pp. 605–617, Apr. 2023, doi: 10.1016/j.cj.2022.08.006.
35. W. Song, H. Shao, A. Zheng, L. Zhao, and Y. Xu, 'Advances in Roles of Salicylic Acid in Plant Tolerance Responses to Biotic and Abiotic Stresses', *Plants*, vol. 12, no. 19, pp. 1–23, 2023, doi: 10.3390/plants12193475.
36. J. Li et al., 'Underlying mechanism of accelerated cell death and multiple disease resistance in a maize lethal leaf spot 1 allele', *J Exp Bot*, vol. 73, no. 12, pp. 3991–4007, Jun. 2022, doi: 10.1093/jxb/erac116.

37. C. L. Cowling, L. Dash, and D. R. Kelley, 'Roles of auxin pathways in maize biology', 2023. doi: 10.1093/jxb/erad297.
38. K. Yue, L. Lingling, J. Xie, J. A. Coulter, and Z. Luo, 'Synthesis and regulation of auxin and abscisic acid in maize', 2021, Bellwether Publishing, Ltd. doi: 10.1080/15592324.2021.1891756.
39. C. Keswani et al., 'Auxins of microbial origin and their use in agriculture', doi: 10.1007/s00253-020-10890-8/Published.
40. S. M. Kang et al., 'Integrated phytohormone production by the plant growth-promoting rhizobacterium *Bacillus tequilensis* SSB07 induced thermotolerance in soybean', J Plant Interact, vol. 14, no. 1, pp. 416–423, Jan. 2019, doi: 10.1080/17429145.2019.1640294.
41. Y. Liu et al., 'Comparative proteomic analysis revealed maize (*Zea mays* L.) response to *Setosphaeria turcica* early infection', 2020, doi: 10.21203/rs.3.rs-41821/v1.
42. N. Tayyab et al., 'Combined seed and foliar pre-treatments with exogenous methyl jasmonate and salicylic acid mitigate drought-induced stress in maize', PLoS One, vol. 15, no. 5, May 2020, doi: 10.1371/journal.pone.0232269.
43. J. Poveda and F. González-Andrés, 'Bacillus as a source of phytohormones for use in agriculture', Dec. 01, 2021, Springer Science and Business Media Deutschland GmbH. doi: 10.1007/s00253-021-11492-8.
44. J. Barciszewski, F. Massino, and B. F. C. Clark, 'Kinetin-A multiactive molecule', Feb. 28, 2007, Elsevier. doi: 10.1016/j.ijbiomac.2006.06.024.
45. K. A. A. Abdelaal et al., 'Exogenous application of proline and salicylic acid can mitigate the injurious impacts of drought stress on barley plants associated with physiological and histological characters', Sustainability (Switzerland), vol. 12, no. 5, Mar. 2020, doi: 10.3390/su12051736.
46. I. Ahmad et al., 'Seed filling in maize and hormones crosstalk regulated by exogenous application of uniconazole in semiarid regions', Environmental Science and Pollution Research, vol. 25, no. 33, pp. 33225–33239, Nov. 2018, doi: 10.1007/s11356-018-3235-0.
47. N. N. Elahi et al., 'Foliar Application of Gibberellin Alleviates Adverse Impacts of Drought Stress and Improves Growth, Physiological and Biochemical Attributes of Canola (*Brassica napus* L.)', Sustainability (Switzerland), vol. 15, no. 1, Jan. 2023, doi: 10.3390/su15010078.
48. S. H. Shah, S. Islam, F. Mohammad, and M. H. Siddiqui, 'Gibberellic Acid: A Versatile Regulator of Plant Growth, Development and Stress Responses', Dec. 01, 2023, Springer. doi: 10.1007/s00344-023-11035-7.
49. W. Cui, Q. Song, B. Zuo, Q. Han, and Z. Jia, 'Effects of gibberellin (Ga4+7) in grain filling, hormonal behavior, and antioxidants in high-density maize (*zea mays* L.)', Aug. 01, 2020, MDPI AG. doi: 10.3390/plants9080978.
50. A. O. Adesemoye, H. A. Torbert, and J. W. Kloepper, 'Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system', Can J Microbiol, vol. 54, no. 10, 2008, doi: 10.1139/W08-081.
51. M. Dary, M. A. Chamber-Pérez, A. J. Palomares, and E. Pajuelo, '"In situ" phytostabilisation of heavy metal polluted soils using *Lupinus luteus* inoculated with metal resistant plant-growth promoting rhizobacteria', J Hazard Mater, vol. 177, no. 1–3, 2010, doi: 10.1016/j.jhazmat.2009.12.035.
52. J. S. Kim, J. E. Lee, H. Nie, Y. J. Lee, S. T. Kim, and S. H. Kim, 'Physiological and proteomic analysis of plant growth enhancement by the rhizobacteria *Bacillus* sp. JS', Genes Genomics, vol. 40, no. 2, 2018, doi: 10.1007/s13258-017-0615-7.
53. K. B. Kuan, R. Othman, K. A. Rahim, and Z. H. Shamsuddin, 'Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions', PLoS One, vol. 11, no. 3, 2016, doi: 10.1371/journal.pone.0152478.
54. M. Saleem, H. N. Asghar, Z. A. Zahir, and M. Shahid, 'Impact of lead tolerant plant growth promoting rhizobacteria on growth, physiology, antioxidant activities, yield and lead content in sunflower in lead contaminated soil', Chemosphere, vol. 195, 2018, doi: 10.1016/j.chemosphere.2017.12.117.
55. A. Velasco-Jiménez, O. Castellanos-Hernández, G. Acevedo-Hernández, R. C. Aarland, and A. Rodríguez-Sahagún, 'Bacterias rizosféricas con beneficios potenciales en la agricultura', REVISTA TERRA LATINOAMERICANA, vol. 38, no. 2, 2020.

56. F. Fathi, R. Saberi-Riseh, and P. Khodaygan, 'Survivability and controlled release of alginate-microencapsulated *Pseudomonas fluorescens* VUPF506 and their effects on biocontrol of *Rhizoctonia solani* on potato', *Int J Biol Macromol*, vol. 183, pp. 627–634, Jul. 2021, doi: 10.1016/j.ijbiomac.2021.04.159.
57. P. Vasmatakar, K. Kaur, P. P. S. Pannu, G. Kaur, and H. Kaur, 'Unraveling the metabolite signatures of maize genotypes showing differential response towards southern corn leaf blight by ¹H-NMR and FTIR spectroscopy', *Physiol Mol Plant Pathol*, vol. 108, Dec. 2019, doi: 10.1016/j.pmpp.2019.101441.
58. J. Shafi, H. Tian, and M. Ji, 'Bacillus species as versatile weapons for plant pathogens: a review', May 04, 2017, Taylor and Francis Ltd. doi: 10.1080/13102818.2017.1286950.
59. C. M. Wallis and E. R. A. Galarneau, 'Phenolic Compound Induction in Plant-Microbe and Plant-Insect Interactions: A Meta-Analysis', *Front Plant Sci*, vol. 11, no. December, pp. 1–13, 2020, doi: 10.3389/fpls.2020.580753.
60. M. Zhang, C. Zhang, S. Zhang, H. Yu, H. Pan, and H. Zhang, '*Klebsiella jilinsis* 2N3 promotes maize growth and induces resistance to northern corn leaf blight', *Biological Control*, vol. 156, May 2021, doi: 10.1016/j.biocontrol.2021.104554.
61. T. Ding et al., 'An endophytic bacterial strain isolated from *eucommia ulmoides* inhibits southern corn leaf blight', *Front Microbiol*, vol. 8, no. MAY, May 2017, doi: 10.3389/fmicb.2017.00903.
62. B. Chen et al., 'Control of Maize Sheath Blight and Elicit Induced Systemic Resistance Using *Paenibacillus polymyxa* Strain SF05', *Microorganisms*, vol. 10, no. 7, Jul. 2022, doi: 10.3390/microorganisms10071318.
63. Satorre, E. H., Benech, R., Slafer, G. A., De la Fuente, E. B., Miralles, D. J., Otegui, M. E., & Savin, R. (2003). Producción de granos: bases funcionales para su manejo. Universidad de Buenos Aires, Facultad de Agronomía, Buenos Aires, Argentina.

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