ACS AGRICULTURAL SCIENCE & TECHNOLOGY

pubs.acs.org/acsagscitech

Article

Spray-Drying Microencapsulation of *Bacillus megaterium* in PVA/Cationic Starch/Zinc Oxide for Promoting Growth and Zinc Availability in Soybean Plants

Ludimila Araújo Lodi, Roger Borges, Marina Momesso Lopes, Vanessa Araújo Graciano, Ricardo Bortoletto-Santos, Hernane S. Barud, Christiane Abreu de Oliveira-Paiva, Caue Ribeiro, and Cristiane Sanchez Farinas*

Cite This: *ACS Agric. Sci. Technol.* 2024, 4, 1271−1283

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Zinc (Zn) is essential for plant development and its deficiency can reduce agricultural productivity. Nutrient-solubilizing microorganisms offer a promising solution to enhance the zinc availability for plants. However, directly applying these microorganisms in the field presents challenges such as cell viability loss. Here, we developed a formulation using poly(vinyl alcohol) (PVA), cationic starch (CS), and zinc oxide (ZnO) for microencapsulating *Bacillus megaterium* via spray drying. Our results showed that *B. megaterium* effectively solubilizes zinc oxide. The PVACS-ZnO matrix provided a favorable environment for the growth and development of *B. megaterium*, releasing cells in quantities exceeding initial inoculation (10 $\log_{10}$ CFU/g). Additionally, it protected the cells against adverse field conditions, maintaining bacterial viability after heat (50 °C/48 h), UV light (95% after 180 min), and fungicide/insecticide exposure (99% after 2 h), unlike free bacteria. Accelerated shelf life tests indicated prolonged stability of PVACS-ZnO microspheres, with double the estimated shelf life (14 months) compared to free bacteria (6 months). In greenhouse experiments, the formulation increased aerial and root biomass of soybean plants, and enhanced phosphorus and zinc absorption. These findings indicate that PVASC-ZnO formulations offer a promising strategy for encapsulating microorganisms and enhancing zinc availability, resulting in an effective and environmentally friendly biofertilizer product.

KEYWORDS: *Bacillus, spray drying, biofertilizer, zinc solubilization, cationic starch*

1. INTRODUCTION

Zinc (Zn) is a micronutrient essential for healthy plant growth, acting as a cofactor in various enzymes and proteins. Its presence is crucial for photosynthesis, stress resistance, and pathogen defense, promoting crop growth and productivity.[1,2] Zinc deficiency negatively impacts the production and development of various crops, such as soybeans (*Glycine max L.*), cotton (*Gossypium hirsutumL.*), and maize (*Zea mays*), making them more susceptible to pathogen attacks.[3−5] Zinc can be applied to the soil as a fertilizer from various sources, such as zinc oxide (ZnO), zinc sulfate ($ZnSO_4$), and chelated zinc (Zn-EDTA), either as part of multinutrient fertilizers like monoammonium phosphate (MAP), or through foliar spraying ($ZnSO_4.7H_2O$ or Zn-EDTA). Zinc oxide (ZnO) is recognized as one of the most cost-effective options for supplying zinc as a fertilizer.[6] Recent studies have highlighted the potential of microbial strains to solubilize zinc sources, thereby enhancing the growth of crops such as potatoes (*Solanum tuberosum*), wheat (*Triticum aestivum*), cotton (*Gossypium hirsutum L.*), and peppers (*Capsicum annuum L.*).[1,7−9] In the present study, *Bacillus megaterium* was used to evaluate the potential to solubilize ZnO. *B. megaterium* is known for its potential to solubilize phosphorus, with the main mechanism being related to its ability to produce organic acids. Therefore, it is hypothesized that a similar mechanism is involved in zinc solubilization. Besides, this microorganism produces side-rophores, compounds with the ability to act as metal-chelating agents, which adds an extra possibility in the case of zinc solubilization.[8,10−15] *B. megaterium* is also related to other growth-promoting factors, such as phytohormones that contribute to biomass production.[11,16−20]

However, the direct application of microorganisms in the fields faces challenges such as loss of cell viability due to exposure to agrochemicals, heat, and UV radiation. Therefore, it is crucial to develop formulations that ensure the protection and viability of these microorganisms.[21] In this context, microencapsulation emerges as a strategy to increase shelf life, regulate release, and preserve microorganisms against adverse environmental influences.[22] Spray-drying microencapsulation is a promising technique for cell encapsulation and is widely used in various industries due to its efficiency, speed, cost-effectiveness, and scalability.[23,24] Studies have shown success in using spray drying for microencapsulating microorganisms for the food industry, maintaining up to 90% of their viability postprocess.[25,26] In this process, the choice of the

Received: August 7, 2024
Revised: October 30, 2024
Accepted: October 31, 2024
Published: November 11, 2024

encapsulation matrix has a significant impact on the preservation of cellular viability. Polymers such as Poly(vinyl alcohol) (PVA) and Starch (ST) are considered promising for encapsulating microorganisms and enzymes due to their cellular compatibility and their partial or total degradation by various microorganisms, including the *Bacillus* bacteria.[27−30] The biodegradation mechanism more specifically related to *B. megaterium* could be associated with its ability to produce specific enzymes, such as esterases, amylases, and dehydrogenases, related to starch and PVA biodegradation. The microbial enzymatic action promotes the matrix degradation, which acts as a carbon source, essential for microbial growth.[31−36] Another polymer of growing interest is cationic starch (CS), a synthetic material derived from the chemical modification of natural starch (ST), which has amine groups with positive charges in its structure.[37] Cationic starch is highly soluble in water and has excellent film-forming properties. Recent research on PVA and cationic starch-based films has shown promising characteristics in applications such as fruit coating[38] and controlled drug release,[39] highlighting the matrix as a promising option for microorganism encapsulation. Despite the potential of these polymeric matrices to protect microbial cells, research on using spray drying for microencapsulating microorganisms for agricultural applications remains limited.

The aim of this work is to evaluate the microencapsulation of *B. megaterium* using the spray dryer technique in PVA and cationic starch-based formulations, investigating the addition of zinc oxide (ZnO) to assess its effect on the growth and development of soybean plants. To achieve this, we investigated: (1) the maximum concentration of ZnO to be added to the matrix; (2) the encapsulation of *B. megaterium* in matrices with and without ZnO and its effect on bacteria protection under conditions of heat stress, UV light, and exposure to agrochemicals; (3) the estimation of the shelf life of the materials produced using Accelerated Shelf Life Testing (ASLT) methodology; and (4) the performance of the ZnO-containing matrix in a greenhouse trial and its effect on soybean cultivation.

2. MATERIALS AND METHODS

2.1. Materials. Cationic starch (CS, Ingredion), poly(vinyl alcohol) (PVA, molecular weight 89,000−98,000, hydrolyzed to 99+%), and zinc oxide (ZnO) were obtained from Sigma-Aldrich. The fungicide, Maxin, Syngenta Brasil, and insecticide, Cruiser 350 FS, Syngenta Brasil, were used in these studies.

2.2. Microorganism Reactivation for Encapsulation. The bacterium used in this study was *B. megaterium* strain CNPMS B119 from Embrapa Maize and Sorghum.[40] The cells stored at −80 °C were initially cultured on Soy Tryptone broth (TSB) and agar at 30 °C for 12 h. The inoculum was then transferred to a liquid medium and incubated at 30 °C for 72 h to produce the cells for inoculation.

2.3. Solubilization of Different Concentrations of Zinc Oxide by *Bacillus megaterium*. A preliminary test was conducted to determine the optimal concentration of ZnO to be added to the matrix of PVA/cationic starch, aiming to achieve maximum solubility of Zn with minimal toxicity to *B. megaterium*. To investigate the solubility of ZnO and bacterial survival, a liquid culture was conducted for 10 days in TSB medium (100 mL, 250 rpm, 30 °C), starting with an initial count of *B. megaterium* of 10^9 CFU/mL and adding predefined quantities of ZnO (0.5%, 0.75%, or 1% w/v). Samples (2 mL) were collected on days 1, 2, 3, 5, and 10 to assess the solubilized Zn and bacterial growth. Solubilized zinc (Zn^{2+}) was quantified using Flame Atomic Absorption Spectroscopy (FAAS) on a PerkinElmer PinAAcle 900 T spectrometer (wavelength 213.86 nm, slit width 0.7 nm, synthetic air at 10 L/min, acetylene at 2.5 L/min). Bacterial growth was assessed by inoculating aliquots onto TSB and agar plates and then incubating for 24 h at 25 °C. All steps were performed in triplicate.

2.4. Microorganism Encapsulation. Two formulations were evaluated in this study: PVA/Cationic Starch (PVACS) and PVA/Cationic Starch/ZnO (PVACS-ZnO). Initially, PVA (5 g) and CS (5 g) were dissolved in 50 mL of distilled water and stirred in a water bath at 80 °C for 15 min to form PVACS. After the solution was cooled (25−30 °C), a cell suspension volume containing *B. megaterium* (10 $\log_{10}$ CFU/g) was added under stirring for 5 min to ensure complete dispersion. The formulation containing ZnO followed the same base and inoculation procedure as PVACS. However, after determination of the ideal amount of ZnO in the previous step (Section 2.2), 0.5% ZnO (w/v) was added to PVACS at room temperature and stirred for 5 min to ensure complete homogenization of the element, resulting in PVACS-ZnO. Both formulations were subsequently subjected to the microencapsulation and drying process using the Spray Dryer MSD 1.0 (LabMaq), operating with an outlet temperature of 70 °C, flow rate of 0.3 L/h, and gas pressure of 1.2 m^3/h. At the end of the procedure, the resulting microcapsules were recovered and stored in Falcon tubes at 25 °C for future analyses.

2.5. Characterizations. *2.5.1. Morphological and Physicochemical Analysis.* Microparticles were analyzed using a scanning electron microscope (SEM) with a JEOL (JSM-6510) at 5 kV, 10 mm working distance, and equipped with an energy dispersive X-ray analysis system (EDS spectrometer—NSS ThermoScientific). The size of the microspheres was determined from the SEM images using ImageJ software (National Institute of Health). Formulation phases were identified using X-ray diffraction with a LabX XRD-6000 diffractometer (Shimadzu, Japan) operating with Cu−Kα radiation. Diffractograms were recorded from 4 to 70° (2θ) at a scanning speed of 1° min^{-1}. The molecular structure was analyzed using Fourier Transform Infrared Spectroscopy (FTIR) on a Bruker Vertex 70 model with an ATR accessory, covering the 4000−500 cm^{-1} range at a resolution of 4 cm^{-1}.

2.6. Encapsulated Material Assays. *2.6.1. Release Profile of Microorganisms from the Matrices.* The release of encapsulated *B. megaterium* was studied by placing 0.1 g of microspheres in 5 mL of 0.85% NaCl solution at 30 °C and 250 rpm. Samples were taken at 2, 24, and 48 h. The samples were then diluted and cultured on TSB medium and agar at 25 °C for 24 h. Each dilution was tested in duplicate.

2.6.2. Stressful Conditions. Both free *Bacillus* and encapsulated in PVACS and PVACS-ZnO were subjected to tests of thermal resistance, exposure to UV light, and contact with fungicides and insecticides, considering an initial concentration of bacteria of 9 $\log_{10}$ CFU/g or mL.[41,42]

The materials were tested for heat resistance at 50 °C for 2, 24, and 48 h. They were also exposed to UV light from six 15W UV−C lamps (Philips 15W G15T8), with UV−C radiation of 4.9 W, for 10, 30, and 180 min, which corresponds to approximately 0.05, 0.14, and 0.84 J, respectively. After each exposure, free bacteria were plated and encapsulated bacteria were released in 0.85% NaCl solution at 30 °C for 2 h. Samples were then inoculated onto TSB medium and bacteriological agar in triplicate for each dilution. Plates were incubated at 25 °C for 24 h, and colonies were counted.

To test resistance to the fungicide (Maxin) and insecticide (Cruiser 350 FS), free and encapsulated *Bacillus* were separately incubated in 5 mL of the respective solution for 2 h at 30 °C. After exposure, 1 mL samples were diluted and inoculated onto TSB medium and bacteriological agar. Biological and analytical triplicates were prepared for each dilution, and the plates were incubated at 25 °C for 24 h for colony counting.

2.7. Accelerated Shelf Life Test (ASLT). The shelf life of the studied matrices was estimated using an Accelerated Shelf life Test (ASLT).[42] Both free-form and encapsulated Bacillus (10^9 CFU/mL or g) were stored at controlled temperatures (15, 30, and 45 °C) and relative humidity (76% RH) for 28 days. Evaluations were conducted

https://doi.org/10.1021/acsagscitech.4c00476
ACS Agric. Sci. Technol. 2024, 4, 1271−1283

Table 1. Experimental Design of a Soybean Greenhouse Application

code	description	Zn per pot (mg·dm^3)	P per pot (mg·dm^3)	*B. megaterium* per pot ($\log_{10}$ CFU·dm^3)
control	no fertilization	0.00	0.00	0
PVACS-ZnO	PVACS-ZnO without *B. megaterium* and without phosphorus	4.50	0.00	0
PVACS-ZnO + P	PVACS-ZnO without *B. megaterium* and phosphorus	4.50	150	0
PVACS-ZnO + bacteria	PVACS-ZnO with *B. megaterium* and without phosphorus	4.50	0.00	8.64
PVACS-ZnO + bacteria + P	PVACS-ZnO with *B. megaterium* and phosphorus addition	4.50	150	8.64

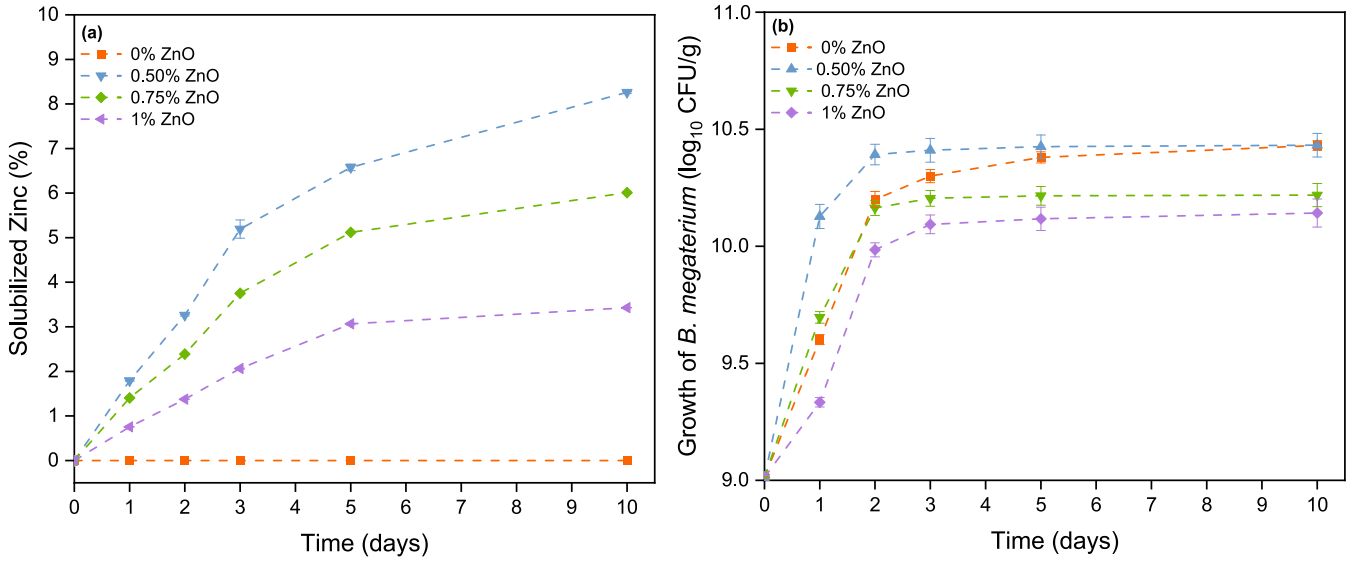

Figure 1. Zinc solubilized (a) and *Bacillus megaterium* growth (b) at different concentrations of ZnO (0−1% w/v) added to the Soy Tryptone broth (TSB) culture medium.

every 7 days after the bacteria were released in NaCl solution (0.85%). The release was done at 250 rpm in an orbital shaker at 30 °C for 2 h, and Colony-forming units (CFUs) were counted using the plate-counting method. The estimated time for ASLT was determined using first-order degradation kinetics and the Arrhenius equation to calculate the *k* value for each temperature. All tests were performed in triplicate.

$$t = \frac{\ln(\text{initial concentration}) - \ln(\text{final concentration})}{k_T} \quad (1)$$

2.8. Greenhouse Application. At this stage, PVACS-ZnO was selected for soybean greenhouse application. One dm^3 of autoclaved (30 min, 121 bar) oxysol soil was placed in plastic pots (four replications each). The soil composition is described in Table S1 (Supporting Information). With the exception of Control treatment, each pot was fertilized with 200 mg of N/dm^3 applied as urea; 185 mg of K applied as potassium sulfate, and 4.5 mg of Zn/dm^3 applied as zinc oxide. Two concentrations of P were also evaluated: 0 mg of P/dm^3 and 150 mg of P/dm^3 applied as monobasic potassium phosphate. Two sets of experiments were performed: with and without *B. megaterium* inoculation (8.64 $\log_{10}$ CFU·dm^3). The experiments were also compared with those of the control treatment without fertilization (Table 1).

The greenhouse experiments were conducted at São Carlos—SP—Brazil (22° 00′ 00″ S, 47° 53′ 27″ O), between the months of November and December, average temperature of approximately 25 °C, and the soil substrate was constantly monitored ensuring adequate water saturation (approximately 50% at the time of watering). Artificial light supplementation was provided for 12 h during the day. Soybean seeds (*Glycine max (L.)*) were germinated in a germination chamber for 6 days before being transplanted into pots already fertilized with macronutrients. The plants were regularly watered to prevent drought stress, and micronutrient fertilization was applied after 2 weeks (Table S2, Supporting Information). Since a nitrogenous source was included, the *Bradyrhizobium* sp. strain was not added to the pots.

The plants were harvested after 4 weeks. Aboveground and roots were collected, dried at 60 °C for 24 h, weighed to measure the dry matter produced, and milled using a SOLAB knife mill. Soil samples of each pot were also collected, dried, and stored for further nutrient analysis. The nutrient composition (mainly focused on P and Zn) of aboveground, roots, and soil substrate was determined by the colorimetric method extracted with ion-exchange resin (P) and extraction with DTPA and determination by atomic absorption spectrophotometry (Zn).[43] The absorption of P and Zn was assessed based on their accumulated concentration (%) in the shoot or dry root matter and the dry matter generated (mg).

2.9. Statistical Analysis. The statistical analysis was conducted using analysis of variance (ANOVA) and Tukey's test at a 95% confidence level to verify the stressful conditions, Accelerated Shelf life Test (ASLT), and greenhouse application, utilizing the Origin 9.8.0.200 software.

3. RESULTS AND DISCUSSION

3.1. Solubility and Survival of *B. megaterium* at Different Zinc Oxide Concentrations. Zinc plays a crucial role in the biochemistry of organisms, including bacterial cells, but high concentrations of this mineral can be toxic due to its antimicrobial activity.[44] Therefore, this preliminary set of experiments aimed to determine the maximum amount of zinc oxide (ZnO), a source of zinc in water-insoluble powder form,

https://doi.org/10.1021/acsagscitech.4c00476
ACS Agric. Sci. Technol. 2024, 4, 1271−1283

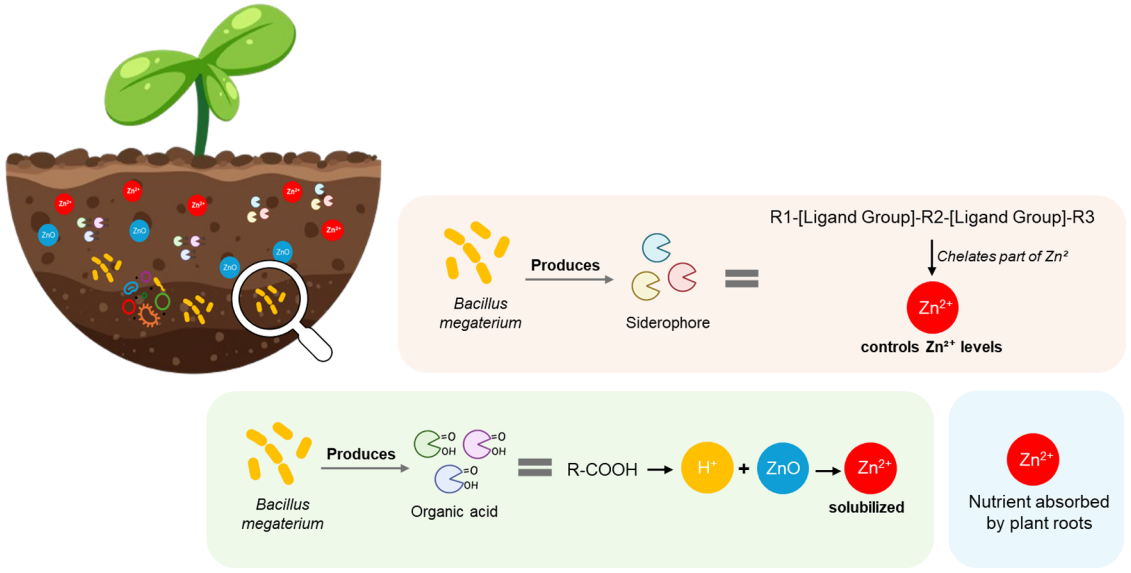

Figure 2. Hypothetical schematic of the main mechanisms potentially involved in the release of Zn^{2+}.

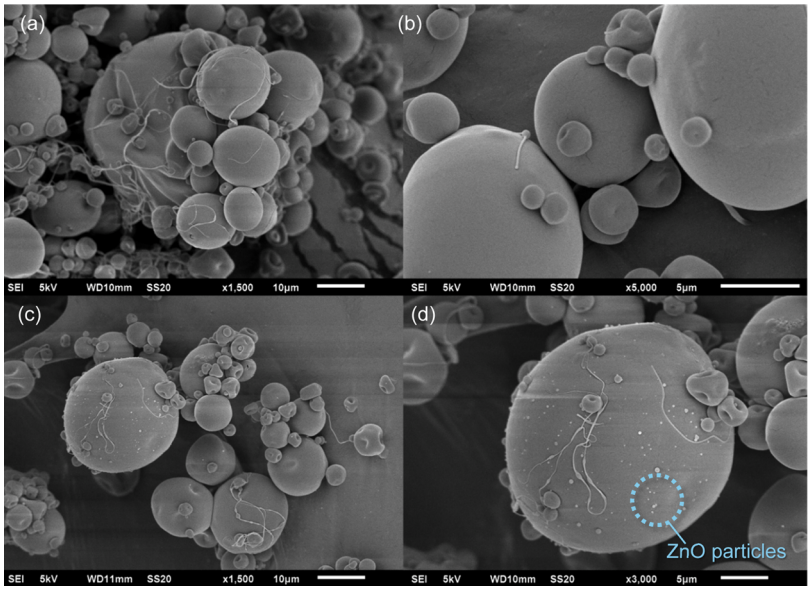

Figure 3. Microspheres by scanning electron microscopy (SEM) micrographs: (a, b) PVACS; (c) and (d) PVACS-ZnO. PVACS (PVA/cationic starch) and PVACS-ZnO (PVA/cationic starch-zinc oxide).

that could be added to the PVA/cationic starch (PVACS) matrix without adversely affecting the encapsulated bacteria. Figure 1 illustrates the percentage of solubilized zinc (Figure 1a) and bacterial growth at different concentrations of ZnO in the medium (Figure 1b).

The optimal concentration among those evaluated was determined to be 0.5% (w/v) ZnO. This concentration showed an increasing trend in zinc solubilization over the 10-day period and initial bacterial growth that was superior to that observed under other conditions. In the literature, it is typically recommended to use a maximum concentration of 0.1% (w/v) zinc sources due to low bacterial tolerance, resulting in solubility below 1% added zinc. However, the B. megaterium strain used here demonstrated promising resistance to higher concentrations of ZnO compared to what is described in the literature, with a solubility of up to 8% in a 10-day assay for the medium containing 0.5% ZnO and showing good development throughout the assay. It is hypothesized that the zinc solubilization mechanisms by *Bacillus* are similar to those observed for phosphorus solubilization (Figure 2). ZnO particles are reactive in acidic medium, generating Zn^{2+} in solution, the ionic form being absorbable by plants. Thus, it is believed that with the growth of *B. megaterium* in the soil substrate, this microorganism releases organic acids into the soil solution that can then act in the bioavailability of zinc from ZnO to Zn^{2+}. Furthermore, *B. megaterium* has been shown to be tolerant to different metals, such as zinc; this behavior could be related to the siderophores production also reported for this microorganism, compounds that act efficiently as metal-chelating agents. In other words, a potential proposal for *B. megaterium* solubilization and tolerance mechanism involving the ZnO compound probably involves the solubilization of zinc through the production of organic acids, then the siderophores produced act to control the levels of Zn^{2+} in

https://doi.org/10.1021/acsagscitech.4c00476
ACS Agric. Sci. Technol. 2024, 4, 1271−1283

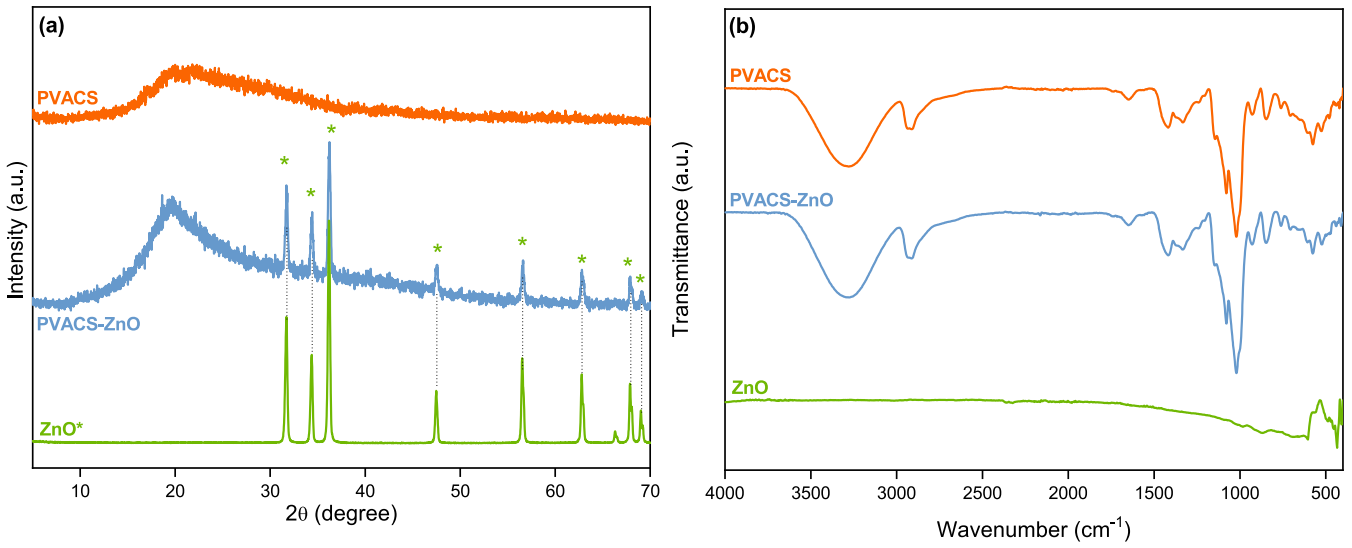

Figure 4. Physicochemical characterizations of the films. (a) X-ray diffraction (XRD); (b) Fourier Transform Infrared Spectroscopy (FTIR). PVACS (PVA/cationic starch) and PVACS-ZnO (PVA/cationic starch-zinc oxide).

the soil solution, ensuring that this nutrient is absorbed by the plants, without producing antimicrobial effects according to the conditions studied.[8,10−15,45−48]

3.2. Physicochemical and Morphological Characterizations. Figure 3 shows the micrographs of spray-dried microcapsules of PVACS and PVACS-ZnO formulations. Both formulations exhibited a similar size distribution (Figure S1, Supporting Information), with average diameters of 5.52 ± 3.23 μm (PVACS) and 5.61 ± 3.72 μm (PVACS-ZnO). However, PVACS microspheres exhibited a smooth surface with only a few other microspheres adhering (Figure 3a,b). In contrast, PVACS-ZnO microspheres displayed dispersed ZnO particles on their surface (Figure 3c,d), as confirmed by energy-dispersive X-ray analysis (Figure S2 in the Supporting Information).

Regarding the physicochemical characteristics of the produced materials, as illustrated in Figure 4a, the XRD pattern of the PVACS matrix is predominantly amorphous. It suggests insufficient time for the orderly packing of the PVA and SC chains using the spray-drying method. However, in the PVACS-ZnO matrix, the peak at 19.4° 2 theta corresponding to PVA could be identified. This behavior indicates that the ZnO particles may favor the formation of the polymer matrix ordered network, even under rapid drying conditions, although qualitatively less crystalline than casting drying.[49] As expected, the PVACS-ZnO sample also shows the characteristic peaks of ZnO,[50] as confirmed by the EDS spectrometer (Figure S2—Supporting Information).

The FTIR results (Figure 4b) show the characteristic bands of PVA overlapped with the starch bands in the case of the PVACS sample and with ZnO in the case of the PVACS-ZnO sample.[38] There was no significant change in the vibrational modes with the addition of ZnO, suggesting that it primarily acts as a filler in the matrix.

3.3. Encapsulated Bacteria. Figure 5 presents the results of tests conducted with the PVACS and PVACS-ZnO microspheres with *B. megaterium* encapsulated. Initially, we conducted an assay to investigate the release profile of the encapsulated bacteria over 48 h, as illustrated in Figure 4a. With an initial inoculation of *B. megaterium* at 10 $\log_{10}$ CFU/g, we observed that within 2 h, the microspheres released approximately 98% of the inoculated bacteria for both PVACS and PVACS-ZnO, maintaining this value up to 24 h. However, after 48 h, the release exceeded 100% of the initially inoculated bacteria, reaching 107% and 105% for PVACS and PVACS-ZnO, respectively. These findings suggest that the matrix may serve as a carbon source for the bacteria, considering its reported ability to promote the microbial enzymatic action matrix degradation,[31−36] aligning with previous studies highlighting the ability of *Bacillus* bacteria to utilize PVA, starch, and its derivatives as carbon sources for growth and multiplication, and a similar behavior could be expected in a plant−soil system.[27,30,51]

The matrix degradation rate directly influences bacterial release into the soil; accelerated degradation results in faster bacterial release, enabling controlled and gradual microorganism delivery that aligns with plant growth requirements. This controlled release facilitates effective rhizosphere colonization, which can boost plant growth through nitrogen fixation, phytohormone production, and nutrient solubilization, such as phosphorus.[52−54] Consequently, progressive matrix biodegradation correlates with a steady bacterial release, sustaining a prolonged interaction between *B. megaterium* and the host plant, enhancing plant growth, as demonstrated in greenhouse trials with other *Bacillus* species.[55−57] Biodegradable matrices like PVA and starch can thus be customized to regulate bacterial degradation and release rates, optimizing the plant performance over the growth cycle.

At this stage, we confirmed the tolerance of *B. megaterium* to ZnO at the previously established concentration (0.5% ZnO), as the release of bacteria was not negatively affected by the presence of zinc compared to that of the formulation without the element. With the successful release and development of encapsulated bacteria in the matrix, it can be concluded that the spray-drying process did not adversely affect the viability of the encapsulated bacteria. Recent studies have shown the effectiveness of microencapsulation of fungi and bacteria using a spray dryer, preserving up to 90% of cell viability.[23,25,26] It aligns with the results observed in this study, where approximately 98% cell viability was maintained.

When considering agricultural applications of the product, it is crucial to consider the potential stresses that cells may

https://doi.org/10.1021/acsagscitech.4c00476
ACS Agric. Sci. Technol. 2024, 4, 1271−1283

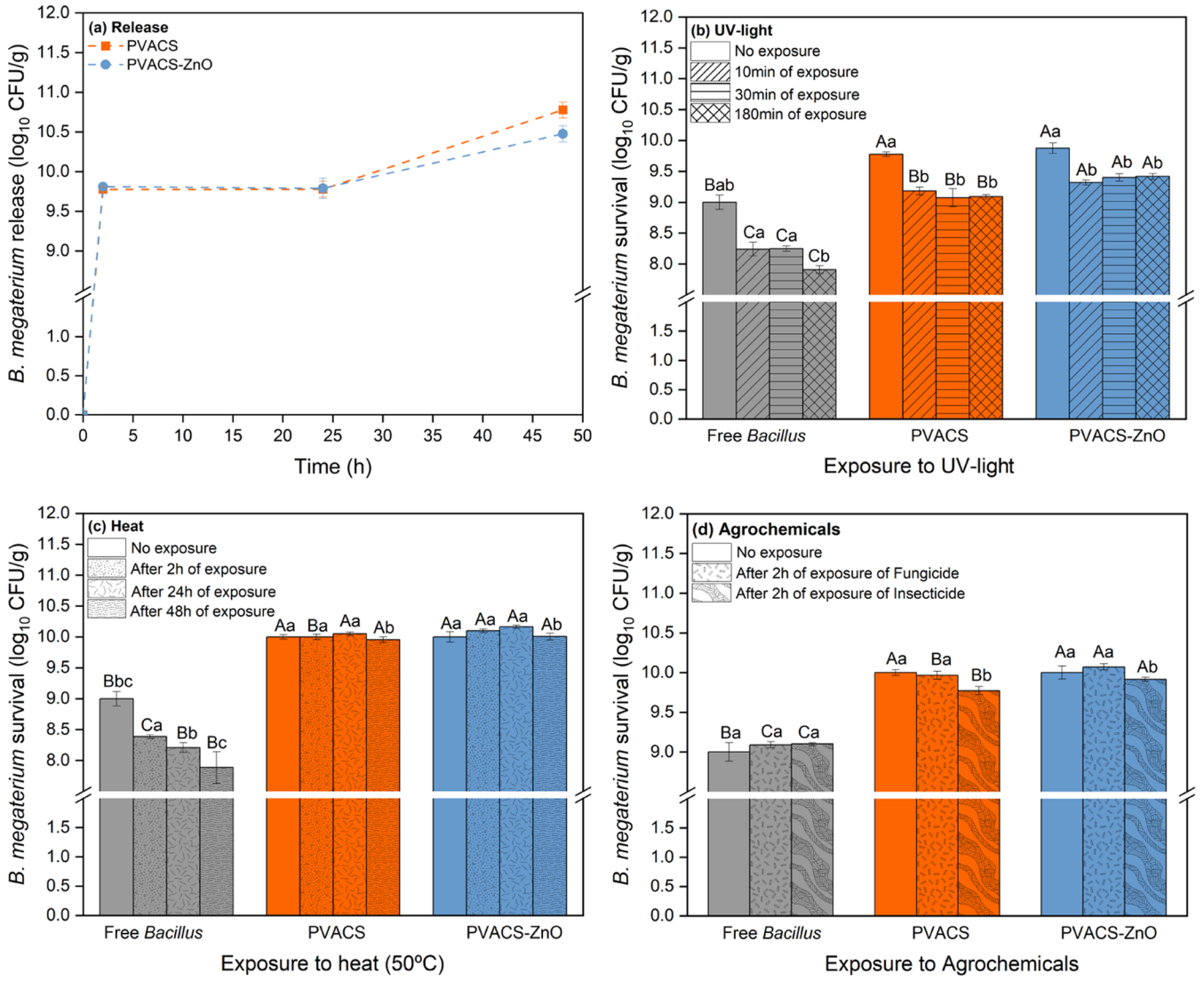

Figure 5. Tests with PVACS and PVACS-ZnO microspheres encapsulated with *B. megaterium*. (a) Release profiles of bacteria in saline medium over 48 h; survival of bacteria before and after exposure (b) to UV light (10, 30, and 180 min); (c) to thermal stress (50 °C for 2, 24, and 48 h); and (d) to contact with the agrochemicals (fungicide and insecticide, 30 °C/2 h). PVACS (PVA/cationic starch) and PVACS-ZnO (PVA/cationic starch-zinc oxide). Capital letters (A, B, C) indicate statistically significant differences between the different matrices (free *Bacillus*, PVACs, and PVACs-ZnO) at the same exposure time. Lowercase letters (a, b) represent significant differences between exposure times within the same matrix. Values sharing the same letter (capital or lowercase) are not significantly different ($p > 0.05$), whereas different letters indicate significant differences ($p < 0.05$).

encounter during field application, such as exposure to pesticides, heat, and UV light.[58−60] Thus, both free bacteria and encapsulated microspheres were exposed to stressful conditions, including UV light (Figure 5b), heat (Figure 5c), and agrochemicals (Figure 5d).

Regarding exposure to UV light, the PVACS-ZnO formulation stood out, maintaining up to 95% bacterial survival over 180 min of exposure when compared to that of the control without exposure. Meanwhile, PVACS experienced a slightly more pronounced reduction with survival of up to 93% over the same period. Free bacteria were the most affected by UV light exposure with up to 88% survival during the experiment. All data are statistically significantly different comparing each exposure (or no exposure) time. Thus, the enhanced protection of the ZnO-containing material is attributed to its recognized ability to filter UV light, commonly used in products such as cosmetics.[61−64] The UV light protective ZnO particles mechanism involves absorb, reflect, and retain of UV light photons, primarily operating in photoprotection through the absorption of UV radiation,[65,66] directly contributing to the barrier protection for *B. megaterium*. Nevertheless, the comparison between free *Bacillus* and PVACS formulations shows the UV protective potential of this proposed matrix, even without ZnO addition. This behavior can be related to two main PVACS matrix main factors. Starch films also present some UV absorption potential, especially in the UV−C region, and it is substantially increased, for example, by ZnO particles incorporation. In addition, both the PVA and starch films present oxygen blocking property, reducing the oxidative stress caused by UV exposure.[66−70]

Thermal stress is another significant factor affecting microorganisms in the field. The rise in temperature due to global warming threatens agricultural production, negatively

https://doi.org/10.1021/acsagscitech.4c00476
ACS Agric. Sci. Technol. 2024, 4, 1271−1283

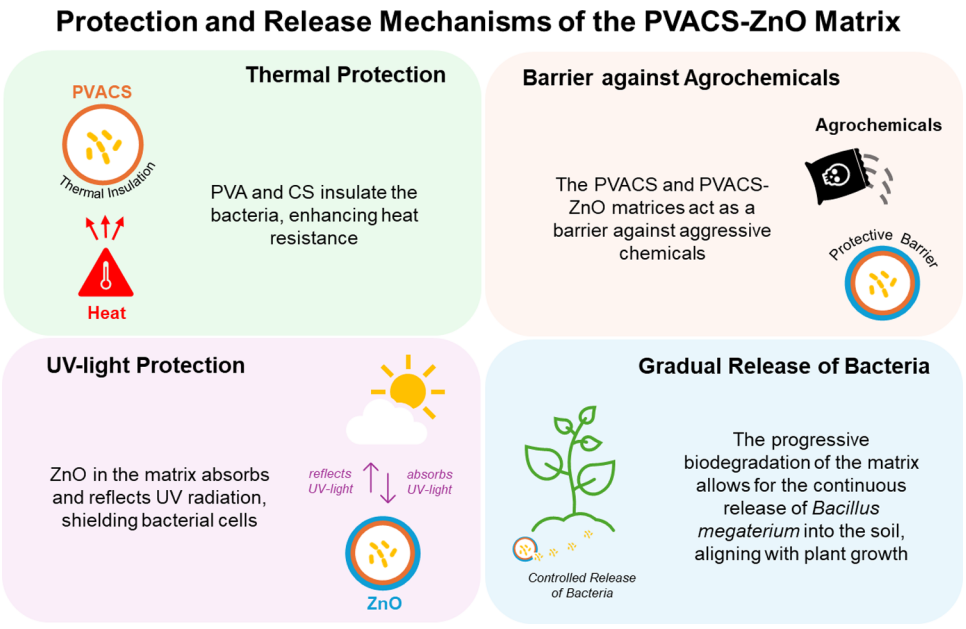

Figure 6. Synthesis of the main mechanisms involved in the protection of the PVA/CS-ZnO matrix to *B. megaterium*.

Table 2. Free and Encapsulated *B. megaterium* to Achieve a Complete Loss of Cell Viability[a]

	free bacteria			PVACS			PVACS-ZnO		
temperature of storage (°C)	*15*	*30*	*45*	*15*	*30*	*45*	*15*	*30*	*45*
months to lose viability	6.6	5.6	4.8	10.4	8.2	6.7	13.7	8.7	5.7

[a]PVACS (PVA/Cationic Starch) and PVACS-ZnO (PVA/Cationic Starch-Zinc Oxide).

impacting the viability of microorganisms and various plant developmental stages.[71] Research on the survival of microorganisms under high temperatures for agricultural applications is limited, with most studies focusing on cells intended for food processing or the elimination of *Bacillus* considered contaminants.[72,73] Both formulations maintained the initial viability of bacteria, even after exposure to 50 °C, with the encapsulated material providing satisfactory protection for *B. megaterium* throughout the experiment. It contrasts with the observed result for free bacteria, which experienced a progressive loss of viability, with survival rates of 93%, 91%, and 88% after 2, 24, and 48 h, respectively. In general, the statistically significant analysis shows that both PVACs and PVACs-ZnO formulations are different from free *Bacillus*. When PVACs and PVACs-ZnO formulations were compared in relation to each time of temperature exposure, after 2 h, PVACs-ZnO proved to be statistically different with a result slightly above PVACs (99 and 100%, respectively). It can be related to the higher thermal stability of ZnO compared to PVA and starch.[74−76]

Lastly, exposure to agrochemicals (fungicides and insecticides), commonly used in modern agriculture to combat pests and plant diseases and potentially affecting the viability of microorganisms,[77] is presented in Figure 4d. Both formulations offered similar protection against the fungicide, with PVACS slightly reducing bacterial survival to 99%, while PVACS-ZnO maintained entire viability after contact with the solution compared to 100% survival of free *Bacillus*. Regarding the insecticide, encapsulated *Bacillus* showed 97 and 99%, survival rates for PVACS and PVACS-ZnO, respectively, compared to 100% survival of free *Bacillus*. The agrochemicals in this assay did not affect the survival of the free bacteria.

The main mechanisms involved in protecting *B. megaterium* against UV radiation, thermal stress, and agrochemicals are illustrated in Figure 6. This figure synthesizes the protective roles of the PVACS-ZnO matrix, highlighting how it serves as a barrier against these environmental challenges while facilitating the controlled release of bacteria into the soil.

3.3.1. Accelerated Shelf life Test (ASLT). In developing matrices for microorganism encapsulation, it is crucial to assess cell viability during storage due to the lack of globally established standards for commercializing these products.[78] In this study, we employed the Accelerated Shelf life Test (ASLT) methodology, commonly used in the food industry, to estimate the shelf life of microspheres containing strain *B. megaterium*, simplifying viability analysis through mathematical models.[42] Table 2 shows the estimated time for bacterial viability loss under various storage temperatures and high humidity conditions to accelerate particle degradation. The time needed for complete viability loss is presented in days for both free and encapsulated bacteria, offering insights into the formulations' effectiveness in preserving bacterial viability. Tables and graphs related to the ASLT shelf life estimation calculations are available in Tables S3, S4, and S5 (Supporting Information).

Temperature plays a crucial role in storage, significantly affecting microorganism viability, as observed in this experiment, where there was a reduction in estimated shelf life for all matrices as the storage temperature increased. Both free and encapsulated bacteria showed a higher estimated shelf life at 15 °C. Among the evaluated matrices, PVACS-ZnO stood out, achieving the best performance with up to 13.5 months for complete viability loss. In the case of PVACS, the total loss reached approximately 10.3 months. In contrast, free *B.*

https://doi.org/10.1021/acsagscitech.4c00476
ACS Agric. Sci. Technol. 2024, 4, 1271−1283

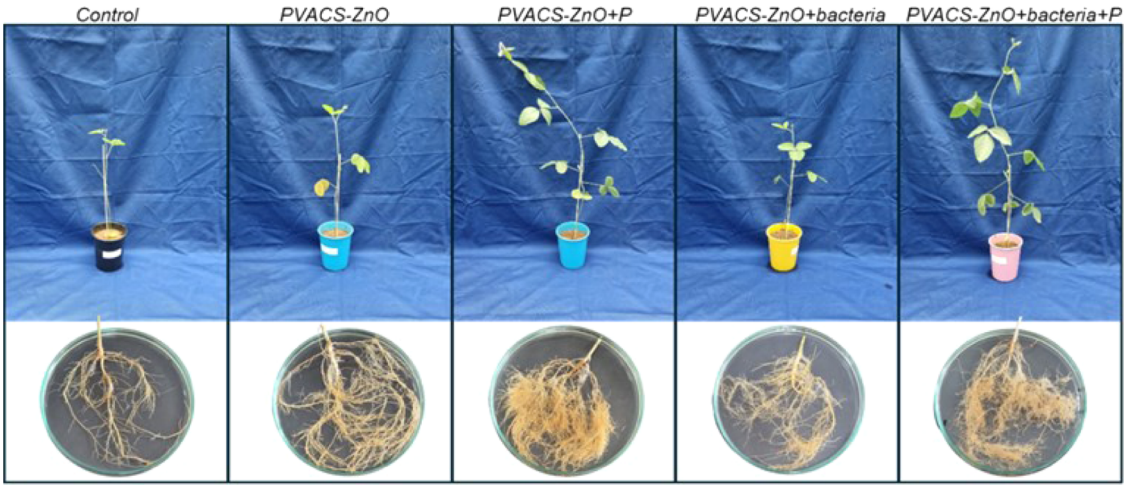

Figure 7. Pot with soybeans at the end of the experiment as follows: control (no fertilization); PVACS-ZnO: PVACS-ZnO without *B. megaterium* and phosphorus; PVACS-ZnO + P: PVACS-ZnO without *B. megaterium* and with phosphorus; PVACS-ZnO + bacteria: PVACS-ZnO with *B. megaterium* and without phosphorus; PVACS-ZnO + bacteria + P: PVACS-ZnO with *B. megaterium* and phosphorus. PVACS (PVA/cationic starch) and PVACS-ZnO (PVA/cationic starch-zinc oxide).

megaterium experienced total viability loss in 6.6 months. The studied matrices, especially PVACS-ZnO, contributed to doubling the shelf life of encapsulated microorganisms compared to free microorganisms, confirming the protection observed in stress tests (Figure 4) and highlighting the protective environment provided for encapsulated *Bacillus*.

3.4. Greenhouse Application. Overall, the greenhouse trial results demonstrated the effectiveness of PVACS-ZnO with encapsulated *B. megaterium* in promoting plant growth, indicating its promising application as a biofertilizer for more sustainable agricultural practices. These favorable outcomes, coupled with the ease of production through atomization, the formulation ability to protect cells against stressful field conditions, and the satisfactory maintenance of viability during storage, highlight the material's potential as a novel and efficient biofertilizer.

The agronomic efficiency of PVACS-ZnO was evaluated in a greenhouse trial to investigate the effect of zinc in soybean plants as a model. The experiment results are presented in Figures 7 and 8. The analysis included treatment *control* (no fertilization) and PVACS-ZnO without *B. megaterium*, both without P (*PVACS-ZnO*) and with phosphorus (P) addition (*PVACS-ZnO + P*), as well as PVACS-ZnO containing *B. megaterium*, again without P (*PVACS-ZnO + bacteria*) and with P addition (*PVACS-ZnO + bacteria + P*).

In terms of composition, a significant increase in aerial biomass production was observed in treatments with bacteria (*PVACS-ZnO + bacteria* and *PVACS-ZnO + bacteria + P*) compared to their respective counterparts without the addition of the microorganism (*PVACS-ZnO* and *PVACS-ZnO + P*) (Figure 8a). Treatment *PVACS-ZnO*, despite receiving fertilization excluding phosphorus, showed biomass production statistically equivalent to the unfertilized control, highlighting the crops high demand for phosphorus.[79,80] However, when comparing treatments with P supplementation, such as *PVACS-ZnO + bacteria + P*, a considerably higher aboveground biomass production was evident than for treatments without P supplementation, as seen in *PVACS-ZnO + bacteria*. The average values for root biomass production exhibited trends similar to those observed for aerial biomass production, with only the *PVACS-ZnO + bacteria + P* treatment showing statistical significance compared to the others (Figure 8b). Although root scanning was not performed, it was observed that the absence of P significantly altered the number of root branches (control, PVACS-ZnO, PVACS-ZnO + bacteria). Nevertheless, even in the absence of P (PVACS-ZnO + bacteria), the presence of *B. megaterium* seems to favor root branching, such as shorter and thinner roots with a hairy effect, while PVACS-ZnO treatment presents longer and thicker roots. These findings agree to the *B. megaterium* ability to also produce phytohormones, such as gibberellin, cytokinin (zeatin), and abscisic acid, but more specifically indole-3-acetic acid, a phytohormone that is related to root grown stimulation.[11,−20] However, further study is needed to provide more details about the effect of the proposed materials on root architecture.

The same discussion applies to aerial biomass production and can be applied to phosphorus analysis (Figure 8c). This behavior may be related to the very low available nutrient concentration in the original soil (Table S1), which can harm the initial development of plants until microorganisms can develop, effectively occupying the entire rhizosphere and then promoting greater availability of P and Zn. The delay in bioavailability slows down the development and loading of the plant with phosphorus and zinc.[81,82] Moreover, in the case of Zn (Figure 6d), as additional evidence of the synergistic effect between the microbiological activity of *B. megaterium* and a minimum concentration of readily available P., the *PVACS-ZnO + bacteria + P* sample demonstrated a significant increase in Zn uptake, for example, compared to the *PVACS-ZnO + P* sample, that is, without inoculation of the microorganism but only with P supplementation.

The analysis of residual P (Figure 8e) and Zn (Figure 8f) in the soil substrate after greenhouse application showed, as expected, that the experiments with P supplementation still present a high content of this nutrient, around 40% (60 mg·dm^3) of the dose initially applied (150 mg·dm^3). The residual soil from the *PVACS-ZnO* sample is statistically equivalent to the *control* without nutritional supplementation or adding *B. megaterium*. However, the *PVACS-ZnO + bacteria* sample showed a subtle increase in available P concentration (~ 2.5 to ~4 mg·dm^3, control and *PVACS-ZnO + bacteria*, respectively)

https://doi.org/10.1021/acsagscitech.4c00476
ACS Agric. Sci. Technol. 2024, 4, 1271−1283

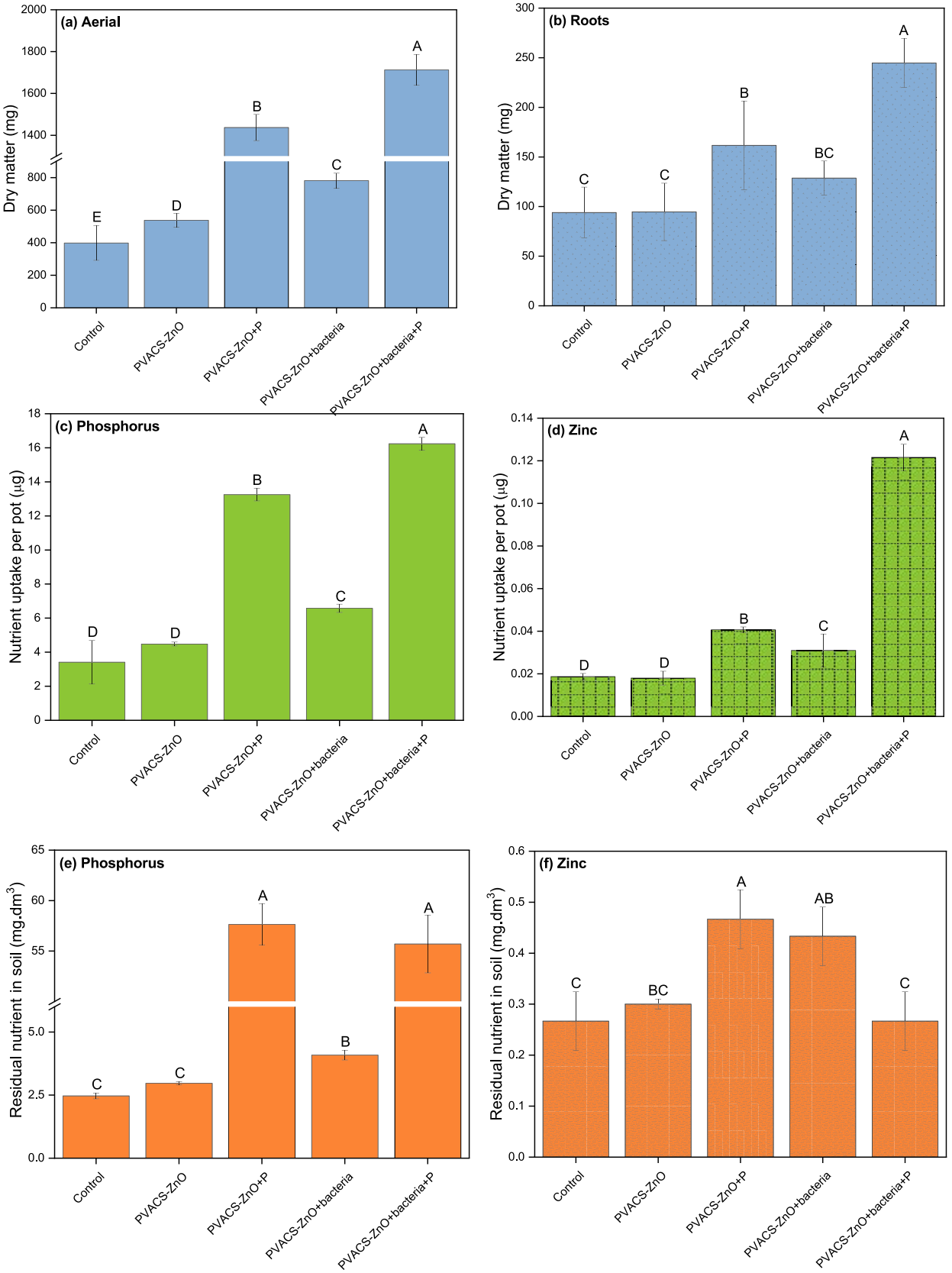

Figure 8. Results obtained from the greenhouse application trial. Dry matter production of aerial (a) and roots (b); nutrient uptake per pot—P (c) and Zn (d); and residual nutrient in soil, P (e) and Zn (f). Control (no fertilization); PVACS-ZnO: PVACS-ZnO without *B. megaterium* and phosphorus; PVACS-ZnO + P: PVACS-ZnO without *B. megaterium* and with phosphorus; PVACS-ZnO + bacteria: PVACS-ZnO with *B. megaterium* and without phosphorus; PVACS-ZnO + bacteria + P: PVACS-ZnO with *B. megaterium* and phosphorus. PVACS (PVA/cationic starch) and PVACS-ZnO (PVA/cationic starch-zinc oxide). [A, B]: Different uppercase letters within the graphs indicate statistical difference by Tukey's test ($P < 0.05$); equal letters do not differ significantly.

https://doi.org/10.1021/acsagscitech.4c00476
ACS Agric. Sci. Technol. 2024, 4, 1271−1283

which indirectly suggests that the microbial activity of *B. megaterium* also contributes to an increase in the concentration of P in the residual soil. A different behavior is observed in the analysis of Zn in the residual soil ($mg \cdot dm^3$). The *PVACS-ZnO + bacteria + P* sample indicates a low Zn content in the residual soil, with values close to the *control*; this can be explained by its significant uptake in aerial biomass compared to the other samples. The *PVACS-ZnO* sample remains statistically equivalent to the *control*. The explanation is that although Zn was supplemented, the absence of *B. megaterium* and the delay in plant development due to the very low P content compromised the solubilization and uptake of this micronutrient. As further proof of the effectiveness of bacteria in Zn solubilization, a significant increase in residual Zn content is observed compared to the other samples except for the *PVACS-ZnO + P* sample. In this last case, the release of biomolecules by soybean roots also synergizes Zn solubilization. However, it appears to be dependent on the concentration of available P.[83]

In overall, this study demonstrated that the *B. megaterium* strain presents a high potential for solubilizing zinc by effectively solubilizing zinc oxide. PVA/cationic starch and zinc oxide microspheres (PVACS-ZnO), produced by spray drying, demonstrated superior cell protection under stressful conditions, such as UV exposure, heat, and agrochemicals. Accelerated Shelf life Tests (ASLT) indicated extended cell viability of PVACS-ZnO microspheres compared to free bacteria, doubling the estimated shelf life of the bacteria and highlighting their effectiveness in preserving bacterial viability during storage. In greenhouse experiments, the presence of zinc and *B. megaterium* in the formulation significantly influenced the increase in aerial and root biomass production of soybean plants, and promoted greater absorption of phosphorus and zinc nutrients by soybean biomass. The observation of a low zinc content in residual soil corroborated the positive effect of zinc. These results suggest that both bacteria and zinc in the matrix benefited plant growth and nutrient absorption, indicating the promising potential of the PVACS-ZnO microspheres as an effective biofertilizer.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsagscitech.4c00476.

> Histogram with the size distribution of microparticles obtained by spray dryer drying. (a) PVASC and (b) PVASC-ZnO (Figure S1); SEM images and energy dispersive spectrometer (EDS) of PVASC-ZnO (PVA/Cationic Starch-Zinc oxide) (Figure S2) and Chemical, physical, and additional analyses of the soil used in the greenhouse trial (Table S1); Micronutrient fertilization was used in the greenhouse trial (Table S2); Tables. calculations. and graphs for determining the life estimation of *B. megaterium* using the ASLT methodology (Table S3); Tables. calculations. and graphs for determining the life estimation of PVASC using the ASLT methodology (Table S4); Tables. calculations. and graphs for determining the life estimation of PVASC-ZnO using the ASLT methodology (Table S5) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Cristiane Sanchez Farinas − *Nanotechnology National Laboratory for Agriculture, Embrapa Instrumentation, São Carlos, SP 13560-970, Brazil; Graduate Program of Chemical Engineering and Graduate Program of Biotechnology, Federal University of Sao Carlos, São Carlos, SP 13565-905, Brazil;* orcid.org/0000-0002-9985-190X; Phone: +55 16 2107-2908; Email: cristiane.farinas@embrapa.br

Authors

Ludimila Araújo Lodi − *Nanotechnology National Laboratory for Agriculture, Embrapa Instrumentation, São Carlos, SP 13560-970, Brazil; Graduate Program of Chemical Engineering, Federal University of Sao Carlos, São Carlos, SP 13565-905, Brazil*

Roger Borges − *Nanotechnology National Laboratory for Agriculture, Embrapa Instrumentation, São Carlos, SP 13560-970, Brazil;* orcid.org/0000-0001-6992-3717

Marina Momesso Lopes − *Nanotechnology National Laboratory for Agriculture, Embrapa Instrumentation, São Carlos, SP 13560-970, Brazil; Graduate Program of Biotechnology, Federal University of Sao Carlos, São Carlos, SP 13565-905, Brazil*

Vanessa Araújo Graciano − *Nanotechnology National Laboratory for Agriculture, Embrapa Instrumentation, São Carlos, SP 13560-970, Brazil; Graduate Program of Biotechnology, Federal University of Sao Carlos, São Carlos, SP 13565-905, Brazil*

Ricardo Bortoletto-Santos − *Postgraduate Program in Environmental Technology, University of Ribeirão Preto (UNAERP), Ribeirão Preto, SP 14096-900, Brazil;* orcid.org/0000-0002-4447-8239

Hernane S. Barud − *University of Araraquara (UNIARA), Araraquara, SP 14801-340, Brazil;* orcid.org/0000-0001-9081-2413

Christiane Abreu de Oliveira-Paiva − *Embrapa Corn and Sorghum, Sete Lagoas, MG 35701-970, Brazil*

Caue Ribeiro − *Nanotechnology National Laboratory for Agriculture, Embrapa Instrumentation, São Carlos, SP 13560-970, Brazil;* orcid.org/0000-0002-8908-6343

Complete contact information is available at: https://pubs.acs.org/10.1021/acsagscitech.4c00476

Funding

The Article Processing Charge for the publication of this research was funded by the Coordination for the Improvement of Higher Education Personnel - CAPES (ROR identifier: 00x0ma614).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Finance Code 001, grant #88887.498020/2020-00), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grants # 20/12210-3, 23/01549-8, 24/09631-8 and 23/09673-0), FINEP (Agreement 01.22.0274.00, and FNDCT/CT-AGRO/FINEP Cooperation Agreement No. 01.22.0080.00, ref 1219/21) and Conselho Nacional de Desenvolvimento

Científico e Tecnológico (CNPq, grants #151189/2023-4, #402713/2023-0, #441573/2023-1, 442575/2019-0-SISNA-NO/MCTI program, and 406925/2022-4-INCT Circularity in Polymer Materials) and FNDCT/CT-AGRO/FINEP (Cooperation Agreement No. 01.22.0080.00, ref 1219/21 - FertBrasil, and FINEP No. 01.22.0274.00 - MATFERT). H.S. Barud thanks (CEMASU) FAPESP-Funding (Process: 2021/11965-3) and (Process: 2017/50334-3). National Council of Scientific and Technological Development/CNPq (Grant: 309614/2021-0), National Institutes of Science and Technology (INCTs), INCT Polysaccharides (Grant: 406973/2022-9), INCT Circularity in Polymer Materials (grant no. 406925/2022-4), INCT-INFO (National Institute of Photonics). Figure 2 used in this work was adapted from Freepik, with original content created by brgfx, available at www.freepik.com. In addition, the authors are grateful to the Agronano Network (Embrapa Research Network) and the Nanotechnology National Laboratory for Agriculture (LNNA) for providing institutional support and facilities.

REFERENCES

(1) Ahmad, I.; Ahmad, M.; Hussain, A.; Jamil, M. Integrated Use of Phosphate-Solubilizing Bacillus Subtilis Strain IA6 and Zinc-Solubilizing Bacillus Sp. Strain IA16: A Promising Approach for Improving Cotton Growth. *Folia Microbiol.* **2021**, *66*, 115−125.

(2) Zeng, H.; Zhang, X.; Ding, M.; Zhang, X.; Zhu, Y. Transcriptome Profiles of Soybean Leaves and Roots in Response to Zinc Deficiency. *Physiol. Plant.* **2019**, *167* (3), 330−351.

(3) Khande, R.; Sharma, S. K.; Ramesh, A.; Sharma, M. P. Zinc Solubilizing Bacillus Strains That Modulate Growth, Yield and Zinc Biofortification of Soybean and Wheat. *Rhizosphere* **2017**, *4*, 126−138.

(4) Pawlowski, M. L.; Helfenstein, J.; Frossard, E.; Hartman, G. L. Boron and Zinc Deficiencies and Toxicities and Their Interactions with Other Nutrients in Soybean Roots, Leaves, and Seeds. *J. Plant Nutr.* **2019**, *42* (6), 634−649.

(5) Xu, J.; Wang, X.; Zhu, H.; Yu, F. Maize Genotypes With Different Zinc Efficiency in Response to Low Zinc Stress and Heterogeneous Zinc Supply. *Front. Plant Sci.* **2021**, *12*, No. 736658, DOI: 10.3389/fpls.2021.736658.

(6) Degryse, F.; da Silva, R. C.; Baird, R.; Cakmak, I.; Yazici, M. A.; McLaughlin, M. J. Comparison and Modelling of Extraction Methods to Assess Agronomic Effectiveness of Fertilizer Zinc. *J. Plant Nutr. Soil Sci.* **2020**, *183* (2), 248−259.

(7) Ali, M.; Ahmed, I.; Tariq, H.; Abbas, S.; Zia, M. H.; Mumtaz, A.; Sharif, M. Growth Improvement of Wheat (Triticum Aestivum) and Zinc Biofortification Using Potent Zinc-Solubilizing Bacteria. *Front. Plant Sci.* **2023**, *14*, No. 1140454, DOI: 10.3389/fpls.2023.1140454.

(8) Bhatt, K.; Maheshwari, D. K. Zinc Solubilizing Bacteria (Bacillus Megaterium) with Multifarious Plant Growth Promoting Activities Alleviates Growth in Capsicum Annuum L. *3 Biotech* **2020**, *10* (2), No. 36, DOI: 10.1007/s13205-019-2033-9.

(9) Gupta, S.; Srivastava, P. K.; Singh, R. P. Application of Plant Growth Promoting Microbes to Enrich Zinc in Potato for Nutritional Security and Sustainable Agriculture. *Rhizosphere* **2023**, *25*, No. 100665.

(10) Wyciszkiewicz, M.; Saeid, A.; Chojnacka, K. Solubilization of Renewable Phosphorus Sources with Organic Acids Produced by Bacillus Megaterium. *J. Renewable Mater.* **2017**, *5* (1), 39−52.

(11) Guzmán-Moreno, J.; García-Ortega, L. F.; Torres-Saucedo, L.; Rivas-Noriega, P.; Ramírez-Santoyo, R. M.; Sánchez-Calderón, L.; Quiroz-Serrano, I. N.; Vidales-Rodríguez, L. E. Bacillus Megaterium HgT21: A Promising Metal Multiresistant Plant Growth-Promoting Bacteria for Soil Biorestoration. *Microbiol. Spectrum* **2022**, *10* (5), No. e00656-22, DOI: 10.1128/spectrum.00656-22.

(12) Ortiz, A.; Sansinenea, E. Succinic Acid Production as Secondary Metabolite from Bacillus Megaterium ELI24. *Nat. Prod. J.* **2020**, *10* (2), 153−157.

(13) Mumtaz, M. Z.; Barry, K. M.; Baker, A. L.; Nichols, D. S.; Ahmad, M.; Zahir, Z. A.; Britz, M. L. Production of Lactic and Acetic Acids by Bacillus Sp. ZM20 and Bacillus Cereus Following Exposure to Zinc Oxide: A Possible Mechanism for Zn Solubilization. *Rhizosphere* **2019**, *12*, No. 100170.

(14) Chuljerm, H.; Deeudom, M.; Fucharoen, S.; Mazzacuva, F.; Hider, R. C.; Srichairatanakool, S.; Cilibrizzi, A. Characterization of Two Siderophores Produced by Bacillus Megaterium: A Preliminary Investigation into Their Potential as Therapeutic Agents. *Biochim. Biophys. Acta, Gen. Subj.* **2020**, *1864* (10), No. 129670.

(15) Wyciszkiewicz, M.; Saeid, A.; Chojnacka, K.; Górecki, H. Production of Phosphate Biofertilizers from Bones by Phosphate-Solubilizing Bacteria Bacillus Megaterium. *Open Chem.* **2015**, *13* (1), 1063−1070.

(16) Naumovich, N. I.; Akhremchuk, A. E.; Valentovich, L. N.; Aleschenkov, Z. M.; Ananyeva, I. N.; Safronova, G. V. Molecular-Genetic Characterization of Halotolerant Strain *Priestia Megaterium* BIM B-1314D. *Dokl. Natl. Acad. Sci. Belarus* **2022**, *66* (1), 55−64.

(17) Rodrigues, V.; Deusdado, S. Meta-Learning Approach for Bacteria Classification and Identification of Informative Genes of the Bacillus Megaterium: Tomato Roots Tissue Interaction. *3 Biotech* **2023**, *13* (8), No. 271.

(18) Bhutani, N.; Maheshwari, R.; Negi, M.; Suneja, P. Optimization of IAA Production by Endophytic Bacillus Spp. from Vigna Radiata for Their Potential Use as Plant Growth Promoters. *Isr. J. Plant Sci.* **2018**, *65* (1−2), 83−96.

(19) Ali, B.; Sabri, A. N.; Ljung, K.; Hasnain, S. Quantification of Indole-3-Acetic Acid from Plant Associated Bacillus Spp. and Their Phytostimulatory Effect on Vigna Radiata (L.). *World J. Microbiol. Biotechnol.* **2009**, *25* (3), 519−526.

(20) Karadeniz, A.; Topcuoğlu, Ş. F.; İnan, S. Auxin, Gibberellin, Cytokinin and Abscisic Acid Production in Some Bacteria. *World J. Microbiol. Biotechnol.* **2006**, *22* (10), 1061−1064.

(21) Berninger, T.; González López, Ó.; Bejarano, A.; Preininger, C.; Sessitsch, A. Maintenance and Assessment of Cell Viability in Formulation of Non-Sporulating Bacterial Inoculants. In *Microbial Biotechnology.*; John Wiley and Sons Ltd, 2018; pp 277−301.

(22) Rojas-Sánchez, B.; Guzmán-Guzmán, P.; Morales-Cedeño, L. R.; Orozco-Mosqueda, Ma. del C.; Saucedo-Martínez, B. C.; Sánchez-Yáñez, J. M.; Fadiji, A. E.; Babalola, O. O.; Glick, B. R.; Santoyo, G. Bioencapsulation of Microbial Inoculants: Mechanisms, Formulation Types and Application Techniques. *Appl. Biosci.* **2022**, *1* (2), 198−220.

(23) Braga, A. B. A. C.; Costa, C. J. M.; Pomella, A. W. V.; Ribeiro, E. J.; Santos, L. D.; Zotarelli, M. F. Evaluation of Lethality Temperature and Use of Different Wall Materials in the Micro-encapsulation Process of Trichoderma Asperellum Conidias by Spray Drying. *Powder Technol.* **2019**, *347*, 199−206.

(24) Piñón-Balderrama, C. I.; Leyva-Porras, C.; Terán-Figueroa, Y.; Espinosa-Solís, V.; Álvarez-Salas, C.; Saavedra-Leos, M. Z. Encapsulation of Active Ingredients in Food Industry by Spray-Drying and Nano Spray-Drying Technologies. *Processes* **2020**, *8* (8), No. 889, DOI: 10.3390/PR8080889.

(25) Seth, D.; Mishra, H. N.; Deka, S. C. Effect of Micro-encapsulation Using Extrusion Technique on Viability of Bacterial Cells during Spray Drying of Sweetened Yoghurt. *Int. J. Biol. Macromol.* **2017**, *103*, 802−807.

(26) Strobel, S. A.; Allen, K.; Roberts, C.; Jimenez, D.; Scher, H. B.; Jeoh, T. Industrially-Scalable Microencapsulation of Plant Beneficial Bacteria in Dry Cross-Linked Alginate Matrix. *Ind. Biotechnol.* **2018**, *14* (3), 138−147.

(27) Bian, H.; Wang, G.; Cao, M.; Wang, Z.; Cui, J. Improved Biodegradation of Polyvinyl Alcohol by Hybrid Nanoflowers of Degrading Enzymes from Bacillus Niacini. *Korean J. Chem. Eng.* **2020**, *37* (6), 1020−1028.

(28) Bian, H.; Cao, M.; Wen, H.; Tan, Z.; Jia, S.; Cui, J. Biodegradation of Polyvinyl Alcohol Using Cross-Linked Enzyme Aggregates of Degrading Enzymes from Bacillus Niacini. *Int. J. Biol. Macromol.* **2019**, *124*, 10−16.
(29) Liu, P.; Chen, W.; Liu, C.; Tian, M.; Liu, P. A Novel Poly (Vinyl Alcohol)/Poly (Ethylene Glycol) Scaffold for Tissue Engineering with a Unique Bimodal Open-Celled Structure Fabricated Using Supercritical Fluid Foaming. *Sci. Rep.* **2019**, *9* (1), No. 9534.
(30) Ullah, M.; Li, H.; Sun, S. W.; Weng, C. H.; Zhang, H.; Zhu, H. Polyvinyl Alcohol Degradation by Bacillus Cereus RA23 from Oil Sludge Sample. *3 Biotech* **2019**, *9* (10), 1−8, DOI: 10.1007/s13205-019-1882-6.
(31) Petrovičová, T.; Markošová, K.; Hegyi, Z.; Smonou, I.; Rosenberg, M.; Rebroš, M. Co-Immobilization of Ketoreductase and Glucose Dehydrogenase. *Catalysts* **2018**, *8* (4), No. 168.
(32) Vaclavkova, T.; Ruzicka, J.; Julinova, M.; Vicha, R.; Koutny, M. Novel Aspects of Symbiotic (Polyvinyl Alcohol) Biodegradation. *Appl. Microbiol. Biotechnol.* **2007**, *76* (4), 911−917.
(33) Hrnčiřík, J.; Pšeja, J.; Kupec, J.; Bernkopfová, S. Anaerobic Biodegradation of Polyvinyl Alcohol Modified by Extracellular Polysaccharides. *J. Polym. Environ.* **2010**, *18* (2), 98−103.
(34) Jana, M.; Maity, C.; Samanta, S.; Pati, B. R.; Islam, S. S.; Mohapatra, P. K. Das.; Mondal, K. C. Salt-Independent Thermophilic α-Amylase from Bacillus Megaterium VUMB109: An Efficacy Testing for Preparation of Maltooligosaccharides. *Ind. Crops Prod.* **2013**, *41*, 386−391.
(35) Zheng, J.-y.; Wang, J.; Zhou, S.; Li, X.; Ying, X.; Wang, Z. A Stereoselective Esterase from Bacillus Megaterium : Purification, Gene Cloning, Expression and Catalytic Properties. *Protein Expression Purif.* **2017**, *136*, 66−72.
(36) Jung, Y.-J.; Lee, J.-K.; Sung, C.-G.; Oh, T. K.; Kim, H. K. Nonionic Detergent-Induced Activation of an Esterase from Bacillus Megaterium 20−1. *J. Mol. Catal. B: Enzym.* **2003**, *26* (3−6), 223−229.
(37) Goswami, B.; Mahanta, D. Starch and Its Derivatives: Properties and Applications. In *Polysaccharides: Properties and Applications*; Inamuddin, I.; Ahamed, M. I.; Boddula, R.; Altalhi, T., Eds.; Scrivener Publishing: Beverly, 2021; pp 253−281.
(38) Petry, J. M.; Pellá, M. C. G.; Silva, O. A.; Caetano, J.; Dragunski, D. C. Plasticizer Concentration Effect on Films and Coatings Based on Poly(Vinyl Alcohol) and Cationic Starch Blends. *Food Chem.* **2024**, *438*, No. 137977.
(39) Nornberg, A. B.; Martins, C. C.; Cervi, V. F.; Sari, M. H. M.; Cruz, L.; Luchese, C.; Wilhelm, E. A.; Fajardo, A. R. Transdermal Release of Methotrexate by Cationic Starch/Poly(Vinyl Alcohol)-Based Films as an Approach for Rheumatoid Arthritis Treatment. *Int. J. Pharm.* **2022**, *611*, No. 121285.
(40) de Sousa, S. M.; de Oliveira, C. A.; Andrade, D. L.; de Carvalho, C. G.; Ribeiro, V. P.; Pastina, M. M.; Marriel, I. E.; de Paula Lana, U. G.; Gomes, E. A. Tropical Bacillus Strains Inoculation Enhances Maize Root Surface Area, Dry Weight, Nutrient Uptake and Grain Yield. *J. Plant Growth Regul.* **2021**, *40* (2), 867−877.
(41) Shahid, M.; Khan, M. S. Cellular Destruction, Phytohormones and Growth Modulating Enzymes Production by Bacillus Subtilis Strain BC8 Impacted by Fungicides. *Pestic. Biochem. Physiol.* **2018**, *149*, 8−19.
(42) Lopes, M. M.; Lodi, L. A.; de Oliveira-Paiva, C. A.; Farinas, C. S. Emulsion/Cross-Linking Encapsulation of Bacillus in Starch/PVA-Based Microparticles for Agricultural Applications. *ACS Agric. Sci. Technol.* **2024**, *4*, No. 490.
(43) Intituto Agronômico de Campinas (IAC). *Análise Química Para Avaliação Da Fertilidade de Solos Tropicais*; Andrade, J. C.; Cantarella, H.; Quaggio, J. A., Eds.; Instituto Agronômico, 2001.
(44) Hutchings, C.; Rajasekharan, S. K.; Reifen, R.; Shemesh, M. Mitigating Milk-Associated Bacteria through Inducing Zinc Ions Antibiofilm Activity. *Foods* **2020**, *9* (8), No. 1094.
(45) Upadhayay, V. K.; Singh, A. V.; Khan, A.; Sharma, A. Contemplating the Role of Zinc-Solubilizing Bacteria in Crop Biofortification: An Approach for Sustainable Bioeconomy. *Front. Agron.* **2022**, *4*, No. 903321, DOI: 10.3389/fagro.2022.903321.
(46) Montoya, R. I. R.; Cota, F. I. P.; de los Santos Villalobos, S. Draft Genome Sequence of Bacillus Megaterium TRQ8, a Plant Growth-Promoting Bacterium Isolated from Wheat (Triticum Turgidum Subsp. Durum) Rhizosphere in the Yaqui Valley, Mexico. *3 Biotech* **2019**, *9* (5), No. 201, DOI: 10.1007/s13205-019-1726-4.
(47) Wyciszkiewicz, M.; Saeid, A.; Chojnacka, K. In Situ Solubilization of Phosphorus-Bearing Raw Materials by Bacillus Megaterium. *Eng. Life Sci.* **2017**, *17* (7), 749−758.
(48) Mehta, N.; Gaëtan, J.; Giura, P.; Azaïs, T.; Benzerara, K. Detection of Biogenic Amorphous Calcium Carbonate (ACC) Formed by Bacteria Using FTIR Spectroscopy. *Spectrochim. Acta, Part A* **2022**, *278*, No. 121262.
(49) Garcia, D. S.; Tavares, M. I. B. Study of the interactions of the nanocomposite formed by poly (vinyl alcohol), clay and zinc oxide nanoparticles *Int. J. Dev. Res.* 2021; Vol. *1*120761 DOI: 10.37118/ijdr.20761.01.2021.
(50) Grace, M. A. L.; Rao, K. V.; Anuradha, K.; Jayarani, A. J.; Kumar, A. A.; Rathika, A. X-Ray Analysis and Size-Strain Plot of Zinc Oxide Nanoparticles by Williamson-Hall. *Mater. Today: Proc.* **2023**, *92*, 1334−1339, DOI: 10.1016/j.matpr.2023.05.492.
(51) Rajashree, P.; Bagde, U. S. Enrichment and Isolation of Microbial Strains Degrading Bioplastic Polyvinyl Alcohol and Time Course Study of Their Degradation Potential. *Afr. J. Biotechnol.* **2015**, *14* (27), 2216−2226.
(52) Santoyo, G.; Urtis-Flores, C. A. A.; Loeza-Lara, P. D.; del Carmen Orozco-Mosqueda, M.; Glick, B. R. Rhizosphere Colonization Determinants by Plant Growth-Promoting Rhizobacteria (PGPR). *Biology* **2021**, *10* (6), No. 475, DOI: 10.3390/biology10060475.
(53) Elhaissoufi, W.; Ghoulam, C.; Barakat, A.; Zeroual, Y.; Bargaz, A. Phosphate Bacterial Solubilization: A Key Rhizosphere Driving Force Enabling Higher P Use Efficiency and Crop Productivity. *J. Adv. Res.* **2022**, *38*, 13−28, DOI: 10.1016/j.jare.2021.08.014.
(54) Timofeeva, A. M.; Galyamova, M. R.; Sedykh, S. E. Plant Growth-Promoting Soil Bacteria: Nitrogen Fixation, Phosphate Solubilization, Siderophore Production, and Other Biological Activities. *Plants* **2023**, *12*, No. 4074, DOI: 10.3390/plants12244074.
(55) Blake, C.; Christensen, M. N.; Kovacs, A. T. Molecular Aspects of Plant Growth Promotion and Protection by Bacillus Subtilis. *Mol. Plant-Microbe Interact.* **2021**, *34* (1), 15−25, DOI: 10.1094/MPMI-08-20-0225-CR.
(56) Malgioglio, G.; Rizzo, G. F.; Nigro, S.; du Prey, V. L.; Herforth-Rahmé, J.; Catara, V.; Branca, F. Plant-Microbe Interaction in Sustainable Agriculture: The Factors That May Influence the Efficacy of PGPM Application. *Sustainability* **2022**, *14* (4), No. 2253, DOI: 10.3390/su14042253.
(57) Bhattacharyya, C.; Bakshi, U.; Mallick, I.; Mukherji, S.; Bera, B.; Ghosh, A. Genome-Guided Insights into the Plant Growth Promotion Capabilities of the Physiologically Versatile Bacillus Aryabhattai Strain AB211. *Front. Microbiol.* **2017**, *8*, No. 411, DOI: 10.3389/fmicb.2017.00411.
(58) Inbaraj, M. P. Plant-Microbe Interactions in Alleviating Abiotic Stress—A Mini Review. *Front. Agron.* **2021**, *3*, No. 667903, DOI: 10.3389/fagro.2021.667903.
(59) Koza, N. A.; Adedayo, A. A.; Babalola, O. O.; Kappo, A. P. Microorganisms in Plant Growth and Development: Roles in Abiotic Stress Tolerance and Secondary Metabolites Secretion. *Microorganisms* **2022**, *10* (8), No. 1528, DOI: 10.3390/microorganisms10081528.
(60) Kumar, P.; Singh, S.; Pranaw, K.; Kumar, S.; Singh, B.; Poria, V. Bioinoculants as Mitigators of Multiple Stresses: A Ray of Hope for Agriculture in the Darkness of Climate Change. *Heliyon* **2022**, *8*, No. e11269, DOI: 10.1016/j.heliyon.2022.e11269.
(61) Bernstein, E. F.; Sarkas, H. W.; Boland, P.; Bouche, D. Beyond Sun Protection Factor: An Approach to Environmental Protection with Novel Mineral Coatings in a Vehicle Containing a Blend of Skincare Ingredients. *J. Cosmet. Dermatol.* **2020**, *19* (2), 407−415.

(62) Elbrolesy, A.; Elhussiny, F. A.; Abdou, Y.; Morsy, R. Facile Synthesis and Biophysical Characterization of Novel Zinc Oxide/Fe3O4 Hybrid Nanocomposite as a Potentially Active Agent in Sunscreens. *Arab J. Sci. Eng.* **2024**, *49* (1), 1083−1093.
(63) Mascarenhas-Melo, F.; Mathur, A.; Murugappan, S.; Sharma, A.; Tanwar, K.; Dua, K.; Singh, S. K.; Mazzola, P. G.; Yadav, D. N.; Rengan, A. K.; Veiga, F.; Paiva-Santos, A. C. Inorganic Nanoparticles in Dermopharmaceutical and Cosmetic Products: Properties, Formulation Development, Toxicity, and Regulatory Issues. *Eur. J. Pharm. Biopharm.* **2023**, 25−40, DOI: 10.1016/j.ejpb.2023.09.011.
(64) Wang, Q.; Cao, L.; Wang, Y.; Qin, M.; Wang, Q. Shell/Core Structure Zinc Oxide/Iron Oxide: A New Sunscreen Material against Blue Light. *Mater. Lett.* **2022**, *322*, No. 132529.
(65) Channa, I. A.; Ashfaq, J.; Gilani, S. J.; Shah, A. A.; Chandio, A. D.; Jumah, M. N. B. UV Blocking and Oxygen Barrier Coatings Based on Polyvinyl Alcohol and Zinc Oxide Nanoparticles for Packaging Applications. *Coatings* **2022**, *12* (7), No. 897.
(66) Cole, C.; Shyr, T.; Ou-Yang, H. Metal Oxide Sunscreens Protect Skin by Absorption, Not by Reflection or Scattering. *Photodermatol., Photoimmunol. Photomed.* **2016**, *32* (1), 5−10.
(67) Suganthi, S.; Vignesh, S.; Sundar, J. K.; Raj, V. Fabrication of PVA Polymer Films with Improved Antibacterial Activity by Fine-Tuning via Organic Acids for Food Packaging Applications. *Appl. Water Sci.* **2020**, *10* (4), No. 100.
(68) Dai, X.; Dong, F.; Dong, Z.; Bai, Z.; Mao, L. Enhanced Antibacterial and Antioxidant Activities of Chlorogenic Acid Loaded Sweet Whey/Starch Active Films for Edible Food Packaging. *LWT* **2024**, *199*, No. 116118.
(69) Zhu, L.; Luo, H.; Shi, Z.-W.; Lin, C.; Chen, J. Preparation, Characterization, and Antibacterial Effect of Bio-Based Modified Starch Films. *Food Chem.: X* **2023**, *17*, No. 100602.
(70) Dou, Y.; Hassan, E. A. M.; Wang, S.; Gibril, M. E.; Kong, F. Enhancing PVA Mulching Films: Leveraging Modified Lignin as a Bio-Based Crosslinking Agent for Improved Mechanical Strength, UV Barrier, and Biodegradability. *Ind. Crops Prod.* **2024**, *222*, No. 119766.
(71) Ahmad, M.; Imtiaz, M.; Nawaz, M. S.; Mubeen, F.; Imran, A. What Did We Learn From Current Progress in Heat Stress Tolerance in Plants? Can Microbes Be a Solution? *Front. Plant Sci.* **2022**, *13*, No. 794782, DOI: 10.3389/fpls.2022.794782.
(72) Cramer, T. A.; Kim, H. W.; Chao, Y.; Wang, W.; Cheng, H. W.; Kim, Y. H. B. Effects of Probiotic (Bacillus Subtilis) Supplementation on Meat Quality Characteristics of Breast Muscle from Broilers Exposed to Chronic Heat Stress. *Poult. Sci.* **2018**, *97* (9), 3358−3368.
(73) Wen, J.; Smelt, J. P. P.; Vischer, N. O. E.; Vos, A. L.; Setlow, P.; Brul, S. Heat Activation and Inactivation of Bacterial Spores: Is There An? *Appl. Environ. Microbiol.* **2022**, *88* (5), No. e02324-21, DOI: 10.1128/aem.02324-21.
(74) Chunduri, L. A. A.; Kurdekar, A.; Pradeep, B. E.; Haleyurgirisetty, M. K.; Venkataramaniah, K.; Hewlett, I. K. Streptavidin Conjugated ZnO Nanoparticles for Early Detection of HIV Infection. *Adv. Mater. Lett.* **2017**, *8* (4), 472−480.
(75) Zarroug, Y.; Boulares, M.; Sfayhi, D.; Slimi, B.; Stiti, B.; Zaieni, K.; Nefissi, S.; Kharrat, M. Structural and Physicochemical Properties of Tunisian Quercus Suber L. Starches for Custard Formulation: A Comparative Study. *Polymers* **2022**, *14* (3), No. 556.
(76) Jinisha, B.; Anilkumar, K. M.; Manoj, M.; Ashraf, C. M.; Pradeep, V. S.; Jayalekshmi, S. Solid-State Supercapacitor with Impressive Performance Characteristics, Assembled Using Redox-Mediated Gel Polymer Electrolyte. *J. Solid State Electrochem.* **2019**, *23* (12), 3343−3353.
(77) Meftaul, I. M.; Venkateswarlu, K.; Dharmarajan, R.; Annamalai, P.; Megharaj, M. Pesticides in the Urban Environment: A Potential Threat That Knocks at the Door. *Sci. Total Environ.* **2020**, *711*, No. 134612, DOI: 10.1016/j.scitotenv.2019.134612.
(78) Kumari, M.; Swarupa, P.; Kesari, K. K.; Kumar, A. Microbial Inoculants as Plant Biostimulants: A Review on Risk Status. *Life* **2023**, *13* (1), No. 12, DOI: 10.3390/life13010012.
(79) Laira, M. D.; Andrade, S. A. L.; Silveira, N. M.; Machado, E. C.; Ribeiro, R. V.; Zambrosi, F. C. B. High Post-Flowering Phosphorus Status Promotes the Tolerance of Soybean to Terminal Heat Stress. *Environ. Exp. Bot.* **2023**, *215*, No. 105501.
(80) Torres, P.; Altier, N.; Beyhaut, E.; Fresia, P.; Garaycochea, S.; Abreo, E. Phenotypic, Genomic and in Planta Characterization of Bacillus Sensu Lato for Their Phosphorus Biofertilization and Plant Growth Promotion Features in Soybean. *Microbiol Res.* **2024**, *280*, No. 127566.
(81) Saleem, M. H.; Usman, K.; Rizwan, M.; Al Jabri, H.; Alsafran, M. Functions and Strategies for Enhancing Zinc Availability in Plants for Sustainable Agriculture. *Front. Plant Sci.* **2022**, *13*, No. 1033092, DOI: 10.3389/fpls.2022.1033092.
(82) Dixon, M.; Simonne, E.; Obreza, T.; Liu, G. Crop Response to Low Phosphorus Bioavailability with a Focus on Tomato. *Agronomy* **2020**, *10*, No. 617, DOI: 10.3390/agronomy10050617.
(83) Bechtaoui, N.; Rabiu, M. K.; Raklami, A.; Oufdou, K.; Hafidi, M.; Jemo, M. Crop Response to Low Phosphorus Bioavailability with a Focus on Tomato. *Front. Plant Sci.* **2021**, DOI: 10.3389/fpls.2021.679916.