



RESEARCH ARTICLE OPEN ACCESS

Plasma Deposition of Coatings for Delivery of Bacterial Spores Active Against *Fusarium graminearum*

Marianna Roggio¹ | Palmira De Bellis²  | Fabio Palumbo³ | Pasquale Trotti⁴ | Mario Masiello²  | Rim Touati² | Antonio Moretti² | Pietro Favia^{1,3}

¹Department of Chemistry, University of Bari "Aldo Moro", Bari, Italy | ²Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Bari, Italy | ³Institute of Nanotechnology (NANOTEC), National Research Council (CNR), Bari, Italy | ⁴Department of soil, Plant, and Food Science, University of Bari "Aldo Moro", Bari, Italy

Correspondence: Fabio Palumbo (fabio.palumbo@cnr.it)

Received: 8 April 2025 | **Revised:** 6 June 2025 | **Accepted:** 11 June 2025

Keywords: antifungal activity | atmospheric pressure glow discharges | *B. velezensis* | plasma deposition | spore-containing coatings

ABSTRACT

Atmospheric Pressure Plasma Deposition processes have been investigated for the preparation of bio-composite coatings embedding bacterial spores of *Bacillus velezensis* N3.2, a strain known in agriculture for its antimicrobial activity against phytopathogenic microorganisms. More specifically, an organic film was plasma-deposited onto casted spores with the aim of checking their viability and antifungal activity in the film-embedded state. Interestingly, results indicate that the spores retain their viability after the deposition, with the thickness of the coating influencing to some extent the formation of bacteria colonies. It was found that the plasma film superimposition does not limit the potential antifungal activity of the spores and the bio-composite coatings are effective in inhibiting the growth of the toxigenic fungal plant pathogen, *Fusarium graminearum*.

1 | Introduction

The global population is projected to reach nearly 10 billion people by 2050, resulting in a surge of agricultural demand, expected to rise by ~50% compared to 2013 [1]. Cereals, including maize, wheat, and rice, together reach slightly less than one-third of the total agricultural production, and are the primary group of crops cultivated worldwide [2].

In all cereal cultivated areas, several phytopathogenic microorganisms, including mycotoxigenic fungi, can colonize plants during the whole crop season. The most important mycotoxigenic fungi occurring on wheat and associated to plant diseases belong to the *Fusarium* genus. Several *Fusarium* species have been isolated from wheat plants and kernels; *F. graminearum*, one of the most dangerous *Fusarium* species, is the main etiological agent of Fusarium Head Blight (FHB) and involved in

Fusarium Crown Rot (FCR), the most destructive diseases of wheat worldwide. For its ability to colonize wheat during the whole crop season, *F. graminearum* represents both a phytopathological problem and a toxicological risk, due to its ability to produce mycotoxins such as trichothecenes and zearalenone [3–6]. These toxic compounds can accumulate in infected plants before harvest and in stored or processed agricultural raw materials such as flours [7–9].

According to the new strategies of integrated pest management, several agronomic, genetic, and biological techniques, as well as agricultural practices, are now available to prevent or limit fungal diseases and mycotoxin accumulation. Chemical control, however, is still considered a key tool to limit FHB and FCR of wheat. Indeed, the use of seeds treated with fungicides allows to protect plants in the first growing stage reducing the symptoms of FCR, while fungicide treatments at the flowering time reduce

Marianna Roggio and Palmira De Bellis contributed equally to this study.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2025 The Author(s). *Plasma Processes and Polymers* published by Wiley-VCH GmbH.

the contamination of grains by *Fusarium* species and their mycotoxins.

In recent years, the growing global concerns about environment and food safety associated with residue accumulation in food and feed has forced the researchers to select new crops protection strategies from mycotoxigenic fungi.

The Farm to Fork Strategy, an environmental sustainability policy that valorises newer technologies in agriculture and food processing, aims at a safer eco-compatible agri-food system by reducing within 2030 the use of pesticides 50% and fertilizers by 20%, to halve the loss of nutrients in the soil, and promote biological agriculture [10, 11]. Within this framework, plant growth promoting microorganisms and biocontrol agents (BCA), namely, microorganisms capable of counteracting one or more target plant pathogens interfering with their life cycles [12–15], appear to be the most promising natural tools to ensure plant health, as well as quality and safety of agricultural products [16, 17].

To reduce the use of chemicals against plant diseases, the research on BCA is mainly focused on lactic acid bacteria (LAB), that are able to produce organic acids and consequent unfavourable conditions for the growth of fungi in food products. In addition, lytic enzymes produced by LAB are capable of degrading the cell wall of microorganisms and have been demonstrated active in biocontrol mechanisms of plant diseases [18].

Recent studies have highlighted the antimicrobial capabilities of strains of the genus *Bacillus* and their metabolites [19, 20]; their potential biocontrol mechanisms have been investigated [21]. In particular, Keshmirshakan et al. focused on *B. velezensis* [22]. This species is included in Appendix E—the list of Qualified Presumption of Safety (QPS) biological agents (<https://www.efsa.europa.eu/en/topics/topic/qualified-presumption-safety-qps>), and some *B. velezensis* strains are already used in agriculture as biocontrol agents. The practice of coating seeds with external materials is commonly used for a better handling, for their protection, and for improving germination and rooting of the plants [23]. In traditional coating methods, seeds are wrapped in a thin polymer matrix impregnated with pesticides and other additives. During their handling, however, particularly during planting operations, coated seeds undergo to mechanical abrasion, which may result in the fast release of the pesticide. Efforts to reduce the use of agro-chemicals have also boosted the development of treatments with BCA and plant growth-promoting microorganisms. It was reported, for example, that spores of *Bacillus subtilis* have been embedded into a matrix to coat the seeds of maize and canola [24–27].

Cold plasmas are partially ionized neutral gases composed of molecules and their fragments, electrons, ions, atoms, and radicals, generated in non-equilibrium conditions, generally under the action of an electric field. Such systems are utilized in several technological applications since decades, and generate continuously newer processes and products [28]. Low-temperature plasma processes are widely applied, due to their low thermal impact, to modify the surface of thermodegradable materials without altering their bulk properties. Plasma

deposition has been largely investigated for embedding active compounds, such as drugs, both in sandwich configuration and in composite one, for drug delivery purposes [29, 30]. Plasma techniques at low (LP) and atmospheric pressure (AP) can find application in agri-food practices and, in particular, AP plasma treatments can be used at pre-planting, pre-harvest, and post-harvest stages, e.g., for decontamination [31].

Investigation of Plasma technologies for application in agriculture is gaining increasing attention [32], especially for seeds treatments; they have been applied for the decontamination of crops [33] and for enhancing the growth of lentils [34], soybeans [35], peas [36], radishes [37], tomatoes [38], sweet peppers [39], oats, wheat [40], and many other vegetables. Very few papers, though, have been published on the plasma deposition of protective coatings on seeds, among which previous studies dealing with LP plasma deposition processes leading to a bilayer embedding sprayed prothioconazole [7, 41]. Such approach gave interesting results in terms of *Fusarium* fungi control, germination, and reduced dispersion of the pesticide in the soil.

In the latest years, atmospheric pressure plasma deposition processes have gained the attention of academic and industrial researchers, also in agriculture, for the many possible advantages offered by this technique with respect to LP processes, such as easier use and scaling up [42–45].

To the best of the authors' knowledge the present paper reports the first research on the use of plasmas in the preparation of a composite coatings containing living, although dormant, cells, the spores. The bacterial strain utilized in this study was previously selected as a potential BCA against certain microbial plant pathogens [46]. For the first time the effect of a plasma coating on viability and activity of embedded bacterial spores is reported.

2 | Experimental Section

2.1 | Materials

He 99.999%, C₂H₄ 99.95% (Air Liquide) and an aerosol of Milli-Q quality water (Millipore, Bedford, MA) were used as feed in the plasma deposition process. The coatings were deposited on 1 × 1 cm² shards of double face polished p-doped crystalline silicon (100) wafers (MicroChemicals GmbH, Ulm, Germany) for comprehensive chemical and morphological characterization. On the other hand, biological assessments were conducted on 13 mm diameter microscope cover glasses, previously cast with bacterial spores, specifically prepared for bioactivity testing.

2.2 | Bacterial and Fungal Strains and Growth Conditions

The strain *Bacillus velezensis* N3.2 (previously named *B. amyloliquefaciens* N3.2) [46] is part of the Culture Collection of the Institute of Sciences of Food Production of the National Research Council (ISPA-CNR), isolated from Italian durum wheat semolina [47, 48]. The strain was stored at –80°C

under cryoprotection and was cultivated on Plate Count Agar (PCA, Difco, Franklin Lakes, NJ) at 30°C for 5 days before each experiment.

In this study, the biological activity of *B. velezensis* N3.2 has been evaluated against a *F. graminearum* ITEM 6415 strain (<http://server.ispa.cnr.it/ITEM/Collection/>); the fungal strain was grown on Potato Dextrose Agar (PDA, Difco) medium at 25°C for 7 days before each experiment.

2.3 | Preparation of the Spore-Containing Coatings

A spore suspension of *B. velezensis* N3.2 was obtained in a NaCl 0.85% solution from a culture on PCA. The suspension was heated at 90°C for 20 min to kill the bacterial cells present; the suspension was then centrifuged (9000 g; 10 min) to collect the spores, which were then resuspended in sterile distilled water at the final density of 10^6 spores/mL. The number of spores was determined with a Thoma cell counting chamber (Brand, Blaubrand, Germany) and verified by dilution-plating on PCA. Aliquots of 40 μ L of spore suspension were cast on each glass substrate (about 8×10^4 spores/sample) and allowed to dry. To generate the bio-composite layers with the embedded spores, the coatings were plasma-deposited on top of the cast bacterial spores with the substrates positioned onto the ground electrode of a dielectric barrier discharge (DBD) reactor, described in detail elsewhere [49]. Briefly, the reactor consists of two parallel plates silver electrodes 5×8 cm² wide, 3 mm apart, both covered with 0.63 mm thick alumina. The DBD electrodes set up is sealed in a plexiglas chamber connected to an exhaust pump. A blend of water aerosol, ethylene (C₂H₄) and helium (He) was used to feed the plasma. The water aerosol was generated with a pneumatic atomizer (mod. 3076, TSI) activated with 3.5 slm He; the mass flow rate of the atomized water resulted 125 mg/min, as evaluated by weight calibration of the reservoir before and after atomization. Ethylene, precursor of the plasma coating, and additional He were supplied at a flow rate of 50 sccm and 2 slm, respectively. The inlets of C₂H₄ and He were controlled with electronic mass flow meters (MKS instruments). The duration of the discharge was set to the following deposition times: 1, 2, 4, 8, and 16 min. The feed was let from one side of the reactor, passed through the plasma zone between the electrodes, and pumped out by a membrane pump from the opposite side.

The discharge was ignited in Continuous Mode with a power generator system composed of wideband AC power amplifier (Al-1000-HF-A by AMP-LINE Corp., West Nyack, NY, USA) driven by a function generator (Model TG-1000 by TTI). The amplifier was connected to the high-voltage upper electrode with an high voltage transformer (Model AL-T1000, AMP-LINE Corp., West Nyack, NY, USA). Voltage and current delivered to the discharges were measured, respectively, with a high-voltage probe (P6015A, Tektronix, Beaverton, USA) and a resistance-type current probe, both connected to an oscilloscope (TDS 2014C, Tektronix, Beaverton, USA). The applied peak-to-peak voltage was kept at 5 KV_{pp} at a frequency of 20 kHz, resulting in a power density of 1.30 Watt cm⁻². Before and after each process the system was purged for 10 min with 5 slm He.

2.4 | Chemical and Morphological Characterization of the Coatings

The composition of the coatings deposited on the Si substrates has been probed by means of Fourier-Transform Infrared Spectroscopy (FT-IR, transmission mode) with a Vertex 70 V Bruker spectrometer (32 scans, 4 cm⁻¹ resolution). The spectrometer was evacuated to less than 150 Pa for 5 min, to avoid spectral interferences from atmospheric H₂O vapour and CO₂.

The atomic composition of the topmost layer (5–10 nm) of the coating was probed with a X-ray Photoelectron Spectroscopy (XPS) PHI 5000 Versa Probe II spectrometer (Physical Electronics GmbH), using a monochromatic Al K α X-ray source@1486.6 eV (15 kV and 24.8 W), with a spot size of 200 μ m. Wide scan and high-resolution C1s, N1s, and O1s spectra were acquired in Fixed Analyser Transmission mode at a pass energy of 117.40 eV and 29.35 eV, respectively. The surface charge was compensated with a dual beam neutralization system and the C1s photoemission peak at 285.0 eV was used as reference for the Binding Energy (BE) scale. All spectra were collected at 45° with respect to the sample surface. To account for the surface distribution of the O-containing moieties of the coatings, the C1s signals were best-fitted with four peak components at 285.0 eV (C–C, C–H), 286.4 eV (C–OH, C–OC), 287.7 eV (C=O), and 289.0 eV (COOH, COOR), using a 90% gaussian shape and 1.5 eV FWHM [49]. Spectra elaboration was carried out with the MultiPak data processing software (Physical Electronics GmbH).

Static Water Contact Angle (WCA) measurements were carried out with a Ramé-Hart 100-manual goniometer (distilled water, 4 μ L drops, room temperature). WCAs were measured on three different points of three replicates.

The thickness of the coatings was measured on three different points of three replicates with a KLA-Tencor instrument (Milpitas, CA, USA) D-120 profilometer after scratching part of the coating with a scalpel to form a step.

Scanning Electron Microscopy (HITACHI TM 3000 Tabletop, Tokyo, Japan) was utilized for the morphological characterization of uncoated and plasma-coated samples. The analyses were carried out in backscattered electron (BSE) mode, at an acceleration voltage of 15 KV, with 6 mm working distance, with no need of prior metallization of the samples.

2.5 | Biological Characterization of the Spore-Containing Coatings

A viability test of the embedded *B. velezensis* N3.2 spores, and a test of their antagonist activity against *F. graminearum* ITEM 6415 strain were performed. Glass slides drop casted with suspended *B. velezensis* N3.2 spores were coated with a plasma-deposited barrier film of different thickness, as described previously, by changing the duration of the plasma deposition process.

The density of the spores on coated and uncoated samples was evaluated after detaching the spores by shaking the samples (110 rpm, 18 h) in 30 mL of sterile NaCl solution (0.85 g/100 mL)

and Tween 80 (0.25% v/v). After centrifugation (9000 g, 10 min), the detached spores were resuspended in sterile distilled water, plated on PCA, and incubated (24°C h, 30°C) for counting the germinated bacteria. Colony counts were expressed as log of the Colony-Forming Units per slide (CFU/slide). The viability of the spores on the samples was also assessed on PCA after incubation (24, 30, and 48 h at 25°C). After incubation the spores could germinate, and their viability was determined from the bacterial colony formation expressed as the width (w, mm) of the growth annulus around the slides. Slides with uncoated spores were used as control for both tests.

The antagonistic activity of the bacterial strain against *F. graminearum* ITEM 6415 strain was evaluated with the dual culture method. In detail, a mycelial plug (4 mm in diameter) sample was taken from the edge of a 1 week old fungal colony and placed at the center of one of the two halves of a PDA plate; a glass slide with the plasma-coated spores was then placed at the center of the other half of the plate, on the same diameter length of the plug. Plates containing only the *Fusarium* strain and slides with uncoated spores were used as controls. After incubation (25°C, 10 days), the antagonistic activity of *B. velezensis* N3.2 was expressed as the width of the inhibition halo (in mm), measured from the edge of the bacterial growth zone to the edge of the mycelium along the inoculum axis. All experiments were performed in triplicate.

The biological results were presented as mean values \pm standard deviations. The statistical analysis was performed with the Statistica 12.0 software (StatSoft Inc., Tulsa, OK, USA). Biological characterization data were compared with a one-way ANOVA test followed by Tukey's test to determine significantly different values ($p < 0.05$).

3 | Results and Discussion

3.1 | Chemical and Morphological Characterization of the Plasma-Deposited Coatings

The deposition rate of 82 ± 8 nm/min was measured for the coating used to embed the cast *Bacillus* spores in the experimental conditions used; coatings of different thickness could thus be deposited on the spores by changing the deposition time.

Transmission FT-IR analysis allowed the identification of the main functional groups present along the thickness of the plasma deposited layer. In Figure 1 the absorption FT-IR spectrum of films deposited on Si for 8 min is reported. The spectrum shows typical bands of organic films characterized by O-containing polar moieties, namely, the broad OH stretching band at $3600\text{--}3100\text{ cm}^{-1}$, the C-O stretching at 1084 cm^{-1} and the C=O stretching at 1717 cm^{-1} . These polar groups, generated from the interaction of the plasma with the water aerosol, are part of the hydrocarbon network of the coating originated from ethylene fragments. Indeed, the IR bands typical of a hydrocarbon backbone are detected, such as the aliphatic CH_x stretching at $2953\text{--}1870\text{ cm}^{-1}$ and the CH_x bending at 1457 cm^{-1} .

XPS analysis has been performed on the plasma deposited coatings. The resulting surface atomic composition of the coatings,

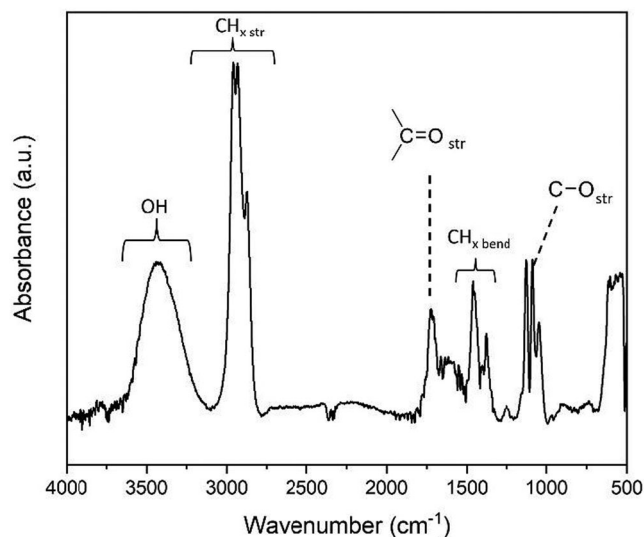


FIGURE 1 | FT-IR transmission spectrum of the plasma-deposited coating utilized for embedding *B. velezensis* N 3.2 spores (8 min discharge duration).

whose corresponding IR spectrum is reported in Figure 1, is C = 88.3%, O = 11.0%, and N = 0.7% respectively. Hydrogen cannot be detected by XPS; the presence of nitrogen is likely due to air remaining in the plasma chamber in spite of the purging with He. The presence of oxygen accounts for the polar groups detected by FT-IR. To identify the nature of the O-containing groups in the coating, the C1s spectrum was best-fitted with four components peaked at 285.0 eV (C1, C-C, C-H, reference), 286.4 eV (C2, C-OH, C-OC), 287.7 eV (C3, C=O), and 289.0 eV (C4, COOH, COOR), respectively, as shown in Figure 2. The relative area of the peaks was 79.7%, 14%, 4.3%, and 1%, respectively, for the components C1 to C4, in good accordance with the FT-IR analysis, and with the structure of coatings deposited in similar conditions discussed in a previous paper [49].

The WCA measurements performed on the plasma-deposited coatings revealed a quite hydrophilic character, with a WCA value of about $52 \pm 2^\circ$. This level of hydrophilicity is compatible with the hydrocarbon network grafted with polar O-containing groups, as revealed by XPS and FT-IR analysis.

In Figure 3 the SEM images of *B. velezensis* N3.2 spores uncoated and coated (for 1 and 8 min) with the plasma-deposited barrier film are reported. The typical shape of the bacillus spores can be observed in the cast but uncoated sample in Figure 3A and B. As a comparison the morphology of the coating without spores is reported in Figure 3C. As it can be appreciated in Figure 3D, a 1 min coating is not enough to uniformly cover the spores, due to their size and shape. When a coating is deposited for 8 min onto the spores, elongated wrinkles about $2\text{ }\mu\text{m}$ wide can be observed, consistent with the presence of the spores underneath the coating. The formation of wrinkles on the coating, evident in Figure 3E and 3F, is most likely induced by the presence of spores, since such coatings deposited onto flat sporeless substrates show smooth morphology with no wrinkles (Figure 3C) [49]. Additionally, it cannot be ruled out that macromolecular residues from cellular material, possibly present at the substrate surface, may reduce the adhesion of the coating, thus leading to film detachment.

3.2 | Biological Characterization of the Spores Embedded in the Coating

3.2.1 | Viability Tests

To evaluate the effect of the plasma deposition process and of the thickness of the coating on the viability of the spores, both the germination of the spores on agar and the quantity of viable spores remaining on the slides were evaluated after the deposition. As shown in Figure 4, after 2 days of incubation on

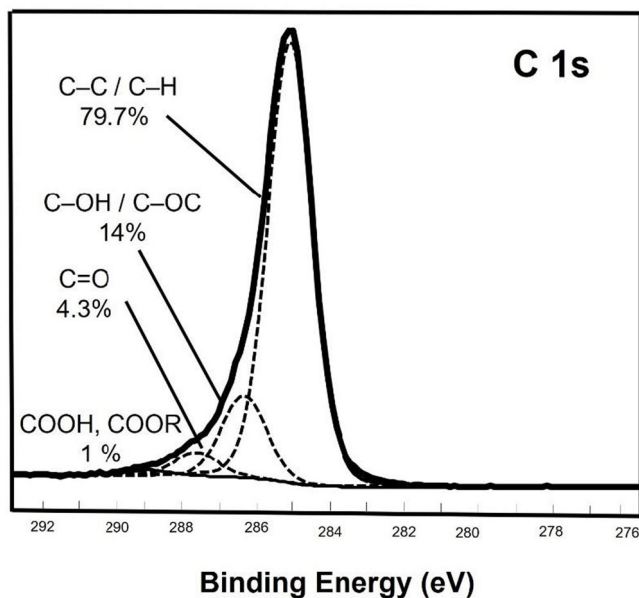


FIGURE 2 | Best fitting of the XPS C1s peak of the plasma-deposited coating utilized for embedding *B. velezensis* N 3.2 spores (8 min discharge duration).

PCA the bacterial growth became clearly visible around each slide (whitish annulus) for all samples, each with a different width of the bacterial growth annulus, depending on the thickness of the barrier film.

As it can be observed in Table 1, the width (w) of the bacterial growth results the same for the uncoated control sample and the one coated with the thinnest (90 ± 10 nm) film, whereas it is found decreasing in the other samples as a function of the increased thickness of the coating, reaching the lowest value of 0.8 mm for spores coated with the thickest (about 1.4 μ m) coating ($p < 0.05$).

To evaluate over time the germination of spore coated with different barrier film thickness, and to better identify the role of the coating onto the spores germination and viability, the bacterial growth has been also observed at different incubation times on spore samples coated with the two thickest barrier films, 700 nm and 1390 nm, respectively, and compared with an uncoated spore sample control. The results reported in Figure 5 show that the uncoated spores germinate only after 24 h giving rise to a growth annulus around sample 35. After 30 h also the spores on sample 33, corresponding to the 700 nm thick coating, germinate while, at last, after 48 h bacterial growth is observed also around sample 34, the one coated with the thickest film.

It is evident, based on the data obtained, that the germination of the spores and the subsequent bacterial growth are somewhat delayed by the presence of the plasma deposited barrier coating. This finding, beneficial for potential applications of plasma deposition approaches on seeds and on other substrates of interest in agriculture, can be due to a limitation of the contact of the spores with the culture medium, that is essential to the germination and formation of the bacterial colonies, or/and to the possible reduced viability of the spores after the interaction with the ionized gas, in spite of the growing protective coating.

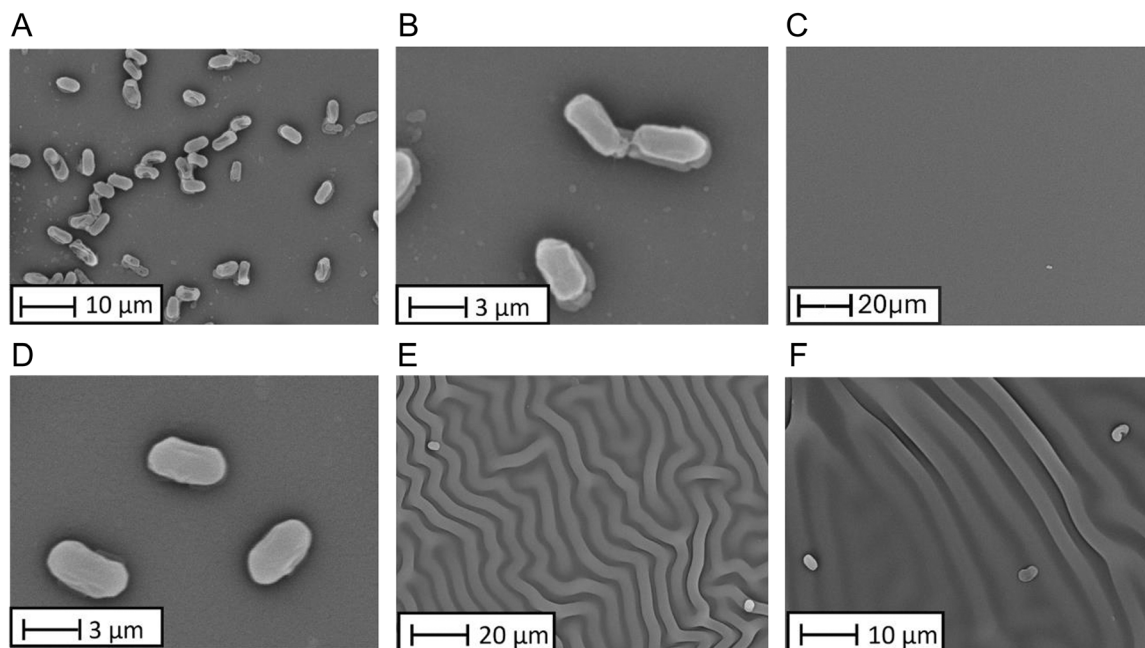


FIGURE 3 | SEM images (BSE mode, 15 kV) at different magnifications of (A, B) *B. velezensis* N3.2 uncoated spores and (C), plasma coating without spores (D) spores coated with 1 min plasma-deposited film, (E, F) spores coated with 8 min plasma-deposited film.

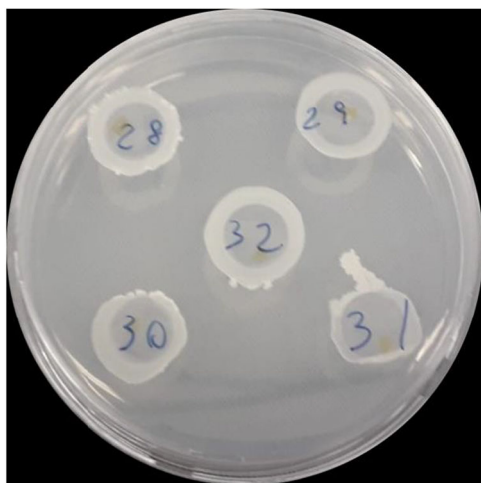


FIGURE 4 | Picture reporting the bacterial growth of *B. velezensis* N3.2 after 48 h incubation on PCA at 25°C. The spores were coated on glass samples with the plasma-deposited barrier film at different thicknesses: 90 nm (sample 28), 170 nm (sample 29), 350 nm (sample 30), 700 nm (sample 31), no film (sample 32, control).

In parallel to the viability test on agar, to obtain information on the adhesion tenacity of the spores to the slides, samples with uncoated spores and coated with the thickest coatings, were subjected to washing, vigorous scraping and subsequent plating to verify the quantity of viable spores present on the slides. As expected, after washing, 5 log CFU/slide were recovered from the uncoated slides, while the quantity of recovered spores decreased with the increased thickness of the film (Table 1). This result could indicate that most spores are more tenaciously protected when the film thickness is higher, making difficult their recovery. In such conditions, however, the inactivation of part of the spores, potentially increasing with the plasma exposure time, cannot be ruled out.

3.2.2 | Antagonistic Activity

To evaluate the antagonistic activity of the N3.2 strain trapped in the coating against *F. graminearum* ITEM 6415, in vitro antagonism tests were carried out. The results are reported in Table 1 and Figure 6. The efficacy of the coating was assessed by observing the growth of the fungal strain after incubation at 25°C.

TABLE 1 | Bacterial growth (w, mm), quantity of spores recovered from coated and uncoated slides (log CFU/slide), and antagonistic activity of *B. velezensis* N3.2 against *F. graminearum* ITEM 6415 (mm), at different thickness of the plasma deposited coating.

Deposition time (min)	Film thickness (nm)	Bacterial growth after 48 (w, mm)*	Recovered Spore/slide (log CFU/slide)	Antagonistic activity against <i>F. graminearum</i> ITEM 6415 (mm)**
0	No Film	4.0 ± 0.1 ^a	4.99 ± 0.09 ^a	10.0 ± 1.2
1	90 ± 10	4.0 ± 0.1 ^a	4.74 ± 0.01 ^b	10 ± 1
2	170 ± 20	3.0 ± 0.1 ^b	3.89 ± 0.01 ^c	9.8 ± 0.5
4	350 ± 50	2.3 ± 0.5 ^c	3.08 ± 0.04 ^c	9.6 ± 0.5
8	700 ± 80	1.8 ± 0.2 ^c	2.38 ± 0.04 ^c	9.5 ± 0.8
16	1390 ± 180	0.8 ± 0.1 ^d	1.14 ± 0.06 ^c	8 ± 1

Note: Data are represented as mean ± standard deviation.

*Expressed as the width (w, mm) of the growth annulus after 48 h of incubation at 25°C.

**Expressed as an inhibition halo (mm) after 10 days of incubation at 25°C.

^{a-d}Values in the same column with different superscript letters are significantly different ($p < 0.05$).

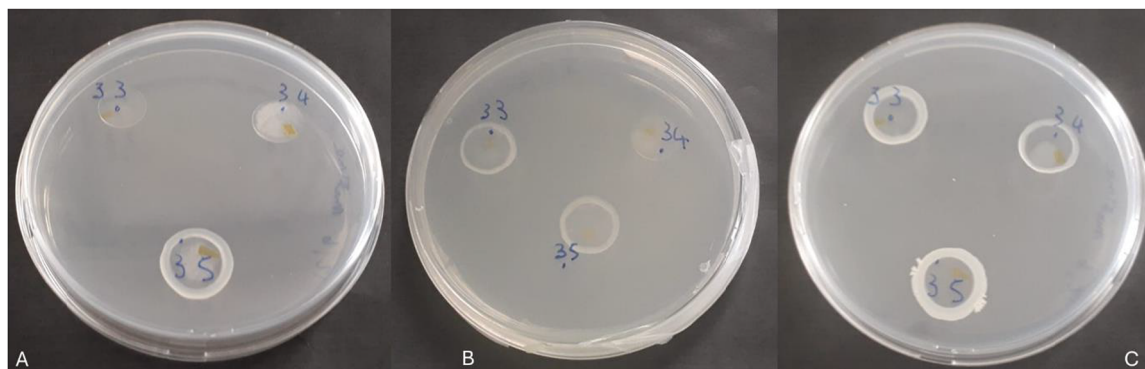


FIGURE 5 | Picture reporting the bacterial growth of *B. velezensis* N3.2 from spores coated with the plasma-deposited barrier film at different thickness, after 24 h (A), 30 h (B) and 48 h (C) of incubation (PCA, 25°C). Thickness: 700 nm (sample 33), 1390 nm (sample 34), no film (sample 35, control).

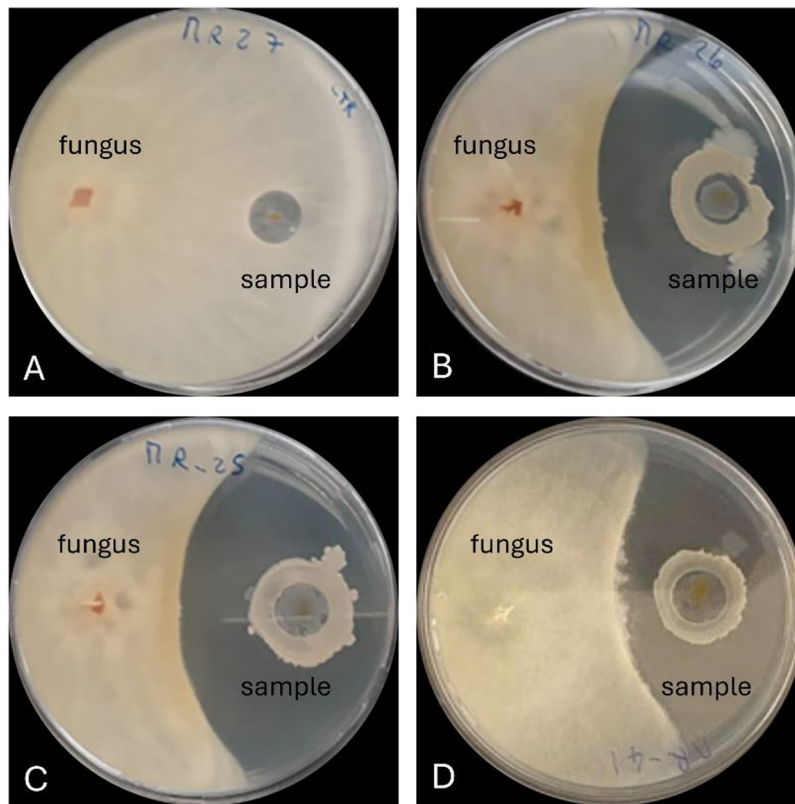


FIGURE 6 | Picture reporting the antagonistic activity of *B. velezensis* N3.2 against *F. graminearum* ITEM 6415. (A) Negative control, plasma coating on slide (w/o spores); (B) Bacterial spores on slide without plasma coating; (C) Barrier film 90 nm thick on bacterial spores; (D) Barrier film 700 nm thick on bacterial spores.

After 10 days, no significant difference was observed between treated and untreated samples, attesting that the treatment did not reduce the antifungal activity of the bacterial strain. Furthermore, the activity remained unchanged as the thickness of the barrier film increased ($p > 0.05$). When the samples used in the viability test were also subjected to the antagonism test after washing, the results showed once more the antagonistic activity of *B. velezensis* N3.2 against the *Fusarium* strain, highlighting again the presence of active spores on the slides, since washing was probably not sufficient for removing all the spores. This result leads to the hypothesis that the lower number of spores recovered from treated slides (Table 1) could be due to the thickness of the film rather than to a reduction in viability due to the plasma process itself.

3.3 | Discussion

Fungal plant pathogens, such as many *Fusarium* species, cause huge losses of crops and are dangerous for human health due to the production of mycotoxins. To replace fungicides researchers are focusing attention onto natural products and biocontrol agents. *Bacillus* species are suitable as biocontrol agents, since in addition to producing antimicrobial substances and stimulating plant-induced systemic resistance, they produce spores that increase their resistance to environmental stress conditions [50]. For effective field distribution, it is useful to incorporate beneficial microorganisms into matrices for seed coating. To be effective, a beneficial microorganism should be protected during storage and field application. At the same time, a good film

should ensure the integrity of the microorganism and the seeds, allowing their germination [51]. Polyvinyl alcohol (PVA)-based films have recently been proposed to deliver *Bacillus megaterium* as seed coatings [51]. Coatings consisting of *Bacillus* strains and gum arabica have also been developed to manage *Fusarium* wilt disease [50].

In the present work, instead, a plasma deposited coating has been considered for embedding spores with antifungal activity. At the best of the authors' knowledge, this paper explores for the first time the plasma deposition of an organic coating onto bacterial spores. This approach greatly differs from most of the recent studies on plasma processes for agriculture, mostly intended for decontamination and not based on plasma deposition. The results well indicate that the plasma deposition of a coating on top of the spores do not alter their germinability characteristics, as illustrated in Figures 4 and 5. Further, the antifungal activity is not reduced with respect to the uncoated spores (Figure 6). On the other hand, when considering the effect of the film thickness, the germination is delayed, and the viability of the spores decreases with increasing deposition time. A possible rationale is the limited contact between the spores and the medium, which is necessary to induce the germination. However, it is known that plasma, especially when fed with air or nitrogen gas and assisted by the associated UV radiation, can inactivate bacterial spores [52]. On the other hand, it can be hypothesized that the increasing thickness of the coating may at least partially shield the spores from aggressive plasma-generated species (e.g., ROS and RNS). Since it can be hypothesized that UV radiation is in part absorbed by the

organic species present in the plasma feed and by the coating itself, the inactivating effect of plasma on spores could be considered negligible. Further biological tests, indeed, are necessary to support this hypothesis.

4 | Conclusions

This study demonstrates, for the first time, the feasibility of incorporating bacterial spores into a plasma-deposited organic coating, introducing a novel strategy for developing active films with antifungal properties, of potential application on seeds. The *Bacillus velezensis* N3.2 strain retained both viability and antifungal activity against *Fusarium* after the plasma deposition process, indicating that the process does not compromise the essential biological functions of the spores. Although the increased film thickness was associated with delayed germination, the embedded spores remained effective against fungal pathogens. This innovative approach may provide a promising alternative to conventional seed coating methods by preserving the protection and functionality of beneficial microorganisms that can act against pathogens or enhance plant growth. Further in-field studies are required to optimize the plasma deposition parameters and assess their direct impact on seed germination and crop protection.

Acknowledgements

This study activity is partly funded by Progetti@CNR “Cold Plasma Processes and Technologies for Sustainable Agriculture and Food Processing”—Prot. 70294 and ON FOODS “Research and Innovation network on food and nutrition Sustainability, Safety and Security—Working ON Food.” Piano Nazionale di Ripresa e Resilienza (PNRR)—Missione 4 Componente 2 Investimento 1.3—Avviso n. 341 del 15/03/2022 del Ministero dell’Università e della Ricerca—“Partenariati estesi alle università, ai centri di ricerca, alle aziende per il finanziamento di progetti di ricerca di base”—NextGenerationEU.- Codice progetto PE00000003. Savino Cosmai (CNR NANOTEC) and Danilo De Benedetti (University of Bari) are kindly acknowledged for their skilled technical support. Open access publishing facilitated by Consiglio Nazionale delle Ricerche, as part of the Wiley - CRUI-CARE agreement.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

References

1. FAO, *The Future of Food and Agriculture—Trends and Challenges* (FAO, 2017).
2. FAO, *World Food and Agriculture—Statistical Yearbook 2022* (FAO, 2022).
3. A. Logrieco, A. Bottalico, G. Mulé, A. Moretti, and G. Perrone, “Epidemiology of Toxigenic Fungi and Their Associated Mycotoxins for Some Mediterranean Crops,” *European Journal of Plant Pathology* 109 (2003): 645–667.
4. European Food Safety Authority (EFSA), “Use of the EFSA Comprehensive European Food Consumption Database in Exposure

Assessment,” *EFSA Journal* 9 (2011): 97, <https://doi.org/10.2903/j.efsa.2011.2097>.

5. E. Balázs and J. S. Schepers, “The Mycotoxin Threat to Food Safety,” *International Journal of Food Microbiology* 119 (2007): 1–2.

6. Z. Kang and H. Buchenauer, in *Mycotoxins in Plant Disease*, eds. A. Logrieco, J. A. Bailey, L. Corazza, and B. M. Cooke (Springer, 2002).

7. M. Masiello, S. Somma, C. Lo Porto, et al., “Plasma Technology Increases the Efficacy of Prothioconazole Against *Fusarium Graminearum* and *Fusarium Proliferatum* Contamination of Maize (*Zea mays*) Seedlings,” *International Journal of Molecular Sciences* 22 (2021): 9301, <https://doi.org/10.3390/ijms22179301>.

8. G. P. Munkvold, C. Watrin, M. Scheller, R. Zeun, and G. Olaya, in *Plant Pathology in the 21st Century*, eds. M. Gullino and G. Munkvold (Springer, 2014), 6.

9. A. Moretti, G. Panzarini, S. Somma, et al., “Systemic Growth of *F. graminearum* in Wheat Plants and Related Accumulation of Deoxynivalenol,” *Toxins* 6 (2014): 1308–1324.

10. European Commission, *A European Green Deal: Striving to be the First Climate-Neutral Continent* (European Commission, 2021), 1–6.

11. United Nations, *Sustainable Development Goals* (United Nations, 2015).

12. J. Köhl, R. Kolnaar, and W. J. Ravensberg, “Mode of Action of Microbial Biological Control Agents Against Plant Diseases: Relevance Beyond Efficacy,” *Frontiers in Plant Science* 10 (2019): 845.

13. A. T. K. Corke, “Biological Control of Plant Pathogens. by Kenneth F. Baker and R. James Cook. San Francisco: Freeman (1974), pp. 433, \$5.90,” *Experimental Agriculture* 11 (1975): 159.

14. K. Raymaekers, L. Ponet, D. Holtappels, B. Berckmans, and B. P. A. Cammue, *Biol. Control* 2020, 144, 104240.

15. D. Palmieri, G. Ianiri, C. Del Grosso, et al., “Advances and Perspectives in the use of Biocontrol Agents Against Fungal Plant Diseases,” *Horticulturae* 8 (2022): 577.

16. M. Tariq, A. Khan, M. Asif, et al., “Acta Agriculturae Scandinavica Section B,” *Soil and Plant Science* 2020, 70: 507.

17. J. Kumar, A. Ramlal, D. Mallick, and V. Mishra, “An Overview of Some Biopesticides and Their Importance in Plant Protection for Commercial Acceptance,” *Plants* 10 (2021): 1185.

18. H. Chen, H. Ju, Y. Wang, et al., “Antifungal Activity and Mode of Action of Lactic Acid Bacteria Isolated From Kefir Against *Penicillium Expansum*,” *Food Control* 130 (2021): 108274.

19. E. Xu, K. Wang, H. Wang, et al., “Evaluation of the Biocontrol Potential of *Bacillus* sp. WB Against *Fusarium oxysporum* f. sp. *ni-veum*,” *Biological Control* 147 (2020): 104288.

20. K. Stumbriene, R. Gudiukaite, R. Semaskiene, P. Svegzda, A. Jonaviciene, and S. Suproniene, “Screening of New Bacterial Isolates With Antifungal Activity and Application of Selected *Bacillus* sp. Cultures for Biocontrol of *Fusarium Graminearum* Under Field Conditions,” *Crop Protection* 113 (2018): 22–28.

21. N. Khan, P. Martínez-Hidalgo, T. A. Ice, et al., “Antifungal Activity of *Bacillus* Species Against *Fusarium* and Analysis of the Potential Mechanisms Used in Biocontrol,” *Frontiers in Microbiology* 9 (2018): 2363.

22. A. Keshmirshakan, L. M. de Souza Mesquita, and S. P. M. Ventura, “Biocontrol Manufacturing and Agricultural Applications of *Bacillus Velezensis*,” *Trends in Biotechnology* 42 (2024): 986–1001.

23. S. Pedrini, D. J. Merritt, J. Stevens, and K. Dixon, “Seed Coating: Science or Marketing Spin,” *Trends in Plant Science* 22 (2017): 106–116.

24. C. Accinelli, H. K. Abbas, N. S. Little, J. K. Kotowicz, M. Mencarelli, and W. T. Shier, “A Liquid Bioplastic Formulation for Film Coating of Agronomic Seeds,” *Crop Protection* 89 (2016): 123–128.

25. A. G. Taylor, P. S. Allen, M. A. Bennett, K. J. Bradford, J. S. Burris, and M. K. Misra, "Seed Enhancements," *Seed Science Research* 8 (1998): 245–256.
26. D. Foqué, J. G. Pieters, and D. Nuyttens, "Characterization of Different Pneumatic Sowing Machines." *Aspects of Applied Biology: International Advances in Pesticide Application* (Association of Applied Biologists, 2014). 122, ed., 77–84.
27. D. Nuyttens, W. Devarrewaere, P. Verboven, and D. Foqué, "Pesticide-Laden Dust Emission and Drift From Treated Seeds During Seed Drilling: A Review," *Pest Management Science* 69 (2013): 564–575.
28. K. D. Weltmann, J. F. Kolb, M. Holub, et al., "The Future for Plasma Science and Technology," *Plasma Processes and Polymers* 16 (2019): e1800118, <https://doi.org/10.1002/ppap.201800118>.
29. K. Vasilev, N. Poulter, P. Martinek, and H. J. Griesser, "Controlled Release of Levofloxacin Sandwiched Between Two Plasma Polymerized Layers on a Solid Carrier," *ACS Applied Materials & Interfaces* 3, no. 12 (2011): 4831–4836.
30. C. Lo Porto, F. Palumbo, J. Buxadera-Palomero, et al., "On the Plasma Deposition of Vancomycin-Containing Nano-Capsules for Drug-Delivery Applications," *Plasma Processes Polymers* 15 (2018): e1700232, <https://doi.org/10.1002/ppap.201700232>.
31. S. Somma, M. Masiello, M. Haidukowski, et al., "Decontamination of Maize Kernels and Degradation of Mycotoxins by Means of Cold Plasmas," *LTW* 215 (2025): 117205, <https://doi.org/10.1016/j.lwt.2024.117205>.
32. P. Favia, E. Sardella, and H. Tanaka, "Special Issue: Advanced Applications of Plasmas in Life Sciences," *Plasma Processes and Polymers (Print)* 17 (2020): 2070028, <https://doi.org/10.1002/ppap.202070028>.
33. A. Mitra, Y.-F. Li, T. G. Klämpfl, et al., "Inactivation of Surface-Borne Microorganisms and Increased Germination of Seed Specimen by Cold Atmospheric Plasma," *Food and Bioprocess Technology* 7 (2014): 645–653.
34. J. J. Zhang, J. O. Jo, D. L. Huynh, et al., "Growth-Inducing Effects of Argon Plasma on Soybean Sprouts via the Regulation of Demethylation Levels of Energy Metabolism-Related Genes," *Scientific Reports* 7 (2017): 41917.
35. L. Li, J. Jiafeng, J. Li, et al., "Effects of Cold Plasma Treatment on Seed Germination and Seedling Growth of Soybean," *Scientific Reports* 4 (2014): 5859, <https://doi.org/10.1038/srep05859>.
36. T. Stolarik, M. Henselová, M. Martinka, O. Novák, A. Zahoranová, and M. Černák, "Effect of Low-Temperature Plasma on the Structure of Seeds, Growth and Metabolism of Endogenous Phytohormones in Pea (*Pisum sativum* L.," *Plasma Chemistry and Plasma Processing* 35 (2015): 659–676.
37. L. Sivachandiran and A. Khacef, "Enhanced Seed Germination and Plant Growth by Atmospheric Pressure Cold Air Plasma: Combined Effect of Seed and Water Treatment," *RSC Advances* 7 (2017): 1822–1832.
38. B. Sera, P. Spatenka, M. Sery, and N. Vrchotova, "Influence of Plasma Treatment on Wheat and Oat Germination and Early Growth," *IEEE Transactions on Plasma Science* 38 (2010): 2963, <https://doi.org/10.1109/TPS.2010.2060728>.
39. J. Jiang, X. He, L. Li, et al., "Effect of Cold Plasma Treatment on Seed Germination and Growth of Wheat," *Plasma Science and Technology* 16 (2014): 54–58.
40. D. Dobrin, M. Magureanu, N. B. Mandache, and M. D. Ionita, "The Effect of Non-Thermal Plasma Treatment on Wheat Germination and Early Growth," *Innovative Food Science & Emerging Technologies* 29 (2015): 255–260.
41. C. Lo Porto, F. Palumbo, S. Somma, et al., "Plasma-Assisted Deposition of Fungicide Containing Coatings for Encapsulation and Protection of Maize Seeds," *Plasma Processes and Polymers* 16, no. 10 (2019): 1900022.
42. Y. W. Yang, J. Y. Wu, C. T. Liu, et al., "Fast Incorporation of Primary Amine Group Into Poly lactide Surface for Improving C2C12 Cell Proliferation Using Nitrogen-Based Atmospheric-Pressure Plasma Jets," *Journal of Biomedical Materials Research Part A* 102A (2014): 160, <https://doi.org/10.1002/jbm.a.34681>.
43. Y. W. Yang, J. Y. Wu, M. H. Chiang, and J. S. Wu, "Highly Effective Large-Area Inactivation of *B. Subtilis* Spore Using a Planar Dielectric Barrier Discharge Jet With CF_4 /Air Mixture," *IEEE Transactions on Plasma Science* 40 (2012): 3003–3006.
44. M. H. Chiang, J. Y. Wu, Y. H. Li, J. S. Wu, S. H. Chen, and C. L. Chang, "Inactivation of *E. Coli* and *B. Subtilis* by a Parallel-Plate Dielectric Barrier Discharge Jet," *Surface and Coatings Technology* 204 (2010): 3729–3737.
45. G. Da Ponte, E. Sardella, F. Fanelli, R. d'Agostino, and P. Favia, "Trends in Surface Engineering of Biomaterials: Atmospheric Pressure Plasma Deposition of Coatings for Biomedical Applications," *European Physical Journal Applied Physics* 56 (2011): 24023.
46. S. Zicca, P. De Bellis, M. Masiello, et al., "Antagonistic Activity of Olive Endophytic Bacteria and of *Bacillus* Spp. Strains Against *Xylella Fastidiosa*," *Microbiological Research* 236 (2020): 126467.
47. F. Valerio, P. De Bellis, M. Di Biase, et al., "Diversity of Spore-Forming Bacteria and Identification of *Bacillus Amyloliquefaciens* as a Species Frequently Associated With the Ropy Spoilage of Bread," *International Journal of Food Microbiology* 156 (2012): 278–285.
48. P. De Bellis, F. Minervini, M. Di Biase, F. Valerio, P. Lavermicocca, and A. Sisto, "Toxicogenic Potential and Heat Survival of Spore-Forming Bacteria Isolated From Bread and Ingredients," *International Journal of Food Microbiology* 197 (2015): 30–39.
49. Y.-W. Yang, G. Camporeale, E. Sardella, et al., "Deposition of Hydroxyl Functionalized Films by Means of Water Aerosol-Assisted Atmospheric Pressure Plasma," *Plasma Processes and Polymers* 11 (2014): 1102–1111.
50. A. Raza, A. Hassan, W. Akram, T. Anjum, Z. H. Aftab, and B. Ali, "Seed Coating With the Synthetic Consortium of Beneficial *Bacillus* Microbes Improves Seedling Growth and Manages *Fusarium* Wilt Disease," *Scientia Horticulturae* 325 (2024): 112645.
51. L. A. Lodi, R. Borges, R. Bortoletto-Santos, C. A. Oliveira-Paiva, C. Ribeiro, and C. S. Farinas, "Biodegradable Pva-Based Films for Delivery of *Bacillus Megaterium* as Seed Coating," *Journal of Environmental Chemical Engineering* 12 (2024): 114539.
52. Z. Zhu, A. P. Bassey, T. Huang, Y. Zhang, I. Ali Khan, and M. Huang, "The Formation, Germination, and Cold Plasma Inactivation of Bacterial Spore," *Food Chemistry Advances* 1 (2022): 100056.