RESEARCH PAPER

Enhancement of Post-Harvest Quality and Shelf Life of Fresh Produce Using EPS from Bacillus mycoides: Physicochemical and Functional Assessment

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ABSTRACT

This study evaluates the potential of exopolysaccharide (EPS) produced by Bacillus mycoides as a biodegradable edible coating for fresh fruits and vegetables. The EPS was extracted, purified, and characterized before being applied to tomato, lady's finger, guava, and strawberry. Coated samples were analyzed to assess physicochemical, microbiological, and functional properties. The evaluations included swelling ratio, titratable acidity, pH variation, nutritional retention, toxicity assessment, antibacterial activity, antioxidant capacity, and shelf stability analysis based on pH changes. The EPS coating exhibited an optimal swelling ratio, indicating good moisture barrier properties and structural integrity. Toxicity studies confirmed its non-toxic and food-safe nature. Compared to uncoated controls, EPS-coated samples showed reduced titratable acidity fluctuations and slower pH decline, indicating moderated metabolic activity during storage. Antibacterial assays demonstrated significant inhibitory effects against common spoilage microorganisms, while antioxidant analysis revealed enhanced free radical scavenging activity in coated samples. Shelf stability assessment based on pH monitoring under ambient conditions indicated improved maintenance of quality parameters in EPS-treated produce. Overall, EPS from Bacillus mycoides exhibits strong functional, antimicrobial, and antioxidant properties, supporting its potential as a natural and sustainable edible coating for post-harvest preservation of fresh produce.

Keywords: Bacillus mycodies, Exopolysaccharides, Natural preservative, Vegetable coating, Shelf life extension

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1.INTRODUCTION

Post-harvest deterioration of fruits and vegetables remains a significant global challenge, contributing to economic losses and reduced nutritional quality. Spoilage is primarily influenced by physiological respiration, microbial contamination, moisture loss, enzymatic degradation, and biochemical changes such as pH decline and titratable acidity variation during storage. Perishable commodities, including tomato, guava, strawberry, and lady's finger (*Abelmoschus esculentus*) are particularly susceptible to rapid quality deterioration under ambient conditions. Increasing consumer demand for safe, minimally processed, and chemical-free food products has intensified research into natural and biodegradable preservation strategies.

Edible coatings have emerged as an effective post-harvest technology due to their ability to act as semi-permeable barriers, regulating gas exchange, reducing moisture migration, and slowing oxidative degradation. Polysaccharide-based coatings are widely studied because of their biodegradability, film-forming capacity, and compatibility with fresh produce systems (Rhim et al., 2006). However, limitations such as water solubility and mechanical weakness in plant-derived polymers have encouraged the exploration of alternative bio-based materials, including microbial exopolysaccharides (EPS) (Sharma & Rajput, 2023).

Microbial EPS are extracellular high-molecular-weight polymers known for their structural diversity, water-binding properties, viscosity enhancement, and biological functionality. In addition to physicochemical stability, several EPS exhibit antioxidant and antimicrobial activities, making them suitable candidates for food preservation applications. Among EPS-producing

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bacteria, species of the genus *Bacillus* are of particular interest due to their industrial relevance, adaptability, and safety profile. (Talbi et al., 2023)

Optimization and characterization studies have demonstrated that exopolysaccharides produced by *Bacillus mycoides* possess favourable swelling behavior, structural stability, and functional groups suitable for film formation (Dhivya et al., n.d.). Furthermore, application-based investigations have reported that *Bacillus mycoides*-derived EPS can function as a natural preservative coating, reducing microbial load and enhancing quality parameters in vegetables during storage. These findings highlight the potential of microbial EPS as sustainable alternatives to synthetic preservatives.

Swelling ratio is a critical parameter for assessing the moisture absorption capacity and barrier properties of edible films. Monitoring pH and titratable acidity provides insight into biochemical stability and metabolic changes occurring during storage. Antioxidant activity plays a vital role in reducing oxidative stress and preserving nutritional components, while antibacterial efficacy limits surface contamination and spoilage. Additionally, toxicity evaluation is essential to ensure food safety and regulatory compliance for edible applications.

Despite growing interest in microbial EPS-based coatings, comprehensive studies integrating physicochemical characterization, antioxidant and antibacterial activities, toxicity assessment, swelling behavior, and pH-based shelf-life evaluation under different temperature conditions across multiple fruits and vegetables remain limited. Therefore, the present study investigates the multifunctional properties of *Bacillus mycoides*-derived EPS as a biodegradable edible coating applied to tomato, lady's finger, guava, and strawberry, with detailed evaluation of its functional, safety, and preservation efficiency parameters. (Jhandai. et al., 2019)

2. MATERIALS AND METHODS

2.1 Preparation of EPS coating:

The exopolysaccharide (EPS) used in this study was obtained from the soil-derived bacterial strain identified as *Bacillus mycoides*. The crude EPS extracted through alcoholic precipitation was used for solution preparation. The precipitated crude EPS was re-dissolved in sterile phosphate-buffered saline (PBS) adjusted to pH 7.2 to obtain a uniform EPS solution. To eliminate low-molecular-weight impurities, residual salts, and unreacted components, the re-solubilized EPS solution was subjected to dialysis against fresh PBS using a dialysis membrane with an appropriate molecular weight cut-off. Dialysis was performed at 4°C with periodic replacement of the buffer to ensure effective purification. Following dialysis, the purified EPS solution was collected and stored at 4°C for subsequent coating formulation and analytical studies (Upadhyaya et al., 2024)

2.2 Coating of Vegetable Samples

Fresh tomatoes, lady's finger, guava and Strawberry were procured from a local market and selected based on uniform size, maturity, and absence of visible defects or mechanical damage. The samples were washed thoroughly with sterile distilled water to remove surface contaminants and air-dried under aseptic conditions (Dhivya et al., 2025).

EPS coatings were applied using a sterile swabbing method to ensure uniform surface coverage. Control samples remained untreated throughout the study. The coating procedure was performed following previously reported methods with minor modifications (Khatoon et al., 2025).

2.3 Swelling Ratio of Control and EPS Coated Fruits and Vegetables

The swelling ratio (SR) of control and EPS-coated fruits and vegetables was determined using a gravimetric method adapted for edible coatings and biopolymer films. All samples were stored under identical room temperature conditions. Swelling ratio was assessed after 5 days of storage to evaluate the moisture uptake and structural stability of the coating. Initially, the weight of each sample was recorded (W_0). After 5 days, surface moisture was gently removed using blotting paper, and the final weight (W_t) was measured.

$$\text{Swelling Ratio (\%)} = \frac{Wt - W0}{W0} \text{ X } 100$$

This measurement provided insight into the moisture retention capacity and barrier efficiency of the EPS coating (Liu et al., 2025).

2.4 Titratable Acidity of Control and EPS Coated Fruits and Vegetables

Titratable acidity (TA) of control and EPS-coated fruits and vegetables, 10 g of each sample was homogenized with distilled water and filtered through muslin cloth. An aliquot of 10 mL of the filtrate was titrated against standardized 0.1 N sodium hydroxide (NaOH) using phenolphthalein as an indicator until a persistent light pink endpoint was obtained (Adjouman et al., 2018).

2.5 Total Antioxidant Activity for Uncoated and EPS Coated Fruits and Vegetables

Total antioxidant activity of purified exopolysaccharide (EPS) derived from *Bacillus mycoides* and EPS-coated vegetable samples was determined using the phosphomolybdenum method (Prieto et al., 1999) with slight modifications. For EPS analysis, purified EPS was dissolved in distilled water to obtain appropriate concentrations. For vegetable samples, 5–10 g of control and EPS-coated samples were homogenized with distilled water and filtered through muslin cloth. The filtrates were used for antioxidant evaluation. Antioxidant activity of coated and control samples was assessed on the 5th, 15th, and 30th day of storage. The pH of the extracts was recorded separately to correlate antioxidant stability with pH changes during storage.

Briefly, 0.5 mL of sample (EPS solution or vegetable extract) was mixed with 0.5 mL of reagent solution containing 0.6 M sulfuric acid (H_2SO_4), 28 mM sodium phosphate, and 4 mM ammonium molybdate. The reaction mixture was incubated at 50°C for 90 minutes in a water bath. A reagent blank was prepared simultaneously. After incubation, samples were cooled to room temperature and absorbance was measured at 695 nm using a UV–Visible spectrophotometer. Ascorbic acid was used as the reference standard, and a calibration curve was prepared using different concentrations. The antioxidant capacity of EPS-coated samples was compared with uncoated controls to evaluate the effect of EPS coating and storage conditions on antioxidant retention (Upadhyaya et al., 2024).

2.6 Antibacterial Activity for Control EPS-Coated Fruits and Vegetables:

Antibacterial activity was assessed using the agar well diffusion method, following standard protocols with minor modifications (Perez et al., 1990; Balouiri et al., 2016). Nutrient broth cultures of the test bacteria were incubated at 37 °C for 24 h. After incubation, 70 µL of each bacterial suspension was uniformly spread on Mueller–Hinton agar (MHA) plates to obtain a confluent lawn. Mueller–Hinton agar was prepared by dissolving 39 g of medium in 1000 mL of distilled water, followed by sterilization in an autoclave at 121 °C for 15 minutes.

Wells were aseptically punched into the agar using a sterile cork borer. A fixed volume of the sample extract was added to each well. Dimethyl sulfoxide (DMSO) was used as the negative control, while a standard antibiotic disc served as the positive control. The plates were allowed to pre-diffuse at room temperature and then incubated at 37 °C for 24 h. After incubation, antibacterial activity was evaluated by measuring the diameter of the zone of inhibition (mm) around each well. Measurements were performed in triplicate, and the mean inhibition zone was recorded

The antibacterial effectiveness of EPS-coated samples stored at room temperature was compared with uncoated controls. Enhanced inhibition zones in EPS-coated samples, were interpreted as improved microbial suppression associated with EPS barrier properties and pH stability. (Gundappa et al., 2024)

2.7 Total Viable count for Control EPS Coated Fruits and Vegetables:

For each sample, 5 g of fruit or vegetable tissue was aseptically weighed and transferred into a sterile conical flask containing 45 mL of sterile 0.85% physiological saline solution to obtain a 10^{-1} dilution. The mixture was homogenized for 20 seconds using a sterile homogenizer to ensure uniform microbial suspension. The homogenate was allowed to settle briefly under aseptic conditions.

From the initial suspension, 0.5 mL of the supernatant was taken and subjected to serial tenfold dilutions using sterile saline solution to obtain appropriate dilution levels for plating. From selected serial dilutions, 1 mL aliquots were aseptically pipetted into sterile Petri plates. Approximately 12–15 mL of molten nutrient agar, previously sterilized and cooled to 45 ± 1

°C, was poured into each plate. The contents were gently mixed by swirling in a circular motion to ensure uniform distribution of microorganisms. The plates were allowed to solidify at room temperature under aseptic conditions. The solidified plates were incubated in an inverted position at 37 ± 2 °C for 24–48 hours. After incubation, plates containing 30–300 colonies were selected for enumeration, as recommended for standard plate count methods (Sivalingam et al., 2024).

Colonies were counted manually using a colony counter, and the total viable count was calculated and expressed as colony-forming units per milliliter (CFU/mL) using the formula:

$$\text{TVC (CFU/mL)} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume Plated (mL)}}$$

3. RESULTS AND DISCUSSION

3.1 Preparation of EPS coating:

The exopolysaccharide (EPS) extracted from *Bacillus mycoides* was successfully obtained through alcoholic precipitation and subsequently purified by dialysis. The crude EPS appeared as a whitish, gelatinous precipitate, indicating the presence of high-molecular-weight polysaccharide fractions. Re-dissolution in sterile phosphate-buffered saline (PBS, pH 7.2) resulted in a clear to slightly opalescent viscous solution, suggesting good solubility and uniform dispersion of the polymer matrix. Dialysis against fresh PBS at 4°C effectively removed residual salts, unreacted metabolites, and low-molecular-weight impurities, as evidenced by improved clarity and consistency of the EPS solution after purification. The cold dialysis condition minimized structural degradation and preserved the functional integrity of the polysaccharide chains.

The purified EPS solution exhibited moderate viscosity, which is advantageous for coating applications, as it facilitates uniform film formation on fruit and vegetable surfaces. The neutral pH (7.2) of the final EPS solution ensures compatibility with fresh produce and prevents pH-induced tissue damage during application (Aqib et al., n.d.).

3.2 Swelling Ratio of EPS-Coated and Uncoated Samples:

The swelling ratio (%) of control and *Bacillus mycoides* EPS-coated fruits and vegetables was evaluated after 5 days of storage to assess moisture absorption and structural stability. A marked reduction in swelling ratio was observed in all EPS-coated samples compared to their respective controls. In tomato, the swelling ratio decreased from 43.6% in the control to 22.4% in the EPS-coated sample. Similarly, lady's finger exhibited a reduction from 40.8% (control) to 20.9% (coated). Guava showed swelling values of 46.3% in the control and 24.1% in the coated sample, while strawberry recorded 49.9% in the control and 24.6% following EPS coating. Across all tested commodities, EPS treatment resulted in approximately 45–55% reduction in swelling ratio compared to uncoated samples.

Samples	Swelling Ratio for Uncoated Samples in (%)	Swelling Ratio for EPS Coated Samples in (%)
Tomato	43.6	22.4
Lady's Finger	40.8	20.9
Guava	46.3	24.1
Strawberry	49.9	24.6

Table 1 – Swelling Ratio for Uncoated and EPS coated Samples

The significant decrease in swelling ratio in EPS-coated samples indicates improved moisture barrier properties imparted by the *Bacillus mycoides* exopolysaccharide coating. Swelling ratio is commonly associated with water uptake capacity and structural integrity; higher swelling reflects increased moisture absorption and cellular breakdown during storage.

Uncoated control samples exhibited higher swelling percentages, suggesting greater water migration and tissue softening. In contrast, the EPS-coated samples demonstrated substantially lower swelling ratios, indicating that the coating formed a semi-permeable protective film over the surface. This film likely reduced water vapor transmission and limited external moisture interaction, thereby maintaining structural stability. Among the tested samples, strawberry and

guava showed the highest swelling in control groups, which may be attributed to their softer tissue matrix and higher intrinsic moisture content. However, EPS coating effectively reduced swelling even in these highly perishable fruits, as critical role in extending shelf life and maintaining post-harvest quality (Wang et al., 2023).

3.3 Titratable Acidity of Control and EPS Coated Fruits and Vegetables:

Titratable acidity (TA), expressed as percentage citric acid, was evaluated in control and *Bacillus mycoides* EPS-coated fruits and vegetables during storage (5, 15, and 30 days). A gradual decline in titratable acidity was observed in all samples over the storage period; however, the reduction was more pronounced in uncoated control samples compared to EPS-coated samples. In tomato, titratable acidity decreased from 3.05% (day 5) to 1.85% (day 30) in the control, whereas EPS-coated samples showed a lower and more stabilized decline from 1.36% to 0.88%. Similarly, lady's finger exhibited a decrease from 2.93% to 1.73% in control samples, while coated samples showed values ranging from 1.28% to 0.81% over the same period. Guava recorded titratable acidity values decreasing from 2.21% to 1.31% in controls, compared to 0.85% to 0.54% in EPS-coated samples. Strawberry showed a decline from 2.67% to 1.47% in control samples, whereas coated samples maintained relatively lower but more stable values from 1.03% to 0.62% across storage. Overall, EPS-coated samples demonstrated a slower and more controlled reduction in titratable acidity compared to uncoated controls.

Titratable acidity is an important quality parameter that reflects organic acid content and is closely associated with flavor, metabolic activity, and ripening progression. The observed decrease in acidity during storage is consistent with normal post-harvest physiological processes, where organic acids are utilized as substrates for respiration. The semi-permeable barrier formed by *Bacillus mycoides* EPS likely reduced oxygen permeability and respiration rate, thereby conserving organic acids for a longer duration. By regulating gas exchange and minimizing moisture loss, the EPS coating appears to delay biochemical degradation associated with storage (Shrestha et al., 2020).

3.4 Total Antioxidant Activity for EPS and EPS-Coated and Uncoated Samples:

The total antioxidant activity (TAA) of *Bacillus mycoides* EPS and EPS-coated fruits and vegetables was determined and expressed as µg/g. The purified EPS exhibited a high intrinsic antioxidant activity of 334.5 µg/g, indicating strong free radical scavenging potential. Among the tested commodities, control samples showed comparatively lower antioxidant activity than EPS-coated samples stored at room temperature. In tomato, antioxidant activity increased from 125.6 µg/g in the control to 139.7 µg/g in EPS-coated samples. Guava showed a substantial increase from 231.2 µg/g (control) to 286.4 µg/g following EPS coating. Lady's finger exhibited an increase from 221 µg/g to 243.5 µg/g, while strawberry demonstrated the highest antioxidant activity overall, increasing from 265.4 µg/g in the control to 312.6 µg/g in coated samples.

Name of sample	*µg* /g
Eps	334.5

Name of sample	Tomato *µg* /g	Guava *µg* /g	Lady's Finger *µg* /g	Strawberry *µg* /g
Control	125.6	231.2	221	265.4
EPS-coated samples stored at room temperature	139.7	286.4	243.5	312.6
EPS-coated samples stored at 4 °C	140.2	289.2	245.1	318.9

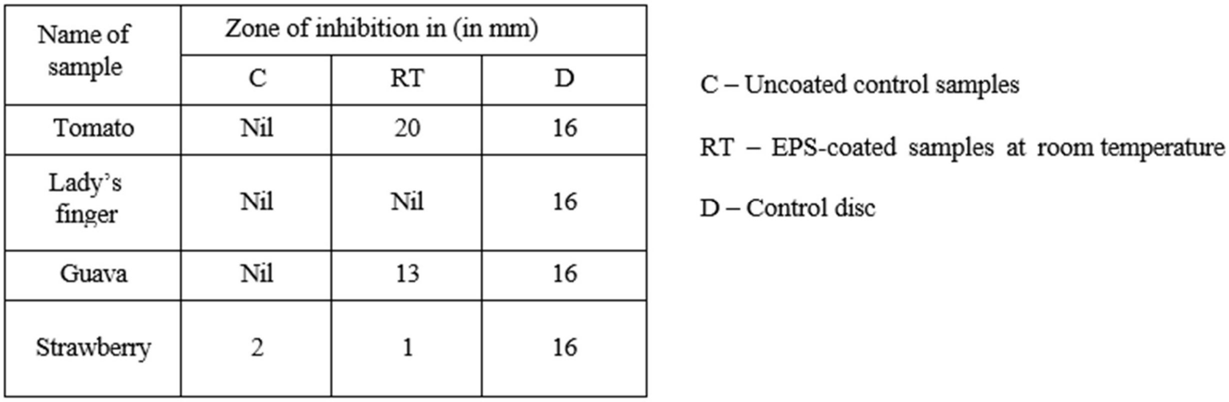
Table 2 – Total Antioxidant Activity for EPS and EPS-Coated and Uncoated Samples

The results indicate that *Bacillus mycoides* EPS possesses significant antioxidant potential, which may contribute directly to the increased antioxidant levels observed in coated fruits and vegetables The improvement in antioxidant retention correlates with earlier observations of reduced swelling ratio, moderated titratable acidity decline, and lower microbial proliferation in EPS-coated samples. Reduced oxidative stress and slower metabolic activity may have contributed to better preservation of bioactive compounds thereby supporting its potential use as a natural bio-preservative in food systems. (Johney et al., 2018)

3.5 Antibacterial Activity for Control EPS Coated Fruits and Vegetables:

The antibacterial activity of fruits and vegetables coated with exopolysaccharides (EPS) derived from *Bacillus mycoides* was evaluated using the agar well diffusion method. The results were expressed as the diameter of the zone of inhibition (mm), and the findings are summarized in Table 22.

The uncoated control samples (C) showed no inhibition zone in tomato, lady's finger, and guava, indicating the absence of significant inherent antibacterial activity in these samples under the tested conditions. Strawberry exhibited a minimal inhibition zone of 2 mm, which may be attributed to naturally occurring bioactive compounds such as organic acids and phenolics known for their mild antimicrobial properties.



Name of sample	Zone of inhibition in (in mm)		
	C	RT	D
Tomato	Nil	20	16
Lady's finger	Nil	Nil	16
Guava	Nil	13	16
Strawberry	2	1	16

C – Uncoated control samples

RT – EPS-coated samples at room temperature

D – Control disc

Table 3 – Antibacterial Activity - Zone of inhibition for Control, Uncoated and EPS Coated Fruits and Vegetables.

EPS-coated samples stored at room temperature (RT) demonstrated enhanced antibacterial activity compared to the control. Tomato exhibited a pronounced inhibition zone of 20 mm, which was even higher than that of the standard control disc (16 mm), indicating strong antimicrobial efficacy of the EPS coating. Guava also showed a notable inhibition zone of 13 mm, suggesting moderate antibacterial activity. In contrast, lady's finger did not exhibit any inhibition, implying that the effectiveness of EPS coating may vary depending on surface characteristics or compositional differences of the produce. Strawberry showed only marginal activity (1 mm), indicating limited enhancement by EPS under room temperature conditions.

The control disc (D) consistently produced a 16 mm zone of inhibition across all samples, confirming the susceptibility of the test microorganism and validating the reliability of the experimental procedure. The observed antibacterial activity of EPS from *Bacillus mycoides* may be attributed to its ability to form a protective film on the surface of produce, thereby restricting microbial growth. EPS are known to interfere with bacterial cell membranes, alter permeability, and reduce nutrient availability, ultimately inhibiting microbial proliferation. The significantly higher inhibition observed in tomato suggests better diffusion or interaction of EPS compounds with the test microorganism, possibly due to surface texture or moisture content facilitating release of active components (Selvakumar et al., 2014).

3.6 Total Viable Count for Control EPS Coated Fruits and Vegetables:

The total viable count (TVC) of microorganisms in tomato, lady's finger, guava, and strawberry during storage is presented in Table X. A progressive increase in microbial load was observed in all control samples from the 5th day to the 30th day of storage, indicating continuous microbial proliferation and deterioration of the produce. In contrast, fruits and vegetables coated with EPS produced by Bacillus mycoides and stored at room temperature (RT) showed significantly lower microbial counts throughout the storage period. In tomato, the microbial count in the control sample increased from 1×10^3 CFU/g on the 5th day to 3.5×10^3 CFU/g on the 30th day, indicating rapid microbial growth during storage. However, the EPS-coated sample showed no detectable microbial growth (Nil) on the 5th and 15th days and only 0.7×10^3 CFU/g by the 30th day, demonstrating strong microbial suppression.

A similar trend was observed in lady's finger. The control sample increased from 1.4×10^3 to

$\times 10^3$ CFU/g over the storage period, whereas the EPS-coated sample showed no microbial growth up to the 15th day and reached only 1.2×10^3 CFU/g by the 30th day. For guava, the control sample showed an increase from 0.9×10^3 CFU/g on the 5th day to 3.6×10^3 CFU/g on the 30th day, indicating significant microbial proliferation. In comparison, the EPS-coated sample remained microbially undetectable until the 15th day and showed only 0.8×10^3 CFU/g by the 30th day. Strawberry showed the highest microbial growth among the control samples, increasing from 1.2×10^3 to 5.1×10^3 CFU/g by the 30th day. In contrast, the EPS-coated strawberry sample showed no detectable microbial growth up to the 15th day and reached only $\times 10^3$ CFU/g at the end of storage.

Name of sample – Tomato	5th day	15th day	30th day
Control	1×10^3	2.1×10^3	3.5×10^3

EPS Coated Stored in Room temperature (RT)	Nil	Nil	0.7×10^3

Name of sample – Lady's Finger	5th day	15th day	30th day
Control	1.4×10^3	2.9×10^3	3.7×10^3
EPS Coated Stored in Room temperature (RT)	Nil	Nil	1.2×10^3

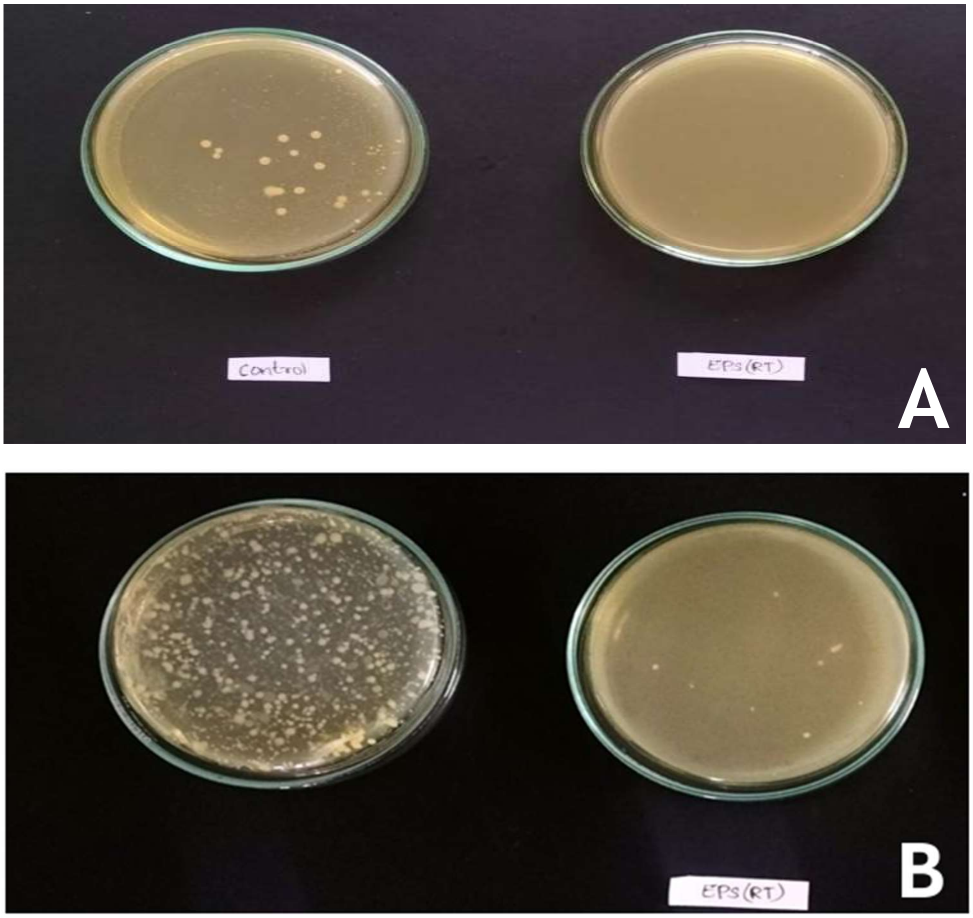


Fig 1 – Total Viable count for control, EPS coated for Tomato –

A)– 5th day storage B) – 30th Day Sto

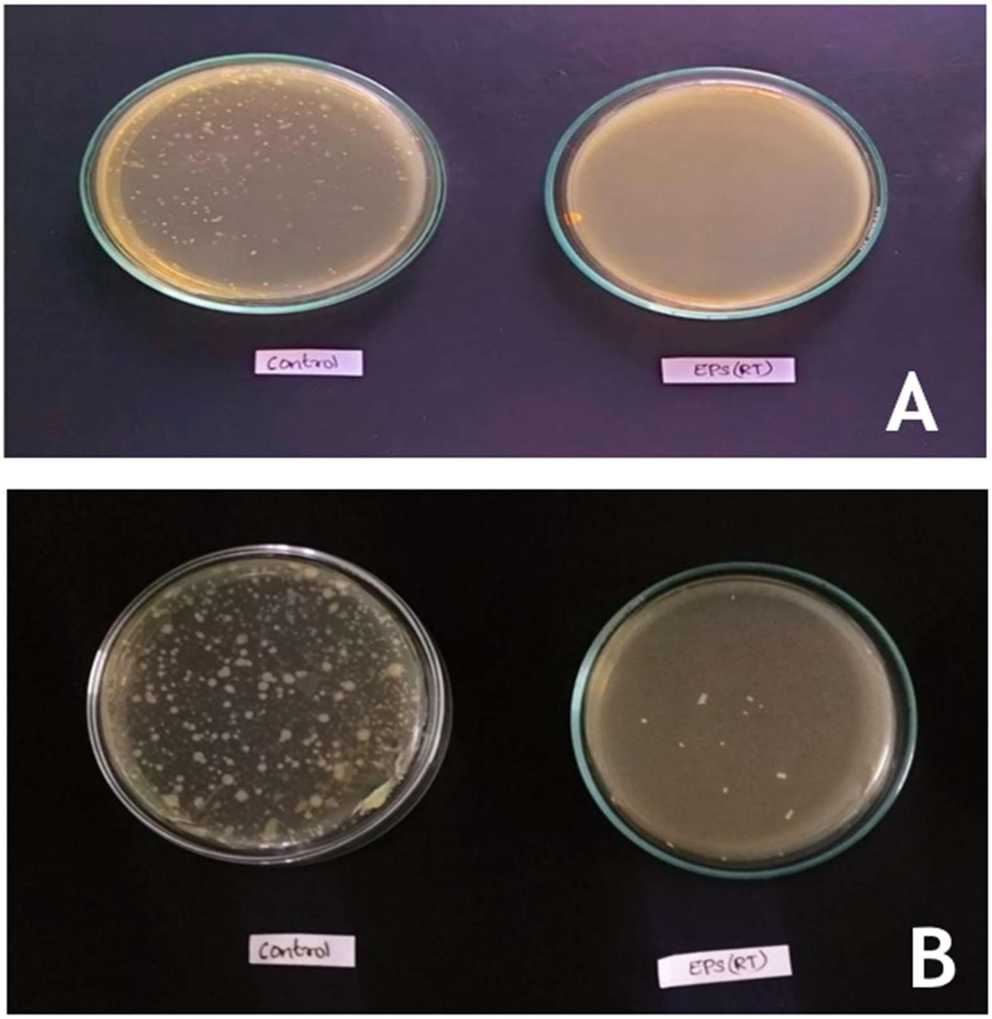


Fig 2 – Total Viable count for control, EPS coated for Lady's Finger –

A) – 5th day storage B) – 30th Day Storage

Name of sample – Guava	5th day	15th day	30th day
Control	0.9×10^3	1.8×10^3	3.6×10^3
EPS Coated Stored in Room temperature (RT)	Nil	Nil	0.8×10^3

Name of sample – Strawberry	5th day	15th day	30th day
Control	1.2×10^3	2.6×10^3	5.1×10^3
EPS Coated Stored in Room temperature (RT)	Nil	Nil	1.1×10^3

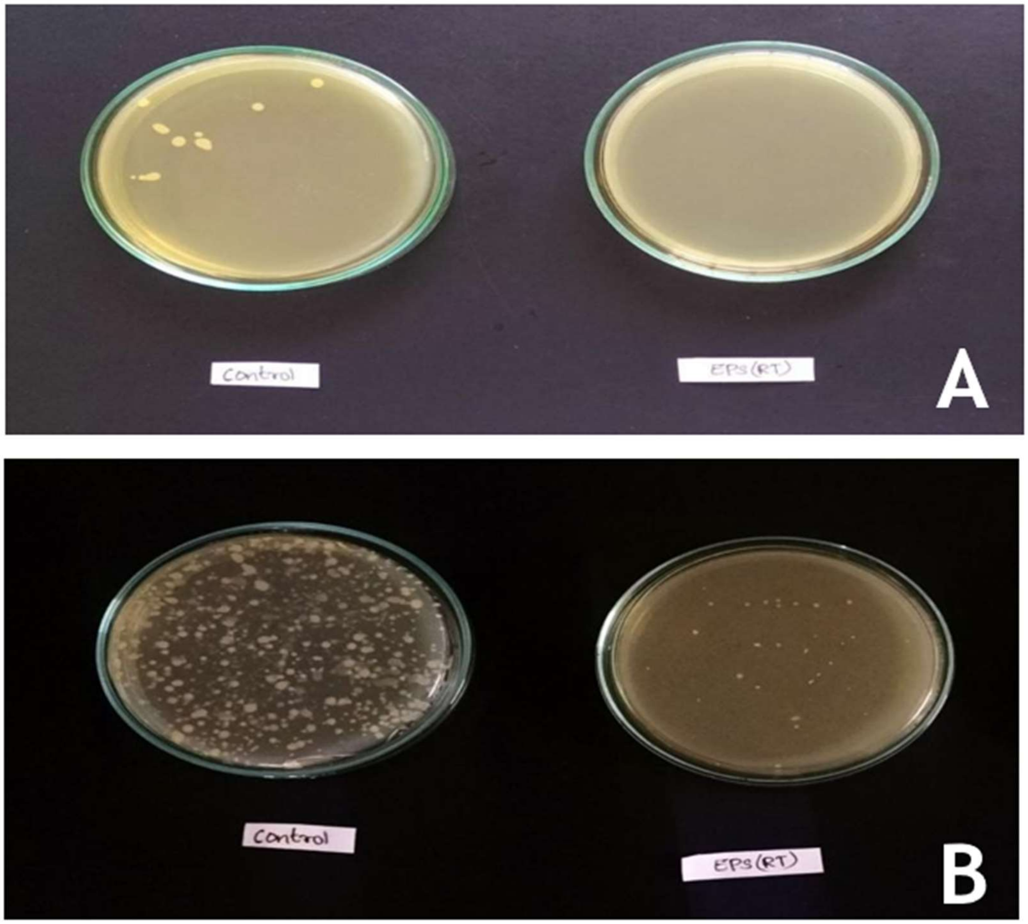
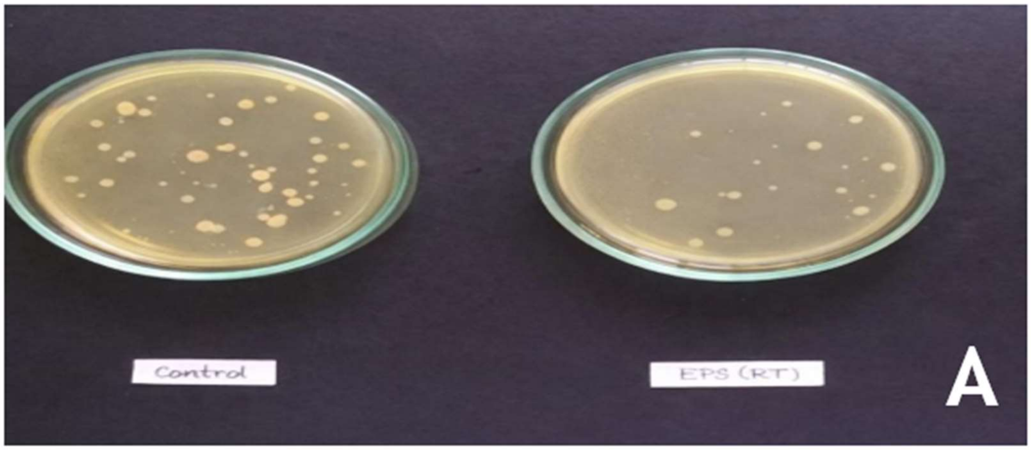


Fig 3 – Total Viable count for control, EPS coated for Guava –

A) – 5th day storage B) – 30th Day Storage



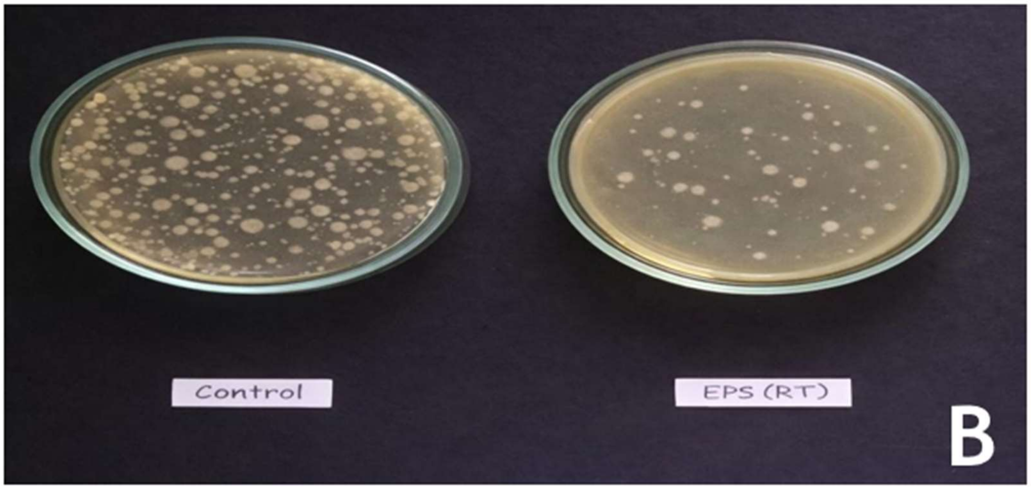


Fig 3 – Total Viable count for control, EPS coated for Strawberry –

A) – 5th day storage B) – 30th Day Storage

The significantly lower microbial counts observed in EPS-coated samples can be attributed to the protective barrier formed by the exopolysaccharide coating derived from *Bacillus mycoides*. This edible coating likely acts as a semi-permeable film that limits oxygen availability, reduces surface moisture, and restricts microbial colonization and proliferation. Additionally, EPS may exhibit antimicrobial properties that inhibit the growth of spoilage microorganisms on the surface of fresh produce (Erazo Anacona et al., 2026).

6. CONCLUSION

This study demonstrated the successful extraction of exopolysaccharides (EPS) from *Bacillus mycoides* and their application as an edible coating for fruits and vegetables. The purified EPS showed good solubility, moderate viscosity, and suitability for coating applications. Cytotoxicity assessment confirmed that the EPS was non-cytotoxic and biocompatible, maintaining high cell viability in L929 fibroblast cells. EPS coating significantly reduced the swelling ratio, indicating improved moisture barrier properties and structural stability. A slower decline in titratable acidity was observed in coated samples, suggesting delayed metabolic activity during storage. EPS also exhibited strong antioxidant activity, contributing to improved antioxidant retention in coated produce. Antibacterial analysis revealed effective microbial inhibition, particularly in tomato and guava. EPS-coated samples showed controlled changes in total soluble solids, indicating delayed ripening. Microbial load and total viable count were significantly lower in coated samples compared to controls. Overall, *Bacillus mycoides* EPS demonstrated strong potential as a natural edible coating for improving quality and extending the shelf life of fresh fruits and vegetables .

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ETHICS STATEMENT: This article does not contain any studies on human participants or animals performed by any of the authors.

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