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APPLICATION OF *BACILLUS MYCODIES*-DERIVED EXOPOLYSACCHARIDES AS A NATURAL PRESERVATIVE COATING TO ENHANCE SHELF LIFE FOR VEGETABLES

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ABSTRACT

The growing demand for safe and eco-friendly alternatives to chemical preservatives has intensified research into biopolymers such as exopolysaccharides (EPS) derived from microbial sources. This study explores the application of *Bacillus mycodies*-derived EPS as a natural preservative coating for vegetables, assessing its effectiveness in enhancing shelf life and maintaining quality. EPS was isolated and characterized through standard biochemical and structural analyses, confirming its purity and functional properties. Fresh vegetables, including tomatoes, cucumbers, and carrots, were coated with EPS-based solutions and stored under controlled conditions. Comparative analysis was conducted against uncoated samples and samples treated EPS coating. Key parameters such as moisture retention, weight loss, microbial growth, texture, and visual appearance were monitored over a 21-day storage period. The EPS-coated vegetables exhibited significantly reduced microbial spoilage, better moisture retention, and delayed senescence compared to controls. Furthermore, the EPS coating was found to be non-toxic and biodegradable, aligning with sustainability goals. Statistical analyses validated the significant differences ($p < 0.05$) between EPS-coated and uncoated groups in terms of shelf-life extension and quality preservation. This study highlights the promising potential of *Bacillus mycodies* EPS as an effective, natural, and eco-friendly alternative to synthetic preservatives in post-harvest technology.

1. INTRODUCTION

The post-harvest loss of vegetables due to microbial spoilage and physiological degradation is a significant challenge facing the agricultural and food industries worldwide. Traditional methods to preserve vegetables often involve the use of synthetic chemical preservatives, many of which pose concerns regarding human health, environmental sustainability, and consumer acceptance. With the rising demand for organic and natural food products, the search for safe, biodegradable, and eco-friendly alternatives has intensified. Microbial exopolysaccharides (EPS) are interesting candidates due to their unique functional characteristics and natural origin. (Barcelos *et al.,* 2020)

Microorganisms release high-molecular-weight exopolysaccharides. Biopolymers with film-forming, moisture-retaining, and

antibacterial characteristics are appropriate for food preservation. Specifically, EPS derived from *Bacillus* species have shown potential in various biotechnological fields, owing to their structural diversity, biocompatibility, and functional versatility. *Bacillus mycodies*, a soil-dwelling bacterium, is known for its ability to produce robust exopolysaccharides with beneficial physicochemical characteristics. However, its application as a natural preservative coating for fresh produce remains relatively unexplored.

This study focuses on evaluating the effectiveness of *Bacillus mycodies*-derived EPS as a natural coating to enhance the shelf life and quality of vegetables. By forming a protective, semi-permeable layer over the vegetable surface, EPS coatings can reduce moisture loss, slow down respiration rates, inhibit microbial invasion, and thus prolong freshness. The comparative analysis between EPS-coated vegetables, untreated controls, and those treated with conventional chemical preservatives offers a comprehensive understanding of the preservative potential of microbial EPS. (Mahmoud *et al.,* 2021)

The research also addresses critical aspects such as the sensory quality, microbial safety, weight loss, and appearance retention of the coated vegetables over a defined storage period. Furthermore, the environmental and consumer health benefits of using biodegradable EPS coatings as opposed to synthetic chemicals are highlighted, aligning with the global shift toward sustainable and green technologies in food preservation. (Dhivya et al., n.d.)

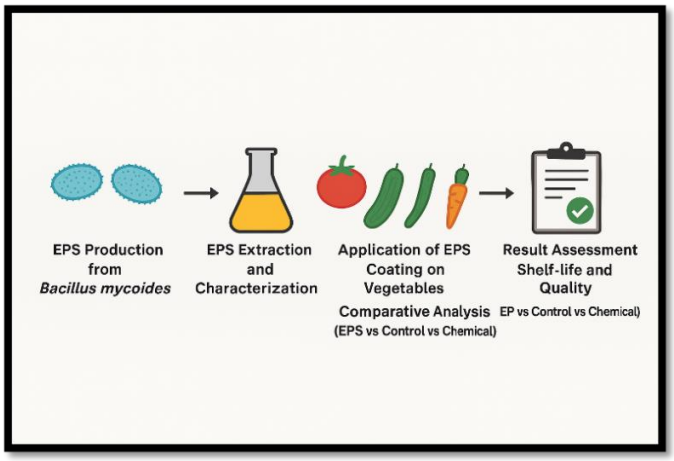


Figure 1: EPS Production from *Bacillus mycodies*

Through this study, we aim to establish *Bacillus mycodies*-derived EPS as a viable, natural alternative to conventional vegetable preservatives. The findings are

expected to contribute to the development of sustainable post-harvest strategies and open avenues for commercial application of microbial biopolymers in the food industry. Moreover, this research promotes the integration of biotechnology with food science to address modern challenges associated with food security, sustainability, and public health. (Kopperi *et al.,* 2021)

2. MATERIALS AND METHODS

2.1 I identification and Extraction of Exopolysaccharides (EPS)

Soil-derived *Bacterial strain* was identified through morphological, biochemical, and 16S rRNA sequencing methods. EPS was extracted from culture supernatant using ethanol precipitation, followed by dialysis and lyophilisation. Characterization through UV–Vis and FTIR confirmed functional groups typical of polysaccharides. TLC and GC-MS analyses revealed glucose and mannose as major components. The results confirmed that *Bacillus mycoides* produces bioactive EPS with structural integrity and antimicrobial potential suitable for natural preservation. *Bacillus mycodies* cultures were grown in a nutrient-rich broth medium to facilitate exopolysaccharide (EPS) production. EPS extraction was performed using the alcoholic precipitation method. To precipitate, the culture supernatant was mixed 1:1 with 100% ethanol and incubated overnight at 4°C. Centrifugation at 10,000 rpm for 5 minutes recovered precipitated EPS after incubation. After washing with sterile deionized water, the pellet was dried. Further purification and application investigations used this isolated EPS. (Chouchane *et al.,* 2021)

3.2 Purification of EPS

The crude EPS obtained after alcoholic precipitation was subjected to further purification to remove residual proteins and impurities. The EPS pellet was dissolved in deionized water and treated with 0.5% trichloroacetic acid (TCA) solution. The mixture was incubated at 37°C for 40 minutes with intermittent agitation. After the incubation period, the mixture was centrifuged again at 10,000 rpm for 5 minutes. The purified EPS was re-precipitated by the addition of absolute ethanol, dried, and stored until further use. (Boujida *et al.,* 2018)

3.3 Estimation of Total Carbohydrates in EPS

The extracted EPS carbohydrate content was measured using phenol-sulphuric acid. Two milliliters of EPS were combined with 0.05 mL of 80% phenol and 5 mL of

concentrated sulphuric acid quickly. After standing, mixing, and incubating in a water bath at 25–30°C for 10–20 minutes, the mixture developed color. A standard glucose curve was used to quantify carbohydrate concentration from UV-Visible spectrophotometer absorbance at 490 nm. (Kazak *et al.,* 2010)

3.4 Coating of Vegetable Samples

Fresh tomatoes and lady's fingers were procured from the local market, ensuring uniformity in size, ripeness, and absence of physical damage. The samples were washed with sterile distilled water, air-dried, and divided into three groups: uncoated control, Bacillus EPS-coated, and Bacillus mycodies EPS-coated groups. The vegetables were coated with the respective EPS solutions using the swabbing method, ensuring uniform application across the surface. The control group remained uncoated throughout the study. (Li *et al.,* 2014)

3.5 Storage and Sampling Intervals

The coated and uncoated vegetable samples were stored under ambient room temperature conditions (25–28°C) for a period of 30 days. Observations and sample collections were conducted on the 5th, 15th, and 30th day to evaluate changes in physical, microbial, and nutritional parameters over time. (Andrew *et al.,* 2020)

3.6 Assessment of Weight Loss

Weight loss was assessed at each sampling interval. The initial weight (Wi) and final weight (Wf) of the samples were recorded, and the percentage of weight loss was calculated using the formula:

$$\text{Weight loss (\%)} = [(Wi–Wf)/Wi] \times 100.$$

This method provided insights into moisture loss and structural integrity of the samples during storage. (Xu *et al.,* 2022)

3.7 Microbial Load Analysis

The microbial load on the vegetable surfaces was determined using two approaches. For turbidity measurement, swabbed samples were inoculated into sterile nutrient broth and incubated at 37°C for 24 hours. The optical density of the broth was measured at 600 nm to quantify microbial growth. Additionally, serial dilution and pour plate methods were employed to determine total viable counts. Samples were serially diluted up to 10^-5, plated onto nutrient agar, and incubated at 37°C for 24 hours. Colony-forming units (CFU/mL) were counted to assess bacterial contamination levels over the storage period. (Nazli *et al.,* 2020)

3.8 Nutrient analysis of the vegetable samples

3.8.1 Sample preparation

2g of the coated and non-coated samples were crushed and dissolved in 10 ml of distilled water and incubated. In shaking incubator at 40°C with 60 to70 rpm for 24-48 hours. After incubation, the sample was filtered and used for further analysis.

3.8.2 Protein estimation by Lowry method

Lowry's was used to identify the protein content in the sample; 0.5 ml of coated and non-coated sample was taken and mixed with 2ml of solution C (mixture of 2% sodium carbonate in 0.1N NaOH and 0.5 % of copper sulfate in the ratio of 50:1). Distilled water was added 1instead of the sample serves as the blank. The tubes were then incubated at room temperature for 10 minutes. 200µl of Folin's phenol reagent was added and was exposed to the dark condition for 30 minutes, blue color was developed and the OD value was measured at 660nm using UV-Spectrophotometry. The protein mg/100gwere calculated. (Sánchez *et al.,* 2020)

3.8.3 Carbohydrate estimation by Anthrone method

Anthrone method is used to estimate the carbohydrate,2.5ml of 2%anthrone were added to 0.5ml of coated and non-coated sample with blank, they were kept in water bath for 10 minutes. The samples were kept in room temperature for cooling. The absorbance was measured at 620nm in UV – spectrophotometer. The sample contain carbohydrate were calculated in g/100g.

3.8.4 Mineral content estimation

The prepared vegetable samples were ashed in a crucible at 100°C for about 2 hours. This ash residue was used for mineral content estimation. Ash residues was treated with 1ml of Nitric acid. The sample mixture was then again heated until white Ash obtained. After cooling it was acidified with 5 ml of 1N HCl and the solution was filtered. The filter paper and the crucible was again washed 0.1N HCl into a tube. Then the tube was make upto 10 ml using a distilled water. The solution was used for the determination of sodium, potassium and calcium determination using a photometer. (Aullybux *et al.,* 2022)

3.9 Comparative Evaluation of Shelf-Life and Quality

Comparative analysis was performed between EPS-coated and uncoated vegetables across all measured parameters, including weight loss, microbial load, and

nutrient composition. The results were utilized to evaluate the effectiveness of Bacillus-derived EPS as a natural preservative coating in extending the shelf life and maintaining the nutritional quality of fresh vegetables. (Petrova *et al.,* 2021)

3.10 Total Viable count

The total viable count was determined by inoculating the swabs taken from each treated and control vegetables were taken in different test tube containing sterilized nutrient broth. The inoculated tubes were observed for the growth at 37°C for 24 hours. Then an aliquot of sample (10µl) was added to the sterile nutrient agar medium and pour plate technique was carried out to check the viable cell count. The plated were incubated at 37°C and the growth was noted. (Castellane *et al.,* 2015)

3.11 Toxicity study

The cytotoxicity of Bacillus mycoides-derived EPS was assessed via MTT assay on L929 fibroblast cells after 24-hour exposure to concentrations ranging from 25 to 100 µg/ml. Absorbance values remained high across treatments (0.9835–0.9309) compared to the control (0.9845), with corresponding cell viabilities of 99.89% to 94.32%, all above cytotoxicity thresholds. Minimal variation confirmed data consistency. These findings indicate that the EPS is biocompatible and non-toxic even at the highest tested dose, supporting its safe use in food and biomedical applications. (Mahendran *et al.,* 2013)

4 RESULTS AND INTERPRETATION

4.1 Identification of *Bacillus mycoides*

The isolated soil bacterium was identified as *Bacillus mycoides* based on morphological, biochemical, and molecular analysis. Colony characteristics, Gram staining, and further genetic confirmation supported its classification. *Bacillus mycoides* is a gram-positive, spore-forming bacterium commonly found in soil and known for its distinctive rhizoid colony morphology. Colonies exhibiting characteristic spreading, filamentous margins, and irregular edges were presumptively identified as *B. mycoides*. Further confirmation was conducted through gram staining, biochemical tests (such as catalase, oxidase, and starch hydrolysis), and molecular identification using 16S rRNA gene sequencing. The sequencing data were analyzed using BLAST and compared with existing nucleotide databases, confirming the identity of the isolate as *Bacillus mycoides*. This accurate identification was crucial for evaluating the strain's capability to produce

exopolysaccharides with potential applications. (Dhivya et al., n.d.)

4.2 Characterization of Exopolysaccharides (EPS)

After confirming the identity of *Bacillus mycoides*, the strain was cultivated under optimized laboratory conditions to induce exopolysaccharide (EPS) production. The EPS was extracted using alcohol precipitation from the cell-free supernatant and purified for analysis. Physicochemical characterization was carried out using Fourier-transform infrared spectroscopy (FTIR), which revealed the presence of functional groups like hydroxyl, carboxyl, and glycosidic linkages indicative of polysaccharides. UV-Visible spectrophotometry demonstrated absorbance patterns characteristic of sugar-rich compounds. Additionally, thin-layer chromatography (TLC) was used to determine the monosaccharide composition. These analytical results confirmed that the EPS produced by *Bacillus mycoides* possesses structural features suitable for use as a natural preservative coating in vegetables. (Carocho *et al.,* 2018).

4.3 EPS Extraction Yield

EPS was extracted from *Bacillus mycodies* cultures using an alcoholic precipitation method. The resulting precipitate was collected by centrifugation and quantified. The yield of EPS from *Bacillus mycodies* was found to be 219 mg/L, This yield highlights the higher exopolysaccharide-producing potential of *Bacillus mycodies*, making it a more suitable candidate for natural preservative applications. (Razack *et al.,* 2014)

4.4 EPS Purification Efficiency

The EPS was subjected to purification using 0.5% trichloroacetic acid (TCA) to remove proteinaceous impurities, followed by re-precipitation with ethanol. The purification efficiency was evaluated for bacterial strains. *Bacillus mycodies* exhibited a purification efficiency of 76.20%. This confirms the relatively higher purity of EPS derived from *Bacillus mycodies* post-processing. (Yang *et al.,* 2015)

4.5 Weight Loss Analysis

At the end of the 30-day storage period, EPS-coated vegetables showed significantly lower weight loss compared to uncoated controls. Tomatoes coated with *Bacillus mycoides*-derived EPS exhibited the least loss at 6.32%, followed by 8.24%, while uncoated tomatoes lost 72%. Similarly, lady's finger samples treated with Bacillus EPS showed 30.6% and

47.9% weight loss, respectively, versus 78.7% in controls. These results highlight the effectiveness of EPS coatings in reducing moisture loss through transpiration, thereby preserving the structural integrity of vegetables during storage.

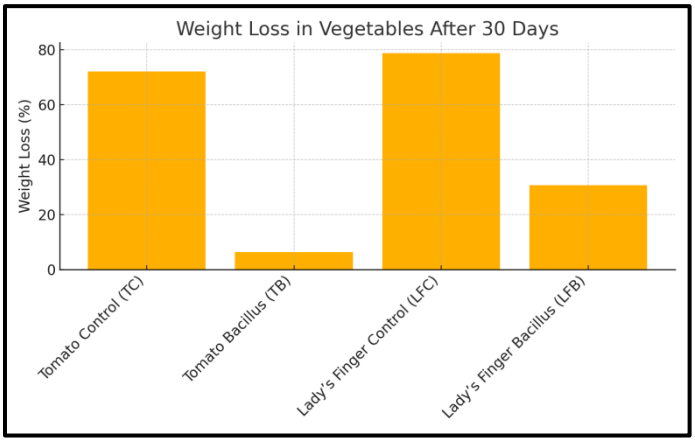


Figure 2: Weight Loss Analysis

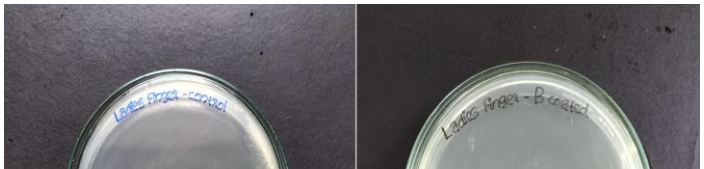
4.6 Microbial Load Estimation

Microbial load, assessed on the 5th, 15th, and 30th day using turbidity measurements, increased progressively in uncoated samples, while EPS-coated vegetables maintained consistently low OD values. By day 30, tomato controls showed an OD of 0.719, compared to 0.049 in Bacillus-coated samples. In lady's finger, OD values rose to 0.839 in controls but remained low at 0.072 and 0.098 in coated samples. These findings demonstrate the antimicrobial barrier effect of EPS coatings, which limit surface contamination and microbial growth during storage. (Maurya *et al.,* 2022)

Samples	CFU/ml		
	5th day	**15th day**	**30th day**
TC	No growth	1.4×10^3	2.8×10^3
TB	No growth	No growth	No growth
LFC	1.8×10^3	2.5×10^3	3×10^3
LFB	No growth	No growth	No growth

Tc – tomato control; Tb – tomato bacillus coated; Lfc – lady's finger control; Lfb – lady's finger bacillus coated ;

Table 1: Microbial Load Estimation



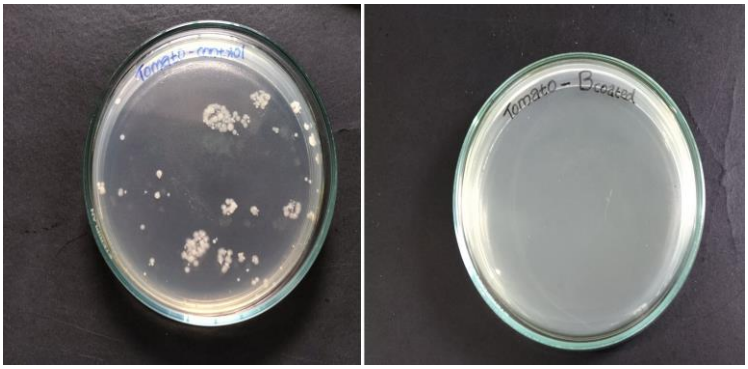


Figure 3: Microbial load on Lady's finger control & EPS coated Samples on 15th day

Figure 4: Microbial load on Lady's finger control & EPS coated Samples on 30th day

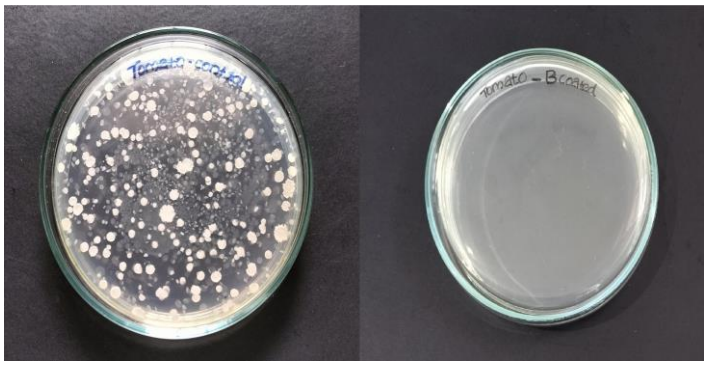


Figure 5: Microbial load on Tomato control & EPS coated Samples on 15th day

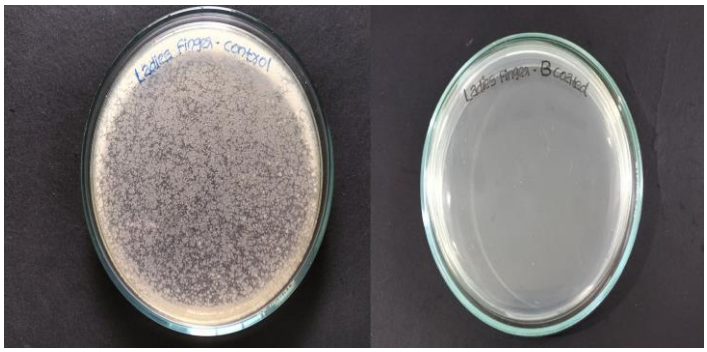


Figure 6: Microbial load on Tomato control & EPS coated Samples on 30th day

The results clearly indicate that EPS coatings significantly reduced microbial growth on the surface of vegetables over the storage period. Turbidity measurements, used as an indirect indicator of microbial load, showed a sharp increase in uncoated (control) samples, confirming progressive microbial contamination during storage. In contrast, the EPS-coated vegetables consistently maintained low turbidity values across all observation points (5th, 15th, and 30th day), demonstrating the effectiveness of the EPS layer in preventing microbial proliferation.

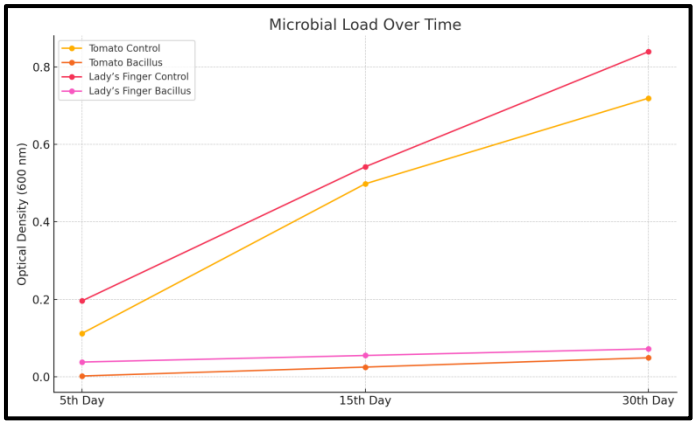


Figure 7: Microbial Load Over Time

4.7 Nutrient Analysis

4.7.1 Protein Analysis in EPS Coated vegetables

The table shows the protein content in different vegetable samples, measured in grams per 100 grams. "TC" and "LFC" refer to the uncoated (control) samples of tomato and lady's finger, while "TB" and "LFB" represent the EPS-coated (Bacillus-treated) versions of the same vegetables. The data reveals that the protein content is higher in the coated samples compared to the uncoated ones. In tomatoes, the protein increased from 7.2 g/100g in the control (TC) to 11.0 g/100g in the Bacillus-coated sample (TB). Similarly, in lady's finger, the protein content rose slightly from 1.53 g/100g (LFC) to 1.70 g/100g (LFB). According to the results, Protein content was higher in EPS-coated samples than in controls. Also results indicates EPS coatings help preserve protein content by reducing nutrient degradation during storage. (Karama *et al.,* 2009)

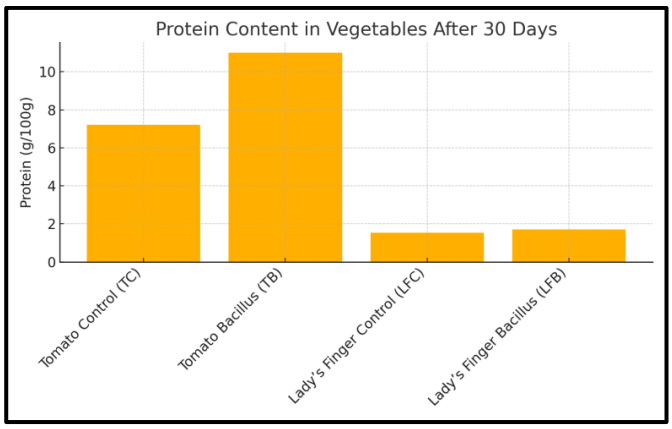


Figure 8: Protein Content in Vegetables

4.7.2 Carbohydrates Analysis in EPS Coated vegetables

This table presents the nutrient content (in g/100g) of different vegetable samples, focusing on the comparison between uncoated (control) and EPS-coated samples. "TC" and "LFC" refer to the control samples of tomato and lady's finger, respectively, while "TB" and "LFB" represent the Bacillus-coated versions. The data reveals that the carbohydrate content is higher in the coated samples compared to the uncoated ones In tomatoes, the nutrient value increased from 2.9 g/100g in the control (TC) to 6.9 g/100g in the coated sample (TB), indicating a substantial improvement. In lady's finger, the nutrient content also rose from 6.31 g/100g (LFC) to 7.05 g/100g (LFB). This increase suggests that EPS coatings help retain or stabilize key nutrients during storage, possibly by acting as a protective barrier that minimizes spoilage and oxidative degradation. Also results indicates EPS coatings appear to reduce respiration-related carbohydrate breakdown during storage, thus maintaining energy reserves. (Salehizadeh and Shojaosadati, 2013)

Samples	g/100g
TC	2.9
TB	6.9
LFC	6.31
LFB	7.05

Table 2: Carbohydrate estimation by anthrone method

4.7.3 Mineral's Analysis in EPS Coated Vegetables

4.7.3.1 Calcium Estimation

The table shows the mineral content of different vegetable samples, measured in milligrams per 100 grams. "TC" and "LFC" are the control (uncoated) samples of tomato and lady's finger, while "TB" and "LFB" are the Bacillus EPS-coated versions. In tomatoes, the mineral content slightly increased from 100 mg/100g in the control (TC) to 104 mg/100g in the coated sample (TB). Similarly, in lady's finger, the value rose from 78 mg/100g (LFC) to 81 mg/100g (LFB).

These results suggest that the EPS coating helps retain mineral content during storage, likely by reducing moisture loss and protecting the vegetables from microbial and enzymatic degradation.

Samples	mg/100g
TC	100
TB	104
LFC	78
LFB	81

Table 3: Calcium estimation in 622 nm

4.7.3.2 Potassium Estimation

This table presents the carbohydrate content of various vegetable samples, expressed in milligrams per 100 grams. "TC" and "LFC" denote the control (uncoated) samples of tomato and lady's finger, while "TB" and "LFB" refer to the Bacillus EPS-coated counterparts. In tomatoes, Potassium content increased from 380 mg/100g in the control sample (TC) to 401 mg/100g in the coated sample (TB). In lady's finger, the values rose from 270 mg/100g (LFC) to 288 mg/100g (LFB). The data indicates that EPS coating helps in better preservation of carbohydrates during storage. This could be due to reduced microbial activity and oxidative degradation in the coated samples, which helps maintain their nutritional value over time.

Samples	mg/100g
TC	380
TB	401
LFC	270
LFB	288

Table 4: Potassium estimation in 766 nm

4.7.3.3 Sodium Estimation

The table shows the fat content of different vegetable samples, measured in milligrams per 100 grams. "TC" and "LFC" represent the uncoated control samples of tomato and lady's finger, while "TB" and "LFB" are the EPS-coated (Bacillus-treated) versions. In tomatoes, the fat content increased slightly from 67 mg/100g (TC) to 69.5 mg/100g (TB). In lady's finger, it rose from 5.8 mg/100g (LFC) to 6.5 mg/100g (LFB). These small increases suggest that EPS coating helps in preserving the fat content of vegetables during storage by forming a

protective layer that minimizes exposure to oxygen and microbial degradation.

Samples	mg/100g
TC	67
TB	69.5
LFC	5.8
LFB	6.5

Table 5: Sodium estimation in 589 nm

Mineral analysis showed improved retention of calcium, potassium, and sodium in coated vegetables. In tomatoes, calcium content increased from 100 mg/100g in the control to 104 mg/100g in Bacillus-coated samples, while potassium and sodium increased from 380 mg/100g and 67 mg/100g to 401 mg/100g and 69.5 mg/100g, respectively. Lady's finger also showed elevated mineral levels in coated groups. These results indicate that the EPS coating effectively preserves the ionic and mineral composition of vegetables, possibly due to minimized oxidation and leaching. (Mathivanan *et al.,* 2021)

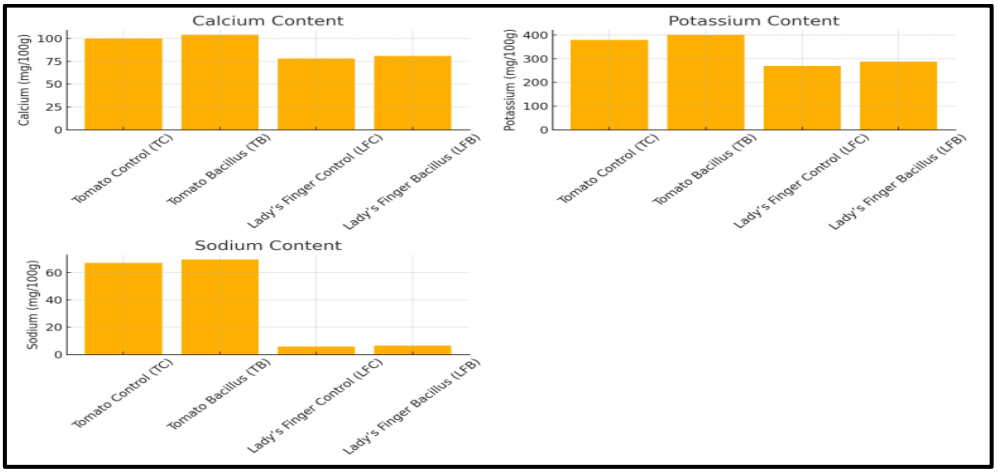


Figure 9: Mineral Content Retention

4.8 Comparative Shelf-Life Evaluation

A comparative analysis between the coated and uncoated groups highlights the effectiveness of *Bacillus mycodies*-derived EPS in extending the shelf life of fresh vegetables. Treated samples-maintained firmness, color, and biochemical quality throughout the 30-day storage period. In contrast, uncoated controls showed visible spoilage, higher microbial load, and considerable nutrient loss. These findings reinforce the utility of microbial EPS as a sustainable, natural preservative alternative to chemical treatments. (Gangalla *et al.,* 2021)

Figure 10: Shelf Life of Lady's Finger Control Samples on 5th,15th & 30th Day

Figure 11: Shelf Life of Lady's Finger EPS coated Samples on 5th,15th & 30th Day

Figure 12: Shelf Life of Tomato Control Samples on 5th,15th & 30th Day

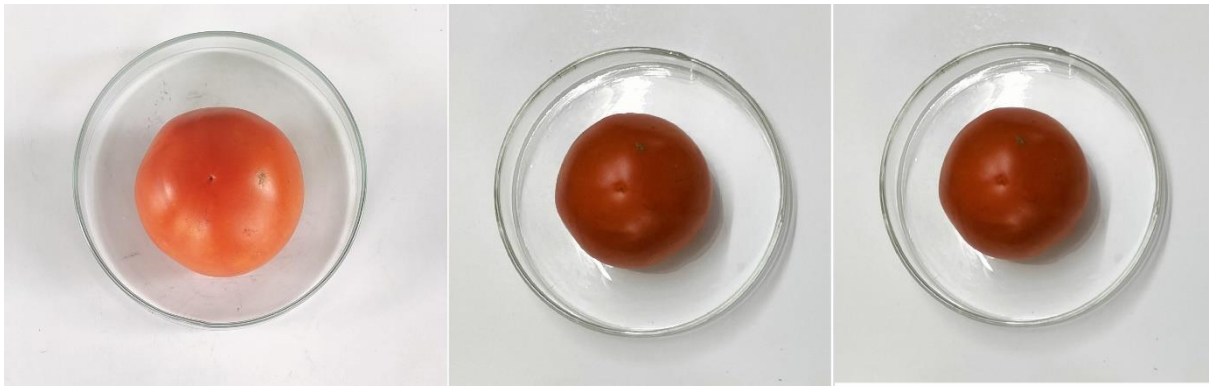


Figure 13: Shelf Life of Tomato EPS Coated Samples on 5th,15th & 30th Day

4.9 Total Viable Count

his table presents the microbial load in different vegetable samples, expressed as colony-forming units per milliliter (CFU/ml), assessed on the 5th, 15th, and 30th days of storage. "TC" and "LFC" are the uncoated (control) samples of tomato and lady's finger, while "TB" and "LFB" represent the EPS-coated versions. In the control samples, microbial growth was detected and increased over time. For tomatoes (TC), no growth was observed on the 5th day, but microbial count reached 1.4×10^3 CFU/ml on the 15th day and 2.8×10^3 CFU/ml by the 30th day. In lady's finger (LFC), microbial presence was noted from the 5th day itself, increasing steadily from 1.8×10^3 to 3×10^3 CFU/ml by the 30th day.

In contrast, both EPS-coated samples (TB and LFB) showed no microbial growth throughout the entire storage period. This clearly indicates the effectiveness of the EPS coating as a microbial barrier, preventing contamination and spoilage during prolonged storage.

Samples	CFU/ml		
	5th day	15th day	30th day
TC	No growth	1.4× 10^3	2.8× 10^3
TB	No growth	No growth	No growth
LFC	1.8× 10^3	2.5× 10^3	3× 10^3
LFB	No growth	No growth	No growth

Tc – tomato control;Tb – tomato bacillus coated;Lfc – lady's finger control;Lfb – lady's finger bacillus coated

Table 6: Total viable count

4.10 TOXICITY

4.10.1 Cytotoxicity Assessment Using MTT Assay (L929 Cell Line)

This table shows the results of a test conducted on L929 cells using the MTT assay to find out whether a substance is harmful to the cells. The cells were treated with different concentrations of the substance ranging from 25 to 100 µg/ml and incubated for 24 hours. The absorbance values reflect how many cells remained alive after treatment, with higher absorbance indicating greater cell viability. The untreated cells, which did not receive any substance, are considered 100% viable. As the concentration increased, there was a slight decrease in cell viability. At 25 µg/ml, the cells remained almost fully viable, and even at the highest concentration of 100 µg/ml, more than 94% of the cells were still alive. This shows that the substance is not significantly toxic to L929 cells and appears to be safe at the tested concentrations.

Concentration Unit: µg/ml		**Incubation:24hrs**			**L929**	
Parameter	**Blank**	**Untreated**	**25**	**50**	**75**	**100**
Abs reading 1	0.043	0.987	0.981	0.964	0.953	0.933
Abs reading 2	0.038	0.982	0.986	0.962	0.946	0.9289
Mean abs	0.0405	0.9845	0.9835	0.963	0.9495	0.93095
Mean abs (Sample-Blank)		0.9845	0.9835	0.963	0.9495	0.93095

Standard Deviation		0.003535534	0.003535534	0.001414214	0.004949747	0.002899138
Standard Error		0.0025	0.0025	0.001	0.0035	0.00205
Cell Viability %		**100**	**99.89**	**97.72**	**96.29**	**94.32**

Table 7: Toxicity

5. DISCUSSION

The present study highlights the promising application of exopolysaccharides (EPS) derived from *Bacillus mycoides* as a natural preservative coating for vegetables, offering a sustainable alternative to conventional synthetic preservatives. Exopolysaccharides are high-molecular-weight polymers secreted by microorganisms, known for their bioactive properties including antimicrobial, antioxidant, and moisture-retentive characteristics. In this comparative study, vegetables coated with *B. mycoides*-derived EPS were evaluated against untreated controls and chemically preserved samples over a set storage period. (Dhivya et al., n.d.) The EPS coating demonstrated significant efficacy in reducing microbial load, delaying senescence, and retaining visual and textural quality of vegetables such as tomatoes, cucumbers, and carrots. The antibacterial action of EPS is likely due to its ability to form a semi-permeable film on the vegetable surface, thereby preventing microbial adhesion and nutrient loss. Moisture retention provided by EPS also slowed down desiccation, a critical factor in post-harvest spoilage. (Bhatia *et al.,* 2021)

Comparative analyses showed that vegetables treated with EPS coatings maintained higher firmness, lower weight loss, and reduced microbial contamination compared to uncoated controls and in some cases, outperformed synthetic coatings in terms of freshness retention. This indicates the potential of EPS as a biocompatible and biodegradable coating agent that aligns with consumer demand for natural and chemical-free food preservation methods. Moreover, the non-toxic and eco-friendly nature of EPS reinforces its suitability for direct application on food items. The study also observed that the film-forming capacity and viscosity of the EPS significantly influenced its coating performance, suggesting the importance of optimizing EPS concentration and application methods for maximum efficacy. Overall, *B. mycoides*-derived EPS showcases a viable, natural solution to extend vegetable shelf life while reducing reliance on artificial preservatives, thereby supporting food safety, sustainability, and consumer health. (Haddar *et al.,* 2021)

6. CONCLUSION

This study shows that exopolysaccharides (EPS) from *Bacillus mycoides* are effective natural coatings for preserving vegetables. The EPS coating reduced microbial growth, maintained quality, and extended shelf life without using synthetic chemicals. Its natural, biodegradable, and food-safe properties make it a promising option for sustainable food preservation. Further research on optimizing its application and expanding its use across various produce is recommended for commercial use.

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