USE OF *Lactobacillus paracasei* Ld3 FOR BIOPRESERVATION OF POST-HARVESTED STRAWBERRY FRUIT

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SUMMARY

Lactic acid bacteria are known for their biopreservative activity. In this study, an endophytic bacterium, *Lactobacillus paracasei* Ld3, was used to maintain the quality of post-harvested strawberry fruit. The sensory (color, taste, texture, flavor, and overall acceptability), physical (% weight loss and decay), microbiological (*L. paracasei* Ld3, and total yeast and mold counts), and chemical (pH, total soluble solids, titratable acidity, and total phenolic content) properties of the strawberries inoculated with *L. paracasei* Ld3 cells and the control samples were evaluated during 10 days of storage at 4^{o}C. Results showed that the inoculated strawberries had higher sensory scores than the control. Inoculation with *L. paracasei* Ld3 on the fruit surface reduced the fruit water loss and decay on day 10 by about 32.9% and 61.5%, respectively. Besides, *L. paracasei* Ld3 sustained high levels on the fruit surface, and yeast and mold fruit proliferation was inhibited in the inoculated strawberries. However, no significant difference ($p > 0.05$) was observed in pH and titratable acidity between the inoculated strawberries and the control samples at the same storage time. Moreover, inoculation with *L. paracasei* Ld3 slowed the deterioration rate of total soluble solids and total phenolic content compared to the control samples. These findings suggest that the application of *L. paracasei* Ld3 is potent for the preservation of post-harvested strawberry fruit.

Keywords: Biological control, biopreservation, endophytes, lactic acid bacteria, strawberry.

INTRODUCTION

Strawberry (*Fragaria* × *ananassa*) is one of the most widely consumed fruits worldwide for its special sensory properties (taste, color) and high nutritional and nutraceutical values. However, this typical non-climacteric fruit appears greatly perishable and susceptible to fungal pathogen infection, and even more so during postharvest storage (Feliziani and Romanazzi, 2016). Chemical fungicides are one of the cheapest and most common approaches to minimizing spoilage of harvested fruit. Considering the chemical methods in terms of the development of pathogen resistance, fungicide residues in fruit, phytotoxicity to other organisms, or environmental and public health problems, microorganisms-based biological control which is safer, naturally eco-friendly and renewable for fruit preservation are needed. Endophytic bacteria are ubiquitous microbes that can naturally colonize the internal tissues of host plants without causing any harmful effects. Like human gut microflora, endophytic bacteria exhibit intimate interactions with their hosts, and have been proven to offer protection against phytopathogens. Endophytic bacteria highlight their status as an interesting source of biological control agents for combating fungal pathogens (Droby and Wisniewski, 2018). Though lactic acid bacteria (LAB) are known for their biopreservative activity, they have not been generally studied as endophytic bacteria on plants. Filling this gap, the present study was undertaken to use an endophytic bacterium, *Lactobacillus paracasei* Ld3 which is antagonistic to spoilage fungi for the preservation of post-harvested strawberries. As a result, the effect on strawberry fruit quality was evaluated by monitoring the sensory, physical, microbiological, and chemical properties of fruits during storage at 4^{o}C for 10 days.

MATERIALS AND METHODS

Fruit source

Fresh strawberries (*Fragaria* × *ananassa*) cv. Tochiotome were obtained from a strawberry farm located in Moc Chau (Son La, Vietnam), taken to the laboratory in a temperature-controlled environment, and kept at 4^{o}C for experimental use. Samples were selected for the experiment on the basis of their uniform size, weight, color, and absence of physical or pathological damage.

Source of microorganisms

An endophytic bacterium, *L. paracasei* Ld3, was isolated from the healthy strawberry fruit on Man, Rogosa, and Sharpe (MRS) agar (Merck, Darmstadt, Germany) and showed antagonistic activity against *Penicilium singorense* Nd4, a pathogenic fungal strain associated with the strawberries' spoilage (Ngo *et al.,* 2023).

Preparation of bacterial inoculum

L. paracasei Ld3 was cultivated in 100 mL of MRS broth at 37°C for 48 hours. The cells were harvested by centrifugation for 15 minutes at 7,000 g at 4°C. Subsequently, the pellets were washed two times with a 0.85% NaCl solution and resuspended in 10 mL of the same solution to obtain a bacterial inoculum (about 8×10^5 CFU/mL).

Preservation procedure

Strawberries were washed with tap water, immersed in distilled water for one minute, and allowed to dry at 25^{o}C for 1 hour. Afterward, they were inoculated by a surface spraying of the bacterial inoculum under a slight agitation to allow a homogenous covering of the product. Subsequently, the strawberries were packaged in a sterile polyethylene bag (five fruits per bag) under ambient pressure and kept at 4°C for 15 storage days. Control samples were not inoculated with *L. paracasei* Ld3 sprayed with distilled water.

Sensory evaluation

The sensory analysis of the strawberry fruit was carried out following the method described by Bai và đồng tác giả (2003), with some modifications. The sensory quality was evaluated by color, taste, texture, flavor, and overall acceptability for all the samples after 0, 3, 5, 7, and 10 days of storage. Samples were randomly presented to ten non-trained panelists for sensory evaluations and were asked to score the differences between the samples where 0–2 represented extreme dislike; 3–5 fair; 6–8 good; and 9 excellent for color, taste, texture, flavor, and overall acceptability.

Physical quality evaluation

Strawberries were weighed using a digital balance (TE612 Satorius-Germany) on day zero of the experiment and at 4 intervals during the 10 days of storage. Total weight loss, expressed as the percentage loss of weight, was compared to the initial weight of the fruit. The decay percentage was calculated as the number of decayed fruit divided by the initial number of all packaged fruit multiplied by 100.

Microbiological quality evaluation

About 10 g of each package was randomly chosen, aseptically grounded, and suspended in 90 mL of sterile peptone saline solution (8.5 g NaCl/l + 1 g peptone/l). The suspension was blended with an Interscience BagMixer 400 S (Stomacher) for 2 minutes, and then a 10-fold serial dilution was made. The dilutions were plated onto Dichloran Rose Bengal Chloramphenicol (DRBC) agar plates, and these plates were incubated at 25^{o}C for 5 days for total yeast and mold count. To determine the viability of *L. paracasei* Ld3, dilutions were plated onto MRS agar plates (Merck, Darmstadt, Germany). These plates were then placed in an anaerobic workstation (Don Whitley Scientific VA500) and incubated at 37^{o}C for 48–72 h. Colonies on the plates were counted with a colony counter (Scan 300 Interscience-France). Results of microbial counts were expressed as log CFU per g of fresh strawberries.

Chemical quality evaluation

The juice of strawberries was extracted using a slow juicer (Tefal ZC600138-Ultra Juice). The pH of the juice was measured using a pH electrode (F51BW Horiba-Japan). The titratable acidity (TA) was determined by titration of 5 mL of juice with 0.1 N NaOH using phenolphthalein as the indicator, and the result was expressed as percent citric acid (Temiz and Ozdemir, 2021). The total soluble solids (TSS) of the juices was obtained with a digital refractometer (HI96802, Hanna-Romania).

The total phenolic content (TPC) of strawberries was measured by the Folin Ciacalteu method (Panico *et al.,* 2009). Briefly, 10 g of strawberries were randomly selected and suspended in 200 mL of 80% methanol with the aid of a blender for 10 minutes. The suspension was then shaken in a 40^{o}C water bath (WTB15 Memmert-Germany) for 2 hours and filtered. To determine TPC, 0.5 mL of the filtered sample was mixed with 2.5 mL of 10% Folin Ciacalteu reagent and 2 mL of 7.5% Na_2CO_3. The sample was vortexed and placed in a 50^{o}C water bath (WTB15 Memmert, Germany) for 5 minutes. Subsequently, the sample was taken from the water bath and kept in the dark until it reached room temperature for 10 minutes. The absorbance of the sample was measured at 765 nm using a spectrophotometer (Evolution 60 Thermo Fisher-USA). The results were given on a fresh weight basis as mg gallic acid equivalents (the concentration of gallic acid was established from a calibration curve) per 100 g (mg GAE/100 g).

Statistical analyses

All experiments were performed in triplicate and are reported as mean ± standard deviations. An analysis of variance ANOVA ($p < 0.05$) was used, and differences between means were determined by Tukey's test with a 95% confidence level. Statistical analysis was performed using Minitab Software (Version 17.1, USA).

RESULTS AND DISCUSSION

Effect of bacterial inoculation on the sensory properties of strawberry fruit

From the results of the sensory evaluation (Table 1, Fig. 1), it can be seen that the strawberry fruit inoculated with *L. paracasei* Ld3 resulted in higher sensory scores than the control for all quality factors tested during storage. Non-inoculated strawberry fruit had decayed due to overripening and fungal infection, exhibited inferior quality in terms of color, taste, texture, flavor, and overall acceptability, and became unacceptable after 10 days of storage.

Table 1. Effect of bacterial inoculation on the sensory properties of strawberry fruit during storage at 4°C

Parameters	Treatments	Storage (days)				
		0	3	5	7	10
Color	Inoculated fruit	8.6 ± 0.19^{Aa}	8.5 ± 0.12^{Ba}	8.2 ± 0.20^{Ca}	7.8 ± 0.28^{Da}	6.2 ± 0.13^{Ea}
	Control	8.9 ± 0.20^{Aa}	8.4 ± 0.10^{Ba}	7.2 ± 0.10^{Cb}	3.5 ± 0.57^{Db}	1.6 ± 0.12^{Eb}
Taste	Inoculated fruit	8.2 ± 0.15^{Ab}	8.1 ± 016^{Aa}	7.8 ± 0.48^{Ba}	7.5 ± 0.17^{Ca}	6.9 ± 0.32^{Da}
	Control	8.9 ± 0.16^{Aa}	8.3 ± 0.22^{Aa}	7.5 ± 0.35^{Bb}	3.6 ± 0.36^{Cb}	2.3 ± 0.39^{Db}
Texture	Inoculated fruit	8.6 ± 0.33^{Ab}	8.5 ± 0.31^{Aa}	8.3 ± 0.52^{Ba}	7.8 ± 0.26^{Ca}	6.1 ± 0.28^{Da}
	Control	8.8 ± 0.15^{Aa}	8.6 ± 0.14^{Aa}	7.7 ± 0.48^{Bb}	4.1 ± 0.42^{Cb}	2.1 ± 0.14^{Db}
Flavor	Inoculated fruit	8.5 ± 0.17^{Aa}	8.4 ± 0.16^{Aa}	8.3 ± 0.10^{Aa}	7.8 ± 0.36^{Ba}	7.0±0.10Ba
	Control	8.7 ± 0.26^{Aa}	8.5 ± 0.45^{Aa}	8.3 ± 0.52^{Aa}	4.0 ± 0.24^{Bb}	1.7 ± 0.24^{Cb}
Overall acceptability	Inoculated fruit	8.6 ± 0.16^{Aa}	8.5 ± 0.25^{Aa}	8.0 ± 0.47^{Ba}	7.4 ± 0.18^{Ca}	6.5 ± 0.39^{Da}
	Control	8.8 ± 0.13^{Aa}	8.4 ± 0.20^{Bb}	7.5 ± 0.35^{Cb}	4.5 ± 0.16^{Db}	1.9 ± 0.12^{Eb}

Different superscript lowercase letters (within each row) show differences in the storage time within the same treatment group ($p < 0.05$). Different superscript uppercase letters (within each column) indicate differences between treatment groups within the same storage time ($p < 0.05$).

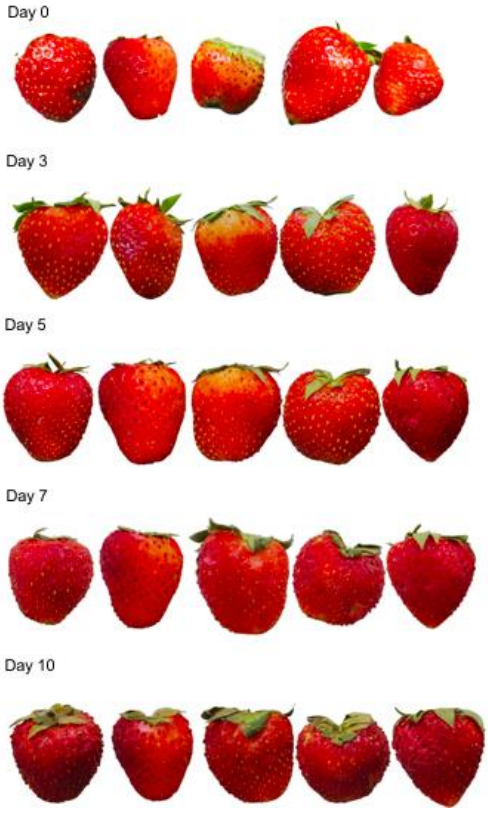

Fig.1. Photograph of inoculated strawberries during storage at 4°C

Effect of bacterial inoculation on the physical properties of strawberries

As it is shown in Table 2, the percentage of weight loss and decay tends to increase continuously during storage. Weight loss is linked to moisture evaporation and respiration on the fruit's surface (Aday and Caner, 2011), whereas the main cause of the decay of strawberries during storage is the development of rot that is caused by fungi (Feliziani and Romanazzi, 2016). On day 10, inoculation with *L. paracasei* Ld3 cells on the fruit reduced the water loss and decay by about 32.9% and 61.5%, respectively. Notably, the efficiency of water preservation and decay inhibition increased in the inoculated strawberries with increasing storage time. In fact, it must be considered that the ability of bacteria to stick effectively to the fruit surfaces for prolonged periods of time is crucial to ensuring their survival on the surface (Marques *et al.* 2002).

Table 2. Percentage of weight loss and decay of strawberries stored at 4°C for 10 days.

Parameters	Treatments	Storage (days)				
		1	3	5	7	10
Weight loss (%)	Inoculated fruit	0.9 ± 0.21^{Ea}	1.7 ± 0.45^{Da}	4.2 ± 0.69^{Cb}	6.6 ± 1.58^{Bb}	9.6 ± 0.51^{Ab}
	Control	1.0 ± 0.15^{Ea}	2.6 ± 0.54^{Da}	5.9 ± 0.72^{Ca}	8.9 ± 1.55^{Ba}	14.3 ± 1.60^{Aa}
Decay (%)	Inoculated fruit	0	6.5 ± 0.5^{Cb}	10.4 ± 0.6^{Bb}	15.6 ± 2.7^{Ab}	18.5 ± 1.8^{Ab}
	Control	0	10.0 ± 1.5^{Da}	27.1 ± 3.2^{Ca}	33.1 ± 4.6^{Ba}	48.0 ± 1.0^{Aa}

Different superscript lowercase letters (within each row) show differences in the storage time within the same treatment group ($p < 0.05$). Different superscript uppercase letters (within each column) indicate differences between treatment groups within the same storage time ($p < 0.05$).

Effect of bacterial inoculation on total yeast and mold counts of strawberry fruit

As it can be seen in Fig. 2, *L. paracasei* Ld3 sustained high levels on the fruit surface, and total yeast and mold counts increased during storage. However, total yeast and mold counts in the inoculated strawberries were found to be lower ($p < 0.05$) compared to the control. This could be related to the production of antimicrobial agents by LAB as well as the competence of LAB and other microorganisms for nutrients and space (Bernatek et al., 2022).

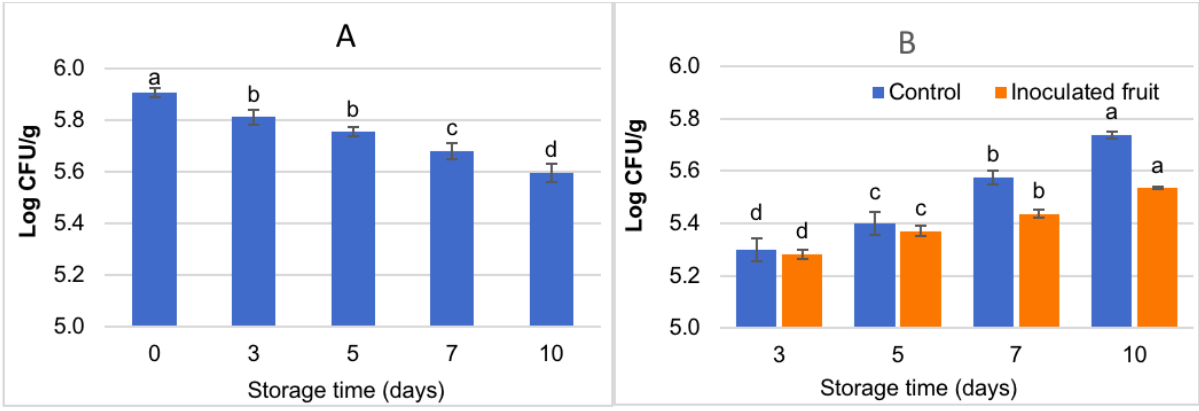

Fig. 2. *L. paracasei* Ld3 viability (A) and total yeast and mold counts (B) of strawberries stored at 4°C for 10 days.

Effect of bacterial inoculation on the chemical properties of strawberries

As indicated in Fig. 3, the pH of the strawberries increased significantly ($p < 0.05$) at the end of the storage. Meanwhile, a slight decreasing trend in TA was observed during storage. Similar to pH, there was no significant difference ($p > 0.05$) in TA between the inoculated strawberries and the control at the same storage time. It may be due to no proliferation of *L. paracasei* Ld3 and no production of acid when adhering to strawberry surfaces.

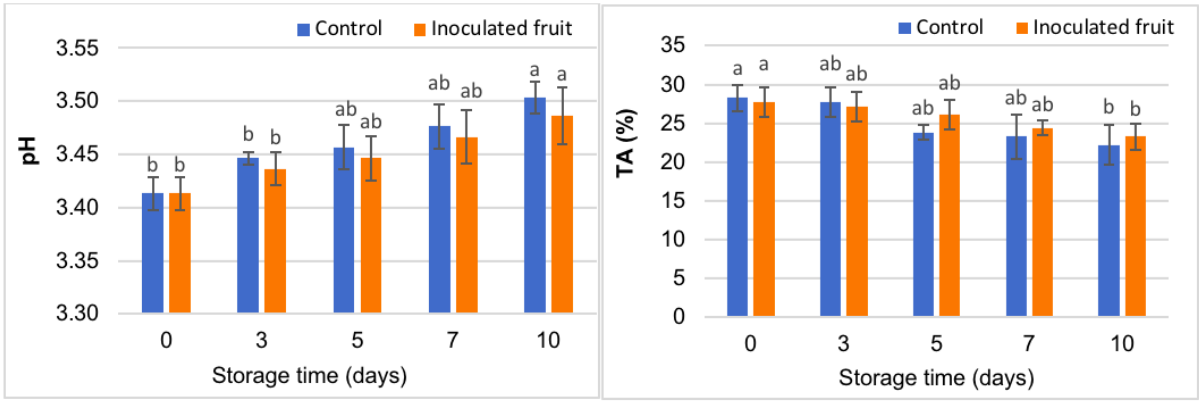

Fig. 3. Changes in pH value and TA (%) of strawberries stored at 4°C for 10 days.

TSS was detected to increase in both treatments during storage (Table 3). The increase in TSS may be shown as a significant amount of water loss by the increase in sugar content in fruit during storage (Hernández-Munoz *et al.,* 2008). However, the TSS of the inoculated strawberries was lower than that of the control. Conversely, the TPC of strawberries decreased progressively during storage (Table 3). Oxidation might be the main reason for TPC reduction (Ali *et al*., 2019). However, the strawberry inoculated with *L. paracasei* Ld3 cells showed a higher phenol content ($P < 0.05$) compared to the control. It can be due to the metabolic process of lactic bacteria. In fact, Avila et al. (2009) found that a lactic bacteria strain might be applied as functional cultures to obtain more bioactive phenolic acids in food products.

Table 3. Chemical quality of of strawberries stored at 4°C for 10 days

Parameters	Treatments	Storage (days)				
		0	3	5	7	10
TSS (%)	Inoculated fruit	$6.60±0.05^{Ea}$	$7.10±0.01^{Da}$	$7.24±0.06^{Ca}$	$7.43±0.02^{Ba}$	$7.62±0.01^{Aa}$
	Control	$6.62±0.07^{Ea}$	$6.81±0.02^{Db}$	$7.09±0.03^{Cb}$	$7.28±0.03^{Bb}$	$7.45±0.02^{Ab}$
TPC (mg GAE/100 g)	Inoculated fruit	$252±10.2^{Aa}$	$217.5±12.6^{Ba}$	$171.9±18.5C^{a}$	$168±.12.1^{Ca}$	$125±9.2^{Da}$
	Control	$258±20.5^{Aa}$	$210±18.7^{Ba}$	$162.5±15.6C^{a}$	$131.3±10.4^{Db}$	$112.5±18.6^{Db}$

Different superscript lowercase letters (within each row) show differences in the storage time within the same treatment group ($p < 0.05$). Different superscript uppercase letters (within each column) indicate differences between treatment groups within the same storage time ($p < 0.05$).

CONCLUSION

Inoculation with an endophytic bacterium, *L. paracasei* Ld3, on the surface of strawberry fruit improved the fruit sensory properties, reduced the total water loss and decay percentages, inhibited the yeast and mold proliferation, and slowed the deterioration rate of TSS and TPC. Therefore, it can be considered an alternative way to preserve post-harvested strawberry fruit.

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NGHIÊN CỨU SỬ DỤNG *Lactobacillus paracasei* Ld3 TRONG BẢO QUẢN SINH HỌC QUẢ DÂU TÂY SAU THU HOẠCH

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TÓM TẮT

Vi khuẩn lactic được biết đến với hoạt tính bảo quản sinh học. Trong nghiên cứu này, một vi khuẩn nội sinh, *Lactobacillus paracasei* Ld3, đã được sử dụng để lưu giữ chất lượng quả dâu tây sau thu hoạch. Các đặc tính cảm quan (màu sắc, mùi vị, kết cấu, hương vị và khả năng chấp nhận tổng thể), vật lý (tỉ lệ % hao hụt khối lượng và thối hỏng), vi sinh vật (mật độ *L. paracasei* Ld3 và tổng nấm men và nấm mốc) và hóa học (pH, tổng chất rắn hòa tan, độ acid chuẩn độ và hàm lượng phenolic tổng) của quả dâu tây được cấy tế bào *L. paracasei* Ld3 và mẫu đối chứng đã được đánh giá trong 10 ngày bảo quản ở 4^{o}C. Kết quả cho thấy quả dâu tây được cấy có điểm đánh giá cảm quan cao hơn so với mẫu đối chứng. Việc cấy *L. paracasei* Ld3 trên bề mặt quả, vào ngày thứ 10, đã làm giảm khoảng 32,9% sự mất nước và 61,5% sự thối hỏng quả. Ngoài ra, mật độ *L. paracasei* Ld3 được duy trì ở mức cao trên bề mặt quả, và sự phát triển của nấm men và nấm mốc bị ức chế ở quả dâu tây được cấy tế bào *L. paracasei* Ld3. Tuy nhiên, không có sự khác biệt có ý nghĩa ($p > 0,05$) về giá trị pH và độ acid chuẩn độ giữa quả dâu tây được cấy tế bào và mẫu đối chứng ở cùng thời gian bảo quản. Hơn nữa, việc cấy *L. paracasei* Ld3 đã làm chậm tốc độ biến đổi tổng chất rắn hòa tan và hàm lượng phenolic tổng so với đối chứng. Qua đó cho thấy tiềm năng của *L. paracasei* Ld3 trong bảo quản quả dâu tây sau thu hoạch.

Từ khóa: Kiểm soát sinh học, bảo quản sinh học, vi khuẩn nội sinh thực vật, vi khuẩn lactic, dâu tây.

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