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Effect of *Bacillus subtilis* applied on soursop fruits (*Annona muricata* L.) in pre-harvest and its effect on physico-chemical properties in post-harvest.

Estudio de *Bacillus subtilis* en frutos de guanábana (*Annona muricata* L.) en precosecha y su efecto en las propiedades fisicoquímicas en postcosecha.

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ABSTRACT

Soursop is a highly perishable climacteric fruit, which limits its commercialization, thus, strategies are being sought to extend its shelf life. With this focus, this research aimed to test the effect of a bacterial consortium of three *Bacillus subtilis* strains applied in pre-harvest to evaluate the fruit quality parameters in the post-harvest stage. Different stages of fruit development on the tree were selected for application (4, 8, and 12 weeks post-anthesis), in addition to fruit at physiological ripeness already harvested. The physicochemical parameters of firmness, titratable acidity, pH, and total soluble solids were evaluated according to AOAC protocols. The expression of the gene coding for the polygalacturonase enzyme was also assessed. The results revealed that the bacterial consortium positively affects shelf life when applied pre-harvest at 12 weeks of fruit development. This study shows that the selection of the stage of fruit development is fundamental to achieving positive effects through the *B. subtilis* application on the quality of fruits with short shelf life, such as soursop.

KEY WORDS: Annona muricata, bacterial consortium, pre-harvest, post-harvest, shelf life.

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RESUMEN

La guanábana es un fruto climatérico altamente perecedero, lo que limita su comercialización y es por ello, que se buscan estrategias para prolongar su vida de anaquel. Considerando esto, el objetivo de esta investigación fue probar el efecto de un consorcio bacteriano de tres cepas de *Bacillus subtilis* aplicado en precosecha para evaluar los parámetros de calidad de los frutos en etapa postcosecha. Se seleccionaron diferentes etapas de desarrollo del fruto en el árbol para la aplicación (4, 8 y 12 semanas post-antesis), además de los frutos en madurez fisiológica ya cosechados. Se evaluaron los parámetros fisicoquímicos de firmeza, acidez titulable, pH, sólidos solubles totales de acuerdo con los protocolos de la AOAC. También se evalúo la expresión del gen que codifica para la enzima poligalactuonasa. Los resultados revelaron que el consorcio bacteriano tiene un efecto positivo en prolongar la vida de anaquel cuando se aplica en precosecha a las 12 semanas de desarrollo del fruto. Este estudio revela que es fundamental la selección de la etapa del desarrollo del fruto para lograr efectos positivos mediante la aplicación *B. subtilis* sobre la calidad de frutos con corta vida de anaquel como la guanábana.

PALABRAS CLAVE: *Annona muricata*, consorcio bacteriano, precosecha, postcosecha, vida útil.

Introduction

The soursop cultivation (*Annona muricata* L.) originates from Mexico and Central America (Nugraha *et al.,* 2021). According to records from the Agri-Food and Fisheries Information Service (SIAP), in 2019, soursop production in Mexico reached 30,790 tons annually. The main producing states are: Nayarit, wit h a production of 23,230 tons; followed by Colima with 2,832 tons, and Michoacán with 2,781 tons. Nationally, it is cultivated on a surface area of 3,612 hectares, resulting in a production value of $248,170.00 (SIAP, 2019). Soursop fruits are highly valued for their edible pulp, which has a soft and fibrous texture with a sweet and sour flavor. They are rich in nutrients and bioactive compounds that may provide health benefits. Additionally, they have great potential for the development of many food products (Villarreal-Fuentes *et al.,* 2020).

The growth of soursop fruits follows a double sigmoid pattern and reaches maturity an average of 160 days after anthesis. The fruits are harvested at the point of physiological ripeness, which coincides with their maximum size, loss of firmness (on the surface and spines), and changes in the color and brightness of the epicarp (Worrell *et al.,* 1994). Once harvested, it is

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essential to establish and maintain proper storage conditions to preserve quality during the post-harvest period (Jiménez-Zurita *et al.,* 2017).

Fruit ripening is a complex process involving a series of genetically programmed events that trigger multiple biochemical and physiological processes, altering their firmness, color, flavor, and texture (Saini *et al.,* 2022). During the ripening of climacteric fruits, there is an ethylene production peak, which regulates the expression of genes encoding proteins that modify the cell wall, leading to softening. Ethylene is an essential hormone that coordinates the ripening processes and activates enzymes that degrade the cell wall, causing fruit softening. As a climacteric fruit, soursop exhibits a high respiration rate and ethylene production, resulting in an accelerated softening process that reduces its shelf life during the post-harvest period (Berumen-Varela *et al.,* 2019). The short post-harvest shelf life of soursop is one of the main limitations for export; therefore, maintaining quality and delaying fruit ripening is of great importance. Among the post-harvest soursop-handling practices, refrigeration at 12–15 °C, residual moisture removal, coatings application, and chemical compounds' use to reduce ethylene production are essential (Moreno-Hernández *et al.,* 2014).

The use of biotic resources, such as plant growth-promoting rhizobacteria (PGPR), has also been explored as an alternative to protect crops and prolong fruit quality during pre-harvest and post-harvest stages (de Andrade *et al.,* 2023; Saebi *et al.,* 2023). *B. subtilis* is one of the most studied PGPRs; this bacterium can promote plant growth and control phytopathogens through several mechanisms, including phosphorus solubilization in soil, biological nitrogen fixation, antimicrobial synthesis, production of the phytohormone indole-3-acetic acid (IAA), and reduction of ethylene levels in plants (Shahid *et al.,* 2023). Initially, *B. subtilis* was only associated with plant growth promotion (Blake *et al.,* 2021), but increasing evidence suggests that applying these bacteria throughout post-harvest can change fruits' physiological activity and overall health (Martínez-Jaime *et al.,* 2019). It is worth noting that most studies have focused on applying PGPR to the rhizosphere of trees or harvested fruits. In the case of soursop, few studies have investigated the application of PGPR, and among those reported, the focus has been on PGPR's abilities as a biological control agent against phytopathogens during post-harvest (Bautista-Rosales *et al.,* 2022; Guardado-Valdivia *et al.,* 2018).

Hitherto, no studies have reported the effects of applying PGPR to soursop fruits during pre-harvest as part of an integrated approach to preserving fruit quality after harvest. Therefore, this study aimed to use a consortium of three *B. subtilis* strains to soursop fruits during pre-harvest and assess their effects on the physicochemical characteristics of the fruits during post-harvest. Additionally, the study evaluated the effect on the PG gene expression, which encodes the enzyme polygalacturonase (PG), involved in firmness loss and textural changes in the fruits.

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Material and Methods

Bacterial strains and biological material

The soursop trees used in the study were situated in El Tonino, a locality in the Compostela municipality, Nayarit, Mexico. Twelve trees were selected, meeting the criteria of being free from pests and physical damage, having uniform height (between 6.5 and 7.5 m) with a conical shape, and being of the same age (20 years, as stated by the producer). The trees bore fruits in several development stages, which were harvested upon reaching physiological ripeness. This ripeness was evidenced by the change in the fruit's epicarp color from shiny dark green to matte light green, as well as the separation of the soft spines on the fruit surface as it ripened. Harvesting was carried out following the producer's experience and instructions. Once harvested, the fruits were transported to the Laboratorio de Agrobiotecnología y Electroquímica at the Instituto Tecnológico de Tepic. The PGPR consortium consisted of three *Bacillus subtilis* (CBs) strains, designated as Bs-05, Bs-16, and Bs-17, which are part of the microbial resource collection at the Microbiology Laboratory of the Universidad de Guanajuato, Mexico.

Preparation of the suspension with the bacterial consortium

Each bacterial strain was cultured in Luria-Bertani medium (Bertani, 1951) at 28 °C for 24 hours. Bacterial cells were harvested by centrifugation at 10,000 rpm for 10 minutes at 25 °C. A bacterial suspension was prepared by combining the three *B. subtilis* strains in equal proportions. The concentration of the bacterial suspension was adjusted to $1x10^7$ CFU/mL using sterile distilled water.

Pre-harvest fruit treatment

For the treatments, three trees from the previously described group of 12 were selected, and five fruits at the same developmental stage were chosen from each tree. For this study, the fruits were classified as follows: P1, fruits at 4 weeks of development after anthesis; P2, fruits at 8 weeks of development; and P3, fruits at 12 weeks of development. The pericarp of the fruits was sprayed with the bacterial consortium suspension. As a control, five fruits were sprayed with sterile water. Once the fruits reached physiological maturity (PM), they were harvested and stored at 25 ºC. On the sixth day of storage, samples were taken for analysis. Fruits treated with the CBs were identified as CBs-P1, CBs-P2, and CBs-P3. Figure 1 outlines the procedure for the treatments.

Post-harvest fruit treatment

For the post-harvest treatment, three trees were also selected from the initial group of 12, and five fruits in the PM stage were chosen from each tree. These fruits were identified as PMF. The fruits were harvested and subsequently treated with the CBs. It is noteworthy that these fruits had not been treated with the CBs during the pre-harvest phase. They were then stored at 25 ºC for 6 days. On the sixth day, samples were taken for analysis. Fruits treated in this post-harvest phase were identified as CBs-PMF (Figure 1).

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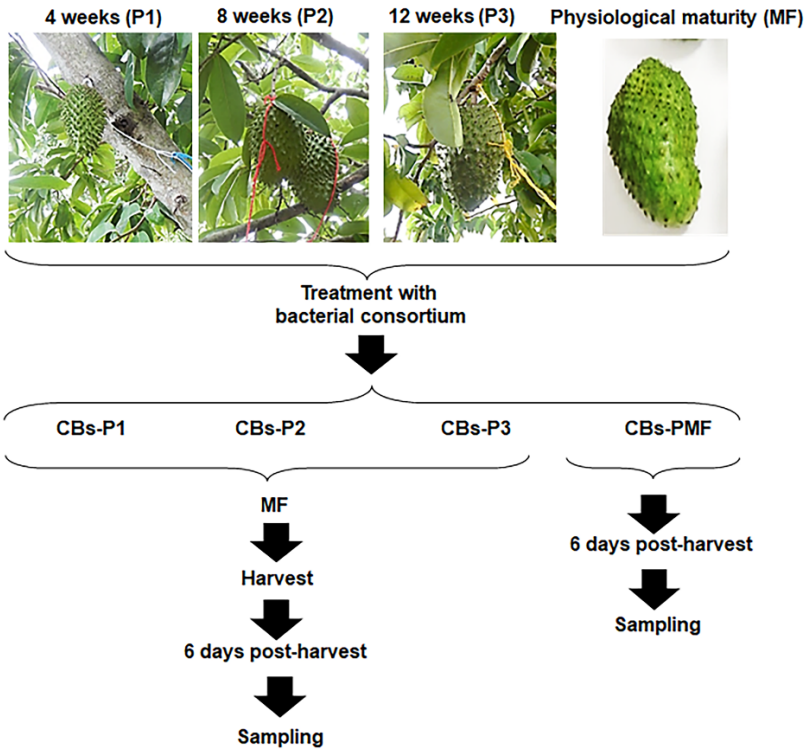

Figure 1. Schematic representation of the treatments applied to soursop fruits in different periods of development.

Source: Own elaboration based on this study.

Physiological weight loss

The physiological weight loss (PWL) evaluation was performed as previously reported (Freitas & Mitcham, 2013). The initial weight was recorded on the first day of harvest, and the final weight was recorded on the sixth day of post-harvest. A digital scale (Ohaus CT600®, USA) was used for this purpose. The results were expressed as the percentage of physiological weight loss (% *PWL*) according to Equation 1.

Equation 1

$$\% PWL = \frac{Initial\ weight - Final\ weight}{Final\ weight} X\ 100$$

Firmness analysis

Firmness was determined using a texture analyzer (StableMycro Systems®, UK) equipped with an 8 mm diameter stainless steel probe. The probe operated at a speed of 1 mm/s, and the penetration test was performed at two points on opposite sides of the fruit's midsection, as

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previously reported (Márquez-Cardozo *et al.,* 2012). A penetration distance of 20 mm was chosen. Firmness was recorded in Newtons (N). All physicochemical analyses related to fruit quality were conducted on the sixth-day post-harvest, with day 0 considered the harvest day.

Analysis of total soluble solids

The determination of total soluble solids (TSS) was carried out following previously reported methodology (AOAC, 1990). Evaluations were performed on the fruit pulp using a refractometer (Atago®, USA). Results are reported in °Brix.

Determination of titratable acidity and pH

The evaluation of titratable acidity, reported as % malic acid, was performed according to previously reported methodology (AOAC, 1990). Five grams of a homogenized sample in deionized water were used. The titration was conducted with a 0.1 N NaOH solution using phenolphthalein as an indicator. Results were calculated using Equation 2.

Equation 2

$$\%\ Malic\ acid = \frac{(mL\ of\ NaOH)(N\ of\ NaOH)(meq.\ Malic\ acid)}{Sample\ (g)}\ X\ 100$$

The pH of the fruit pulp was determined using a pH meter (Hanna Instruments®, USA) (AOAC, 1990).

Statistical analysis

A one-way ANOVA was performed, followed by Tukey's comparison with a significance level of $p \leq 0.05$. Data analysis was conducted using the Statistica software, Version 12.0 (StatSoft®, USA). Three repetitions were performed, using five fruits per repetition.

RNA extraction from soursop fruits

Total RNA was extracted following the methodology proposed by Brasil *et al.* (2008). For this, 600 mg of pulverized tissue was mixed with 1 mL of extraction buffer (0.5 % SDS, 25 mM EDTA, 250 mM NaCl, 145 mM β-mercaptoethanol, 250 mM Tris-HCl) and kept at room temperature for 1 minute. The mixture was then incubated at 60 °C for 15 minutes with constant agitation. Immediately after, it was transferred to an ice-water bath for 10 minutes. Then, 0.15 mL of cold 5 M potassium acetate was added, mixed, and centrifuged at 12,000 rpm for 20 minutes. The supernatant was collected, and 1 volume of isopropanol was added. The mixture was centrifuged again, and the pellet was recovered. It was resuspended in 100 µL of deionized water. To purify the RNA samples, the commercial Pure Link RNA Mini Kit (Ambion®, USA) was used, following the manufacturer's specifications. Subsequently, RNA quantification and quality assessment were performed. The RNA samples were subjected to electrophoresis in 2 % agarose gels. A gel documentation system (BioRad®, USA) was used to visualize the RNA samples in the gel. RNA quantification was conducted using a NanoDrop 2000 (Thermo Fisher Scientific®, USA).

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Gene expression analysis

The expression of the gene encoding the PG enzyme in the fruits treated with the bacterial consortium was analyzed. Samples for genetic expression analysis were taken 10 days after the application of the CBs. As a control, the expression of the PG gene was analyzed in untreated fruits. To amplify a fragment of the gene encoding PG from soursop, the primers PGf (5´-TGCTTGGAAAGATGCTTGTG-3´) and PGr (5´-GCAGTATGCCATCAACCTGA-3´) were designed, which amplify a 134 bp fragment. As an endogenous constitutive expression control, the primers ACTf (5´-ACGAGCTGTTTTCCCTAGCA-3´) and ACTr (5´-TCTTTTGGATTGAGCCTCGT-3´) were designed, which amplify a 593 bp fragment of the ACT gene, encoding actin.

Gene expression analysis was carried out using the Reverse Transcription-Polymerase Chain Reaction (RT-PCR) technique. The commercial SuperScript One-Step RT-PCR with Platinum Taq Kit (Invitrogen®, USA) was used, following the supplier's specifications. For the reactions, 170 ng of purified total RNA was used. Amplification reactions were conducted in a Select Cycler thermocycler (Select Bio Products®, USA) under the following amplification conditions: 50°C for 30 min; 94°C for 2 min; followed by 94°C for 15 s, 56°C for 30 s, 72°C for 1 min, for 35 cycles. Finally, 72°C for 10 min, 1 cycle. PCR products were subjected to electrophoresis for evaluation.

Semi-quantitative gene expression analysis

For the semi-quantitative gene expression analysis, the software ImageJ 1.54d was used to determine the integrated density values of the PCR amplicons from the electrophoresis gel image (Antiabong *et al.,* 2016). The measurements were configured and established for the analysis, and the integrated density was marked. The total pixel intensities (RawIntDen) for each image of the band corresponding to the PCR product were determined. RawIntDen is the sum of the pixel values of the images. The standard error of the mean was determined from three independent trials.

Results and Discussion

Physiological weight loss

Fruits undergo water loss in the form of vapor (transpiration) after harvesting. This is a determining factor in the physiological weight loss (PWL) of the fruit, which is related to the physiological processes of transpiration and respiration, affecting its consistency, quality, and commercial value (Lufu *et al.,* 2020). Figure 2A shows that the application of the CBs on the fruit surface, particularly those where the consortium was applied during the pre-harvest at 12 weeks of development (CBs-P3), significantly decreased PWL compared to the control and to fruits CBs-P1 and CBs-P2. The transpiration rate is closely related to the integrity of the cell wall

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structure (Rossi *et al.,* 2022). In this regard, the treatment with the bacterial consortium on the CBs-P3 fruits likely strengthened certain structures like the cuticle, preventing damage to the cell wall structure that occurs during the ripening process. Therefore, transpiration was reduced, and this allowed the tissue's turgidity to be maintained for a longer period. Regarding the CBs-PMF fruits, it was observed that they behaved similarly to the control, indicating that the treatment with the consortium did not prevent weight loss in the fruits (Figure 2A). These results show that the treatment with CBs did not prevent PWL when applied to already harvested fruits. In a study conducted by Viencz *et al.* (2023), *B. subtilis* was applied to bananas for controlling anthracnose during post-harvest, and quality parameters such as PWL and firmness were evaluated. They observed that the application of the biocontrol agent at this stage did not have a positive effect on quality, as there was an increase in PWL and a decrease in fruit firmness (Viencz *et al.,* 2023).

Fruit firmness

Firmness is a parameter connected to the cell wall structure of fruits and their ripeness stage. This parameter directly relies on the turgidity, cohesion, shape, and size of the cells that make up the cell wall. Loss of firmness in fruits presents multiple problems for their commercialization (Wang *et al.,* 2023). Therefore, maintaining fruit firmness is essential for preserving shelf life and consumer acceptability. In this study, the potential of CBs to maintain firmness in soursop fruits was evaluated. As a result, it was found that the treatment with CBs on P3 fruits achieved the highest firmness values, with an average of 27.82 ± 8.3 N, compared to the control fruits, which showed 2.83 N ± 1.5. This difference corresponds to 89.83 % more firmness in the fruits treated with CBs (Figure 2B). In the CBs-P1 and CBs-P2 fruits, firmness was also maintained more than in the control, reaching 11.01 N ± 5.1 and 11.95 N ± 5.5, respectively. This could be attributed to the action of the enzyme ACC deaminase present in the cytoplasm of some bacteria, including *B. subtilis* (Penrose and Glick, 2003). This enzyme acts on the ethylene precursor (ACC), leading to a decrease in the production of this hormone, which stimulates the ripening process and softening of climacteric fruits. It is interesting to note that the fruit development period is a determining factor in the effect of the bacterial consortium. The application on fruits with 12 weeks of development (CBs-P3) was the most favorable for maintaining firmness, which represents a longer shelf life and, therefore, more time for commercialization. These results were consistent with those obtained in the PFP evaluations on the P3 fruits. Recently, a study on the application of PGPR in cherry fruits during pre-harvest reported that PGPR-treated fruits had higher firmness values compared to control fruits (Küçüker *et al.,* 2023), similar to the observations in this study with soursop. The CBs-PMF fruits did not show a significant difference from the control, indicating that the bacterial treatment applied to the already harvested soursop fruits did not affect firmness (Figure 2B).

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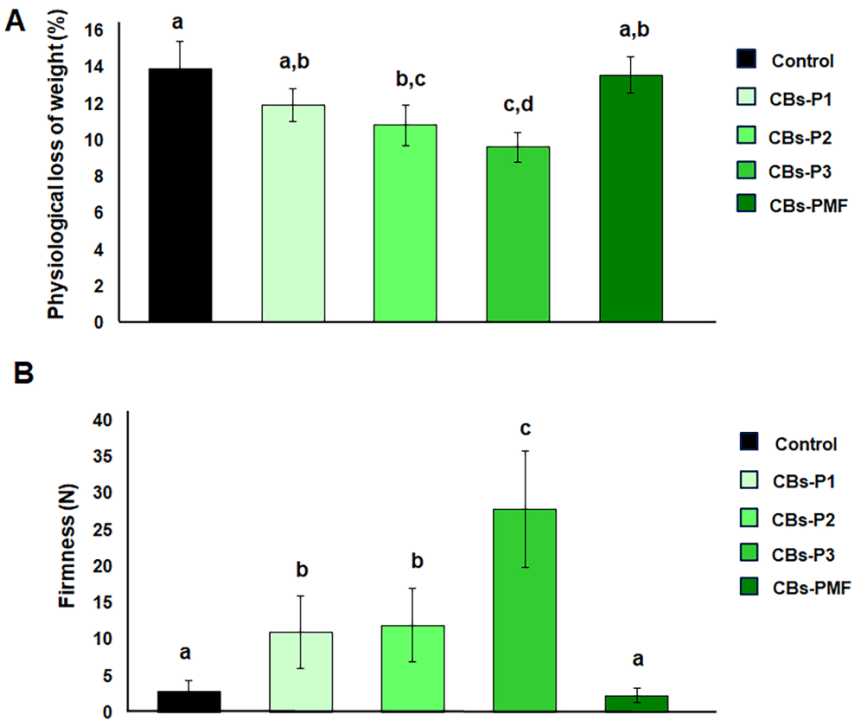

Figure 2. Effect of the *B. subtilis* consortium (CBs)), applied at different periods of soursop development, on the physiological weight loss (A) and fruit firmness (B) six days after harvesting.

Different letters indicate significant differences ($p < 0.05$).

Source: Own elaboration based on the results obtained. The lines on the bars correspond to the standard deviation.

Titratable acidity and pH

During ripening, the acidity of soursop increases due to an increase in the concentration of organic acids, mainly malic, citric, and ascorbic acids (Paull *et al.,* 1983). This is a particular characteristic of soursop fruit, which has a sweet-sour taste, as opposed to sweet fruits where titratable acidity decreases during maturation. Paul *et al.* (2003) reported that during the post-harvest maturation of soursop fruit, the concentration of malic acid can increase up to seven times, contributing to the balance between sweetness and acidity, which enhances flavor perception and the development of the fruit's optimal organoleptic profile. Acidity is commonly expressed in terms of the predominant organic acid, which in soursop is malic acid (Terán-Erazo *et al.,* 2019). Figure 3A shows the results obtained for titratable acidity. Significantly lower values of % malic acid were observed in the CBs-P3 fruits, where a mean of 0.55 ± 0.05 % was recorded, compared

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to the control fruits, which showed a mean of 0.79 ± 0.07 %. Considering that titratable acidity tends to increase during ripening, it is suggested that the CBs treatment modified the starch and carbohydrate catabolism during the maturation process, resulting in a reduction in the malic acid concentration. In this regard, the *Bacillus subtilis* consortium slowed down the maturation process in the soursop fruits CBs-P3 and CBs-P2. The CBs-P1 and CBs-PMF fruits did not show a statistically significant difference compared to the control. This indicates that the effect of the *B. subtilis* consortium on malic acid production in soursop is not achieved when the fruits are in the early development stages, nor when they have already been harvested (Figure 3A).

Obtained data reveal that it is essential to select the appropriate stage of fruit development for PGPR application, as the effects on the physicochemical characteristics differ depending on whether the application is made in the pre-harvest or post-harvest stages. Furthermore, it is important to assess the effect that PGPR has on each fruit in particular, as the response will depend on the specific metabolic interactions between the microorganism and the plant species. For example, in strawberries, PGPR application to the root led to an increase in titratable acidity in the fruit, acting as a ripening enhancer (Nam *et al.,* 2023). In blueberry*, B. subtilis* strains applied to harvested fruits resulted in increased titratable acidity (Martínez-Jaime *et al.,* 2019). In contrast, in cherry, PGPR application to fruits during pre-harvest decreased titratable acidity, suggesting a delay in fruit ripening (Küçüker *et al.,* 2023). Considering this background, it can be advised that the effect of *B. subtilis* depends on factors such as the fruit type and its physiology, the metabolic interactions that may occur between the bacteria and the fruit, as well as the environmental conditions in which the fruit developed and the post-harvest handling. In this study, to achieve an extension of shelf life mediated by PGPR application in soursop, it was more effective to apply the treatment during pre-harvest at 12 weeks of fruit development post-anthesis.

As for pH, it has been determined in soursop that it tends to decrease as the ripening process advances, which is related to the increase in organic acid synthesis (Espinoza *et al.,* 2013). In the CBs-P3 fruits, this decrease in pH was not observed compared to the control (Figure 3B), in line with the results observed for titratable acidity. It is important to highlight that, similar to the firmness and PFP evaluations, the differential effect on titratable acidity and pH was observed in the fruits with 12 weeks of development on the tree to which the CBs were applied. According to the values of these parameters, the post-harvest maturation process was attenuated.

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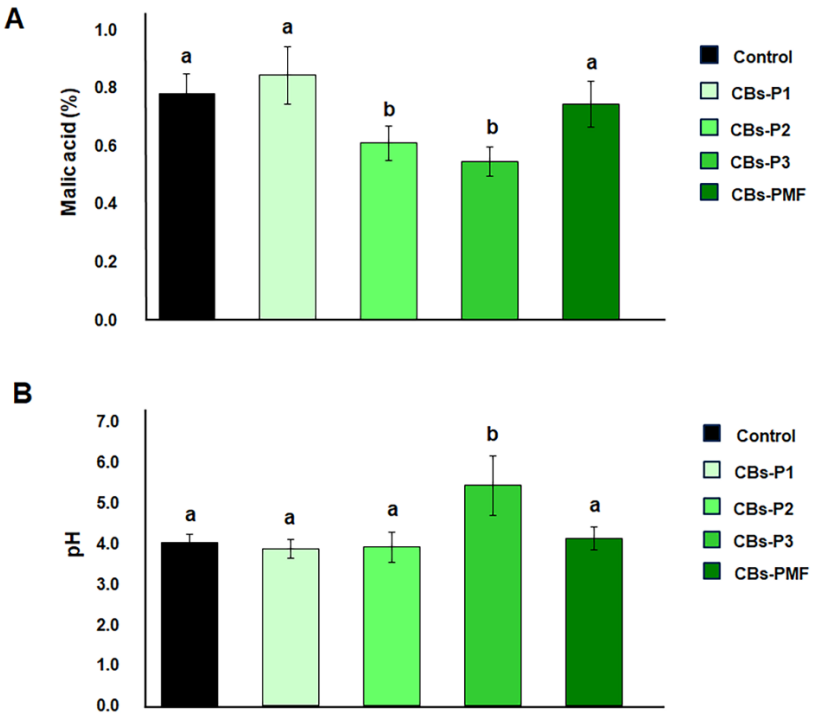

Figure 3. Effect of the *B. subtilis* consortium (CBs) on the content of malic acid (A) and the pH (B) of soursop fruits at different development periods.

Different letters indicate significant differences ($p < 0.05$).

Source: Own elaboration based on the results obtained. The lines on the bars correspond to the standard deviation.

Total soluble solids

As the ripening process progresses, the enzymes α and β amylases hydrolyze starch into simpler carbohydrates such as mono- and disaccharides, primarily sucrose, glucose, and fructose (Márquez-Cardozo *et al*., 2012). This parameter of total soluble solids (TSS), like firmness, PWL, titratable acidity, and pH, is indicative of the fruit's maturation stage. In this study, the control fruits had a TSS of 16.39 ± 2.5 °Brix (Figure 4), which is consistent with previous studies on soursop, reporting a TSS content between 7.1 and 20.1 °Brix (Jiménez-Zurita *et al*., 2016). When analyzing the effect of the bacterial treatment on the CBs-P3 fruits, it was observed that the TSS values were significantly lower compared to the control group and the other study groups. These results indicate that the application of the *B. subtilis* consortium modifies the carbohydrate metabolism in the fruit. There might be a lower expression of genes encoding enzymes involved in the hydrolysis of carbohydrates such as starch and pectin. However, further studies are needed to detail these

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metabolic responses. In the CBs-P1, CBs-P2, and CBs-PMF fruits, no significant changes were observed in the TSS content compared to the control, again supporting the notion that the stage of fruit development is an important factor when applying the bacterial consortium. Based on the results from the analyses of TSS, PWL, firmness, titratable acidity, and pH, the application of the bacterial consortium to fruits at 12 weeks of development, post-anthesis, appears to be the most suitable for extending the shelf life of soursop.

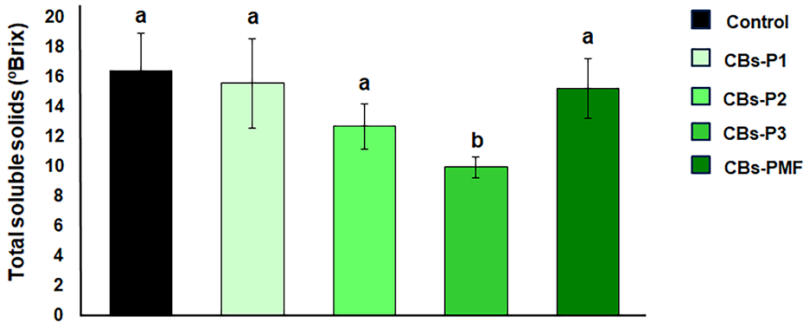

Figure 4. Effect of the *B. subtilis* consortium (CBs) on the total soluble solids content of soursop fruits at different development periods.

Different letters indicate significant differences ($p < 0.05$).

Source: Own elaboration based on the results obtained. The lines on the bars correspond to the standard deviation.

Expression of the gene encoding PG in soursop

During fruit ripening, tissue softening occurs due to the breakdown of interactions among the polymers that constitute the cell wall. The loss of fruit firmness is associated with the degradation of pectin, cellulose, and hemicellulose, which are key components of the cell wall. The metabolism of these carbohydrates involves enzymes such as polygalacturonase (PG), β-galactosidase, endo-1,4-β-glucanase, pectin methylesterase, and α-arabinosidase, among others. In particular, PG activity is highly correlated with the firmness of pericarp tissue in fruits (Ayour *et al.,* 2024). The PG enzyme plays a critical role in this process by hydrolyzing α(1-4) linkages in galacturonan chains, which links PG activity with fruit softening as ripening progresses (Jiang *et al.,* 2019).

In this study, we observed that the application of CBs on soursop fruits affected the expression of the gene encoding PG (Figure 5A). Contrary to expectations, the bacterial treatment significantly reduced the gene transcription in all treated fruits compared to the control (Figure 5B). It has been reported that the *B. subtilis* application in the rhizosphere of tomatoes decreases PG enzyme activity in fruits (Mena-Violante *et al.,* 2009). It is suggested that *B. subtilis* can reduce ethylene levels in plants through the action of the enzyme ACC deaminase, which

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deaminates 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene, diverting ACC away from the ethylene biosynthetic pathway (Misra & Chauhan, 2020). The ripening process in climacteric fruits is ethylene-dependent, as this plant hormone regulates the expression of numerous genes, including the PG gene, which is transcriptionally activated in the presence of ethylene (Bennett & Labavitch, 2008). Consequently, ethylene indirectly influences the physicochemical parameters of fruits (Monsalve *et al.,* 2022). Based on this, it is proposed that the *B. subtilis* consortium, via ACC deaminase synthesis, may have negatively affected ethylene biosynthesis in soursop fruits. However, this effect was more evident in fruits with a development period of 12 weeks post-anthesis. Additionally, other bacterial mechanisms potentially influencing the delayed ripening of soursop fruits cannot be ruled out.

To date, there are no reports on the effects of *B. subtilis* application during the precocious phase on the transcription of the gene encoding PG. Therefore, this study represents the first of its kind, flagging the way for further research on the effects of bacterial consortia applied to fruits during the precocious stage and their impact on the quality of these fruits during the post-harvest phase.

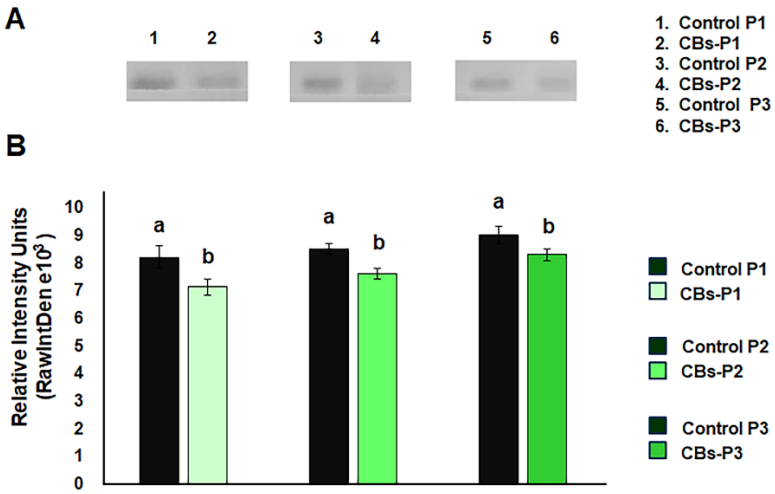

Figure 5. Effect of the *B. subtilis* consortium (CBs) on the transcription of the gene that codes for the enzyme polygalacturonase (PG).

(A) Amplicons subjected to agarose gel electrophoresis. (B) Integrated density values of each image obtained from the amplicons in the electrophoresis gel were estimated using ImageJ software. Relative transcript accumulation was determined by calculating the percentage difference in normalized band intensities. Different letters indicate significant differences ($p < 0.05$).

Source: Own elaboration based on the results obtained. The lines on the bars correspond to the standard deviation.

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Conclusions

Obtained data indicated that strains of *B. subtilis* applied to soursop fruits during the pre-harvest stage, particularly in fruits with a development period of 12 weeks on the tree, effectively delayed the ripening process during post-harvest. The application of *B. subtilis* as a plant growth-promoting rhizobacterium has been widely documented. Furthermore, its abilities in the biological control of phytopathogens during pre-harvest and post-harvest stages have been demonstrated. Regarding the effects of *B. subtilis* on the physicochemical properties of fruits, few studies support the benefits of applying these PGPRs during this stage. Therefore, this study contributes to the development of integrated strategies aimed at improving fruit quality across different developmental stages during pre-harvest and post-harvest.

Author contributions

Methodology development: SAA and JFAS; experimental validation: MRAJ and MACL; results analysis: SAA, EMG, RIOB, and MACL; manuscript preparation, review, and editing: SAA and MACL; project administration and funding acquisition: MACL.

“All authors of this manuscript have read and approved the published version.”

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Conflict of Interest

“The authors declare no conflict of interest.”

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References

Antiabong, J. F., Ngoepe, M. G., & Abechi, A. S. (2016). Semiquantitative digital analysis of polymerase chain reaction electrophoresis gel: potential applications in low-income veterinary laboratories. *Veterinary World,* 9, 935-939. https://doi.org/10.14202/vetworld.2016.935-939

Association of Official Analytical Chemists [AOAC]. (1990). Official methods of analysis of the Association of Official Analytical Chemists.15th ed. Association of Official Analytical Chemists, Washington DC, USA., 2, 685-1298.

Ayour, J., Harrak, H., & Benichou, M. (2024). Cell wall enzymatic activity control: a reliable technique in the fruit ripening process. *IntechOpen,* 1, 1-10. https://doi.org/10.5772/intechopen.113752

Bautista-Rosales, P. U., Ochoa-Jimenez, V. A., Casas-Junco, P. P., Balois-Morales, R., Rubio-Melgarejo, A., Diaz-Jasso, A. E., & Berumen-Varela, G. (2022). *Bacillus mojavensis* enhances the antioxidant defense mechanism of soursop (*Annona muricata* L.) fruits during postharvest storage. *Archives of Microbiology,* 204(9), 578-588. https://doi.org/10.1007/s00203-022-03199-9

Bennett, A. B., & Labavitch, J. M. (2008). Ethylene and ripening-regulated expression and function of fruit cell wall modifying proteins. *Plant Science,* 175(1), 130-136. https://doi.org/10.1016/j.plantsci.2008.03.004

Bertani, G. (1951). A method for detection of mutations, using streptomycin dependence in *Escherichia coli*. *Genetics,* 36(6), 598-611. https://doi.org/10.1093/genetics/36.6.598

Berumen-Varela, G., Hernández-Oñate, M. A., & Tiznado-Hernández, M. E. (2019). Utilization of biotechnological tools in soursop (*Annona muricata* L.). *Scientia Horticulturae,* 245, 269-273. https://doi.org/10.1016/j.scienta.2018.10.028

Blake, C., Christensen, M. N., & Kovacs, A. T. (2021). Molecular aspects of plant growth promotion and protection by *Bacillus subtilis*. *Molecular Plant Microbe Interactions,* 34(1), 15-25. https://doi.org/10.1094/MPMI-08-20-0225-CR

Brasil, I. M., Otoch, M. d. L. O., Costa, J. H., Maia, G. A., Lima, M. d. G. S., Arnholdt-Schmitt, B., & de Melo, D. F. (2008). Isolation of total RNA from ripe and unripe soursop (*Annona muricata* L.) fruit. *African Journal of Plant Science,* 2(9), 094-098. http://hdl.handle.net/10174/2627

de Andrade, L. A., Santos, C. H. B., Frezarin, E. T., Sales, L. R., & Rigobelo, E. C. (2023). Plant growth-promoting rhizobacteria for sustainable agricultural production. *Microorganisms,* 11(4), 1088. https://doi.org/10.3390/microorganisms11041088

Espinoza, I., Ortiz-Basurto, R. I., Tovar, B., Mata, M., & Montalvo, E. (2013). Physiological and physicochemical behavior of soursop fruits refrigerated with 1-methylcyclopropene. *Journal of Food Quality,* 36, 10-20. https://doi.org/10.1111/jfq.12011

Freitas, S. T., & Mitcham, E. J. (2013). Quality of pitaya fruit (*Hylocereus undatus*) as influenced by storage temperature and packaging. *Scientia Agricola,* 70, 257-262. https://doi.org/10.1590/S0103-90162013000400006

Guardado-Valdivia, L., Tovar-Perez, E., Chacon-Lopez, A., Lopez-Garcia, U., Gutierrez-Martinez, P., Stoll, A., & Aguilera, S. (2018). Identification and characterization of a new *Bacillus atrophaeus* strain B5 as biocontrol agent of postharvest anthracnose disease in soursop (*Annona muricata*) and avocado (*Persea americana*). *Microbiological Research,* 210, 26-32.

Revista Bio ciencias
ISSN 2007-3380

https://doi.org/10.1016/j.micres.2018.01.007

Jiang, F., Lopez, A., Jeon, S., de Freitas, S. T., Yu, Q., Wu, Z., Labavitch, J. M., Tian, S., Powell, A. L. T., & Mitcham, E. (2019). Disassembly of the fruit cell wall by the ripening-associated polygalacturonase and expansin influences tomato cracking. *Horticulture Research,* 6, 17-27. https://doi.org/10.1038/s41438-018-0105-3

Jiménez-Zurita, J. O., Balois-Morales, R., Alia-Tejacal, I., Juárez-López, P., Jiménez-Ruíz, E. I., Sumaya-Martínez, M. T., & Bello-Lara, J. E. (2017). Tópicos del manejo poscosecha del fruto de guanábana (*Annona Muricata* L.). *Revista Mexicana De Ciencias Agrícolas,* 8(5), 1155-1167. https://doi.org/10.29312/remexca.v8i5.115

Jiménez-Zurita, J. O., Balois-Morales, R., Alia-Tejacal, I., Juárez-López, P., Sumaya-Martínez, M. T., & Bello-Lara, J. E. (2016). Caracterización de frutos de guanabana (*Annona muricata* L.) en Tepic, Nayarit, México. *Revista Mexicana De Ciencias Agrícolas,* 7(6), 1261-1270.

Küçüker, E., Celik, K., Kızgin Özcengiz, C., Ogurlu, F., & Aglar, E. (2023). Pre-harvest application of aminoethoxyvinylglycine, salicylic acid and plant growth promoting rhizobacteria on fruit quality of 'sweetheart' sweet cherry. *Turkish Journal of Agriculture - Food Science and Technology,* 11(4), 871-875. https://doi.org/10.24925/turjaf.v11i4.871-875.5860

Lufu, R., Ambaw, A., & Opara, U. L. (2020). Water loss of fresh fruit: Influencing pre-harvest, harvest and postharvest factors. *Scientia Horticulturae,* 272, 109519-109529. https://doi.org/10.1016/j.scienta.2020.109519

Márquez-Cardozo, C. J., Villacorta Lozano, V., Yepes Betancur, D. P., Velásquez, C., José, H., & Cartagena Valenzuela, J. R. (2012). Physiological and physico-chemical characterization of the soursop fruit (*Annona muricata* L. cv. Elita). *Revista Facultad Nacional de Agronomía,* 65(1), 6477-6486. https://www.redalyc.org/articulo.oa?id=179924340018

Martínez-Jaime, O. A., Abraham-Juárez, M. R., Saldaña-Robles, A., Cervantes-Díaz, E., Ozuna-López, C., & Pérez-Becerra, L. (2019). Efecto de la aplicación de *Bacillus subtilis* sobre propiedades Físico-Químicas de frutos de arándano (*Vaccinium corymbosum*) almacenados en refrigeración. *Investigación y Desarrollo en Ciencia y Tecnología de Alimentos,* 4, 399-403.

Mena-Violante, H. G., Cruz-Hernández, A., Paredes-López, O., Gómez-Lim, M. A., & Olalde-Portugal, V. (2009). Fruit texture related changes and enhanced shelf–life through tomato root inoculation with *Bacillus subtilis* BEB–13BS. *Agrociencia,* 43(6). https://www.redalyc.org/articulo.oa?id=30215549001

Misra, S., & Chauhan, P. S. (2020). ACC deaminase-producing rhizosphere competent *Bacillus* spp. mitigate salt stress and promote *Zea mays* growth by modulating ethylene metabolism. *3 Biotech,* 10(3), 119. https://doi.org/10.1007/s13205-020-2104-y

Monsalve, L., Bernales, M., Ayala-Raso, A., Álvarez, F., Valdenegro, M., Alvaro, J. E., Figueroa, C. R., Defilippi, B. G., & Fuentes, L. (2022). Relationship between endogenous ethylene production and firmness during the ripening and cold storage of raspberry (*Rubus idaeus* 'heritage') fruit. *Horticulturae,* 8(3), 262. https://doi.org/10.3390/horticulturae8030262

Moreno-Hernández, C. L., Sáyago-Ayerdi, S. G., García-Galindo, H. S., Mata-Montes De Oca, M., & Montalvo-Gonzalez, E. (2014). Effect of the application of 1-methylcyclopropene and wax emulsions on proximate analysis and some antioxidants of soursop (*Annona muricata* L.). *The Scientific World Journal,* 2014, 896853. https://doi.org/10.1155/2014/896853

Nam, J. H., Thibodeau, A., Qian, Y. L., Qian, M. C., & Park, S. H. (2023). Multidisciplinary

evaluation of plant growth promoting rhizobacteria on soil microbiome and strawberry quality. *AMB Express,* 13(1), 18. https://doi.org/10.1186/s13568-023-01524-z

Nugraha, A. S., Haritakun, R., Lambert, J. M., Dillon, C. T., & Keller, P. A. (2021). Alkaloids from the root of Indonesian *Annona muricata* L. *Natural Product Research,* 35(3), 481-489. https://doi.org/10.1080/14786419.2019.1638380

Paull, R. E., Deputy, J., & Chen, N. J. (1983). Changes in organic acids, sugars, and headspace volatiles during fruit ripening of soursop (*Annona muricata* L.). *Journal of the American Society for Horticultural Science,* 108, 931-934. https://doi.org/10.21273/JASHS.108.6.931

Penrose, D. M., & Glick, B. R. 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiologia plantarum*, 118(1), 10-15. https://doi.org/10.1034/j.1399-3054.2003.00086.x

Rossi, F., Manfrini, L., Venturi, M., Grappadelli, L. C., & Morandi, B. (2022). Fruit transpiration drives interspecific variability in fruit growth strategies. *Horticultural Research,* 9, uhac036. https://doi.org/10.1093/hr/uhac036

Saebi, M. R., Moradinezhad, F., & Ansarifar, E. (2023). Quality preservation and decay reduction of minimally processed seedless barberry fruit via postharvest ultrasonic treatment. *Food Science & Nutrition,* 11(12), 7816-7825. https://doi.org/10.1002/fsn3.3698

Saini, M. K., Capalash, N., Varghese, E., Kaur, C., & Singh, S. P. (2022). A Targeted metabolomics approach to study secondary metabolites and antioxidant activity in 'Kinnow Mandarin' during advanced fruit maturity. *Foods,* 11(10), 1410. https://doi.org/10.3390/foods11101410

Shahid, M., Singh, U. B., Khan, M. S., Singh, P., Kumar, R., Singh, R. N., Kumar, A., & Singh, H. V. (2023). Bacterial ACC deaminase: Insights into enzymology, biochemistry, genetics, and potential role in amelioration of environmental stress in crop plants. *Frontiers in Microbiology,* 14, 1132770. https://doi.org/10.3389/fmicb.2023.1132770

Servicio de Información Agroalimentaria y Pesquera [SIAP]. (2019). Cierre de la producción agrícola (1980-2021). Anuario Estadístico de la Producción Agrícola, por cultivo y variedad. https://www.gob.mx/siap/acciones-y-programas/produccion-agricola-33119.

Terán-Erazo, B., Alia-Tejacal, I., Balois-Morales, R., Juárez-Lopez, P., López-Guzmán, G. G., Pérez-Arias, G. A., & Núñez-Colín, C. A. (2019). Caracterización física, química y morfologíca de frutos de guanábana (*Annona muricata* L.). *Agrociencia,* 53, 1013-1027.

Viencz, T., Malmann-Medilha, L. C. B., Marek, J., Duarte-Rios Faria, C. M., & Vasconcelos-Botelho, R. (2023). *Bacillus subtilis* and *Trichoderma harzianum* on the quality and occurrence of anthracnose in bananas. *Communicata Scientiae,* 14, e3701. https://doi.org/10.14295/cs.v14.3701

Villarreal-Fuentes, J. M., Alia-Tejacal, I., Hernández-Salvador, M. A., Hernández-Ortiz, E., Marroquín-Agreda, F. J., Núñez-Colín, C. A., & E., C.-R. (2020). *In situ* characterization of soursop (*Annona muricata* L.) in the Soconusco region, Chiapas, Mexico. *Revista Chapingo Serie Horticultura,* 26(3), 189-205. https://doi.org/10.5154/r.rchsh.2020.05.008

Wang, D., Ding, C., Feng, Z., Ji, S., & Cui, D. (2023). Recent advances in portable devices for fruit firmness assessment. *Critical Reviews in Food Science and Nutrition,* 63(8), 1143-1154. https://doi.org/10.1080/10408398.2021.1960477

Worrell, D. B., Carrington, C. M. S., & Huber, D. J. (1994). Growth, maturation and ripening of soursop (*Annona muricata* L.) fruit. *Scientia Horticulturae,* 57, 7-15. https://doi.org/10.1016/0304-4238(94)90030-2