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Utilization of an exudate from a *Bacillus licheniformis* strain to control *Alternaria alternata* rot on tomato fruits

Utilización de un exudado de una cepa de *Bacillus licheniformis* para el control de la pudrición de frutos de tomate por *Alternaria alternata*

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Abstract

Fungi decay is the most crucial factor leading to postharvest fruit losses. The objective was to test the effect of a bacterium exudate over the growth of *Alternaria alternata* infecting tomato fruit. The biochemical tests catalase, oxidase, motility-indole-ornithine, triple sugar iron, nitrification test, growth at 45°C and 60°C, Gram staining, and sequencing of 16S ribosomal gene, were performed in the bacterium. Tomato fruit in the red ripe stage was either untreated, infected with spores of *A. alternata* (IN), infected and treated with the exudate of bacterium growing in either Luria-Bertani broth (LB+BE+IN) or potato dextrose broth (PDB+BE+IN). The damage index (DI) was measured for 23 days at 25 °C in the tomatoes. The bacterium was identified as a *Bacillus licheniformis* strain and found to

Resumen

La pudrición fúngica es el factor más importante causante de pérdidas de frutas durante la postcosecha. El objetivo fue evaluar el efecto de un exudado bacteriano en el crecimiento de *Alternaria alternata* infectando el fruto de tomate. Las pruebas bioquímicas de catalasa, oxidasa, motilidad-indol-ornitina, hierro y triple azúcar, prueba de nitrificación, crecimiento a 45°C y 60°C, tinción de Gram y secuenciación del gene ribosomal 16S fueron llevadas a cabo en la bacteria. Frutos de tomate en el estado de verde maduro fueron no tratados, infectados con esporas de *A. alternata* (IN), infectados y tratados con el exudado de la bacteria multiplicada ya sea en caldo Luria-Bertani (LB+BE+IN) o en caldo papa dextrosa (PDB+BE+IN). El índice de daño (DI) fue evaluado durante 23 días a 25 °C en

excrete an active chitinase isoform. Potent inhibition of the fungi growth *in vitro* was recorded, and DI was lower in the LB+BE+IN treatment compared to the IN treatment. We concluded that the exudate can control the growth of *A. alternata* infecting tomato fruit without adversely affecting fruit quality.

Keywords: *Bacillus licheniformis*, chitinase, *Alternaria alternata*, tomato fruit, postharvest fruit quality.

los tomates. La bacteria fue identificada como una cepa de *Bacillus licheni*formis que excreta una isoforma activa de quitinasa. Se encontró una inhibición muy potente del crecimiento del hongo *in vitro* y un valor de DI más bajo en el tratamiento PDB+BE+IN comparado con el tratamiento IN. Se concluyó que el exudado puede controlar el crecimiento de *A. alternata* infectando frutos de tomate sin afectar negativamente la calidad del fruto.

Palabras clave: *Bacillus licheniformis*, quitinasa, *Alternaria alternata*, fruto de tomate, calidad postcosecha del fruto.

Introduction

Fruits and vegetables are the primary sources of vitamins, minerals and fiber. Out of the worldwide total food consumption, fruits constitute approximately 7.5%, whereas vegetables plus tubercules account for about 12%. These products are of great importance in the Mexican diet due to the variety of the available products and their importance in the traditional food intake. Furthermore, frequent consumption of fruits and vegetables is associated with a reduced risk of cancer, heart disease, hypertension, and stroke (Abanda-Nkpwatt *et al*., 2006). This positive effect on human health has been related to phytochemicals and antioxidants such as carotenoids, flavonoids, and anthocyanins (Lako *et al*., 2007). Unfortunately, fruits and fresh vegetables are highly perishables due to their inherent susceptibility to loss of quality due to normal physiological changes, mechanical injury, and the invasion of pests and diseases, among other factors (FAO, 2002). There are several causes leading to postharvest fruit losses besides fruit senescence, including pre-harvest factors (Alcaraz-Lopez *et al*., 2003); packaging systems (Sivakumar & Korsten, 2006), unadequate practices of handling (Kadzere *et al*., 2006), unoptimal levels of oxygen and/or carbon dioxide inside

the storage atmosphere (Kader & Ben-Yehoshua, 2000; Malakou & Nanos, 2005), bacterial infections (Richards & Beuchaf, 2005), and fungi infections (Prusky *et al*., 2006). Out of the causes mentioned above that lead to postharvest losses, frequently it does occur the development of some postharvest rots resulting from pre-harvest latent infections, especially in tropical and subtropical regions, whose environmental conditions in the field are particularly conducive to fruit infection. Moreover, controlling rots resulting from pre-harvest latent infections with postharvest treatments is difficult due to the lack of a universally applied method to measure losses and the lack of existing protocols to distinguish between qualitative, quantitative, and nutritional losses, no solid information can be found regarding the precise amount and nature of the losses. Therefore, estimates of total postharvest fruit losses range widely, from about 20% to 51% in developing countries (Kitinoja & Kader, 2015).

Chemical fungicides provide the primary means for controlling postharvest fungal decay, which is the most important cause of postharvest losses of many fruits (Bautista-Baños *et al*., 2006). However, the continuous use of fungicides faces two significant obstacles: increasing public concern regarding the contamination of perishables with fungicidal residues and the proliferation of resistance in pathogenic populations (Tripathi & Dubey, 2004). For this reason, many substances and methodologies had been tested to control fungal diseases, such as induction of natural disease resistance using elicitors (Terry & Joyce, 2004), chitosan application as a natural compound to induce natural defense mechanism (Bautista-Baños *et al*., 2006), curing treatments (Plaza *et al*., 2003), utilization of antifungal compounds derived from plants like isothiocyantes (Troncoso-Rojas *et al*., 2005), plant volatiles compounds (Abanda-Nkpwatt *et al*., 2006), natural compounds (Troncoso-Rojas & Tiznado-Hernández, 2007), phenolic compounds (Ruelas *et al*., 2006), antifungal compounds synthesized by bacteria like gluconic acid (Kaur *et al*., 2006), among others. Nevertheless, some of these treatments are expensive and difficult to apply, and they can inflict injury on the fruit. This situation leads to looking for alternatives to reduce the postharvest fruit losses by fungi infection.

Tomato is an important commercial crop for Mexico and many other countries and its demand continuously increases with its culture, production, and commerce. However, the fruit is highly perishable and the postharvest losses have been ascribed mainly to storage temperature during harvest and marketing. *Alternaria* is the most frequently encountered bacteria genera infecting tomato fruit in the field and before harvest, which causes low yields in the tomato industry (Etebu *et al*., 2013). Biological control agents for plant diseases are currently being examined as alternatives to synthetic pesticides due to their perceived level of safety and minimal environmental impacts. One of the most utilized

biological controls is those that use antagonistic microorganisms. Furthermore, biological control of postharvest diseases has emerged as an effective alternative and an interesting research field because wound-invading necrotrophic pathogens are vulnerable to biocontrol. Because of this, antagonistic microorganisms can be added directly to the fruit wounds using delivery systems such as drenches, line sprayers, and immersion containers, which can significantly reduce fruit decays. Also, many bacteria have been reported to have several positive effects controlling postharvest decay of fruits, like: *Cryptococcus laurentii* which has been demonstrated to reduce the decay increment in peach fruit induced by *Botrytis cinerea*, *Penicillium expansum,* and *Rhizopus stolinifer* (Zhang *et al.*, 2007). *Serratia plymuthica* can control green and blue mold on orange fruit (Meziane *et al.,* 2006), and halophilic bacteria can control grey mold diseases on tomato fruits (Sadfi-Zouaoui *et al.*, 2008) and strawberry fruits (Essghaier *et al.*, 2009). Additionally, several strains and species of *Pseudomonas* have proven to have activity against several fungi, including: *Pseudomonas syringae* to control blue mold of apples (Errampalli & Brubacher, 2006), *Pseudomonas corrugata* and *P. cepacia* to control postharvest brown rot caused by *Monilinia fructicola*, *Pseudomonas corrugata* to control *in vitro Alternaria alternata* and *Fusarium oxysporum* (Trivedi *et al.*, 2008), among others.

We had tested already the pathosystem *Alternaria alternata*-tomato as a model to study the phenomena of fungi infection in fruits (Troncoso-Rojas *et al.*, 2005; Ruelas *et al.*, 2006). While that work was conducted, we grew *Alternaria alternata* fungi on potato dextrose agar (PDA) and observed a contaminating bacterium on the plate. The presence of an inhibition zone surrounding the bacteria colony suggested to us that the bacteria was exudating a fungi toxic compound. In this sense, the present experiment aimed to evaluate the ability of the bacterium to secrete extracellular chitinase and probe the efficacy of the bacterial extract exudates to control *Alternaria alternata* infecting tomato fruit. Microbial identification based on biochemical activity and sequencing of 16S rRNA is also presented.

Materials and Methods

Biochemistry tests to identify the bacterium

Petri dishes containing PDA were inoculated with the bacterium and incubated at 37° C. Then, a 24 h old colony was isolated to carry out the biochemical tests: catalase, oxidase, motility-indol-ornithine (MIO), triple sugar iron (TSI), nitrification test, growth test at both 45°C and 60°C, and Gram staining. Two replicates of all the tests were carried out.

Sequence of the 16S rRNA gene

Genomic DNA extraction and purification were performed with the phenol-chloroform procedure as previously described (Bolado-Martínez & Acedo-Felix, 2009). Amplification of the 16S rRNA was carried out by PCR with a group of universal primers described in Table 1 (Lane, 1991), using a Perkin-Elmer 480 thermocycler (Perkin-Elmer, Wellesley, MA, USA, USA). The conditions for the amplification were: 96°C for 5 min, followed by 36 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 1 min, and a final extension step of 72°C for 5 min. All the amplified PCR products were purified using GFX columns (GE Healthcare, Piscataway, NJ, USA) and sent to the University of Arizona Genetics Core for sequencing. Finally, DNA fragments were assembled using the DNAMAN software version 7 (Lynnon Corporation. 383 av St-Louis, Pointe-Claire, Quebec, Canada H9R 2A3), and aligned with those available at GenBank using BLAST software, available at the national center for biotechnology information NCBI (http://www.ncbi.nlm.nih.gov/BLAST/).

Table 1. Set of primers used for PCR amplification of the 16S rRNA.

Primer name	DNA sequence 5´→ 3´
27f	AGAGTTTGATCMTGGCTCAG
530f	GTGCCAGCMGCCGCGG
926f	AAACTYAAAKGAATTGACGG
685r1	TCTACGRATTTCACCYCTAC
1110r	GGGTTGCGCTCGTTG
1492r	TACGGYTACCTTGTTACGACTT

Phylogenetic tree construction

Using a region of 1413 bases from the 16S rRNA of 15 *Bacillus licheniformis* strains published in the GenBank, an unrooted phylogenetic tree was created using the strain of *Escherichia coli* ATCC 11775T X80725 as an outgroup. The phylogenetic tree was created using MEGA7 software (Kumar *et al.*, 2018) based on neighbor-joining method with 1000 resampled data sets of the observed divergence method.

Alternaria alternata strain

Alternaria alternata was isolated from tomatoes (*Lycopersicon esculentum* Mill.) harvested in commercial orchards at Sinaloa, Mexico. The isolate was cultured on PDA, and the colony and sporulation characteristics were studied based on

published work (Simmons, 1995; Pryor & Michailides, 2002). The isolate was maintained in PDA at 25°C and subcultured into PDA every two months.

Tomato Fruit

Freshly harvested tomatoes cv `Charleston´ at the commercial mature red stage were obtained from a local grower. Then, the tomatoes were immediately transported to the lab and sorted to obtain fruits with uniform size, firmness, ripeness, color and free from bruising and rotting.

Extraction of bacterium toxins

Luria-Bertani broth (LB) and Potato dextrose broth (PDB) were inoculated with the *Bacillus licheniformis* strain isolated and kept in an orbital shaker for 24 h at 37°C and 150 rpm. After the incubation, the broth media was filtered and sterilized to eliminate the bacterium present. Next, the media was mixed with benzene in a proportion of 1:1. Finally, the benzene was eliminated using a rotary evaporator under vacuum and a water bath set at 40°C. The residue after benzene elimination was recovered with a 0.01 N solution of NaOH.

In vitro effect of the bacterium and the bacterial toxins on *Alternaria alternata* growth

The bacterium and the bacterial extract exudates were tested *in vitro* against *Alternaria alternata* growth using the following procedure. Fungi inoculation was carried out by removing a 7 mm diameter disc from the margins of a 10-day-old culture of *A. alternata* and placing it in the center of the PDA petri dish. The bacterium was inoculated in the margins of the Petri dish by using a sterile inoculation loop. All plates were incubated for seven days at 25°C. In the case of bacteria exudates, we used 20, 40, 60 and 80 µL of bacterial extract exudates sterilized by filtration with a 0.2 µM Millipore filter (Millipore Co., MA, USA). The bacterial exudates were placed in the Petri dish inoculated with *A. alternata* through a little hole made with a sterile pipet tip on the PDA in one of the margins of the Petri plates. The control plates were placed on 0.1 N NaOH. Three replicates were performed for the bacterium, and each volume level of bacterial extract exudates was tested, as well as for the control plates.

In vivo effect of the bacterium toxins on *Alternaria alternata* infecting tomato fruit

Tomatoes were divided into four groups of 32 fruits each. The four groups were dipped into a 200 mgL^{-1} of chlorine solution for 30 sec, washed with sterile wa-

ter and air dried at room temperature. Three groups of tomatoes were inoculated with *Alternaria alternata* by immersion of the fruit into a conidial suspension of 6 x 10^4 spores/mL and maintained for three min. After two h, the different groups of inoculated tomatoes were either not treated (IN treatment), or treated by spraying with a solution of PDB with bacterial extract exudate (PDB+BE+IN treatment) or treated by spraying with LB with bacterial extract exudates (LB+BE+IN). Also, one group of tomatoes was sprayed with water as control. After the treatments, tomato fruits were stored for 23 days at 25°C and 85-90% of relative humidity. The solution used for all treatments was sterilized by filtration with a 0.2 μM Millipore filter (Millipore Co., MA, USA).

The severity of infections was evaluated at 0, 3, 5, 7, 11, 15, 19, and 23 days in 8 fruits from every treatment using a hedonic scale. The degree of damage in tomato fruit was calculated using the Towsend-Heuberger formula (Sánchez-Estrada *et al.*, 2009). The fruits were classified by a five-point scale: group 0, 0% of damage; group 1, from 1 to 25% of damage; group 2, from 26 to 50% of damage; group 3, from 51 to 75% of damage and group 4, from 76 to 100% of damage.

The formula used for the calculation was:

$$P = \frac{(n*0) + (n*1) + (n*2) + (n*3) + (n*4)}{5*N} \times 100$$

where:

N = number of evaluated fruits
n = number of damaged fruits in each group
P = percentage of damage

The results were reported as the percentage of damage. In this investigation, confirmation of the fungi infection by *A. alternata* was evaluated by isolating the fungi from the tomato fruit lesions and observing the spore morphology and colony characteristics in PDA under the microscope.

The experimental unit was a tomato fruit and the whole experiment was performed twice.

Analysis of the postharvest physiology and quality of tomato fruit

The effects of the different treatments on the postharvest physiology and quality of tomatoes during storage at 25°C (market conditions) were determined by monitoring the respiration rate daily, measuring carbon dioxide production, ethylene production, and fresh weight loss. Whereas fruit firmness, color analysis, total soluble solids, pH and titratable acidity were determined initially and after 3, 5, 7,

11, 15, 19 and 23 days of storage at 25°C.

Production of chitinase enzyme

The bacterium strain was cultured in 200 mL of PDB and LB medium, and its optical density (580 nm) was monitored after 10 h of incubation at 37°C. After the incubation, the media was filter sterilized to eliminate the bacterium present and two mL of supernatant was harvested and stored at -20°C for the chitinase assays.

Chitinase activity assay

Chitinase activity was determined fluorometrically according to (Cota *et al.*, 2007) with some modifications. The reaction mixture contained 10 µL of bacterial extract exudates, 5 µL of 1.3 µM solution of 4-methylumbelliferyl-N, N', N"-triacetyl-ß-chitotrioside (Sigma Chemical Co.) and 35 µL of 50 mM sodium phosphate buffer pH 7.0 to obtain a total volume reaction of 50 µL. Release of free 4-methylumbelliferone (4-MU) was measured by fluorescence using a TBS-380 fluorometer (Turner Biosystems, Sunnyvale, CA USA) and quantified by using a standard curve developed with different concentrations of 4-MU. Chitinase from *Streptomyces griseus* (Sigma) was used as a positive control and the reaction without the bacterial extract exudates was included as a negative control. Chitinase activity was expressed as nMoles of 4-methylumbelliferone/min/mg of protein.

Statistical Analysis

The effect of the different treatments on respiration rate, ethylene production and fruit quality was studied by one-way variance analysis with a significant level of 5% based on a completely randomized design. Chromas Lite 2.01 software was used to edit sequences and review ambiguities whenever they appeared. DNA fragments were assembled using the DNAMAN 7.0 (Lynnon Corporation. 383 av St-Louis, Pointe-Claire, Quebec, Canada H9R 2A3). Finally, 16S rRNA sequences were compared with those available in the GenBank, EMBL, and DJB databases using the gapped BLASTN 2.0.5 program through the National Center for Biotechnology Information server.

Results

The results of the biochemistry tests showed that the bacterium was catalase and oxidase positive and mobile in MIO semisolid agar. On the other hand, on TSI agar, lactose and glucose were fermented although no production of hydrogen

sulfide gas was recorded. The bacterium grew at 45°C, and the Gram stain test showed it is a bacillus Gram-positive. Besides, BLAST analysis of 1413 bp of the 16S rRNA region of the bacterial sequence, we identified the bacterium as *Bacillus licheniformis*, showing 99% of identity with different sequences of *Bacillus licheniformis* published in the GenBank (Barreras-Bojórquez *et al.*, 2013).

Effects of the bacterium extract on the levels of chitinase activity. Secretion of chitinase was followed by culturing bacterium in either LB or PDB. The results showed that chitinase production reached 150.15 and 246.36 units after 10 hours of incubation in the PDB and LB mediums, respectively.

Effects of the bacterium extract on *in vitro* growth of *A. alternata* growth

The effect of bacterial extract exudates at different volume levels used 20, 40, 60, and 80 mL against *in vitro* growth of *A. alternata* showed that the inhibition effect was higher when using bacteria growing in LB than with PDB. However, it was not possible to quantify the effect, and therefore, data is not included. These results can be ascribed to the more significant chitinase produced in the LB than the PDB media described above. Figure 1 shows the assay results using 80 ml of those assays for illustration purposes only.

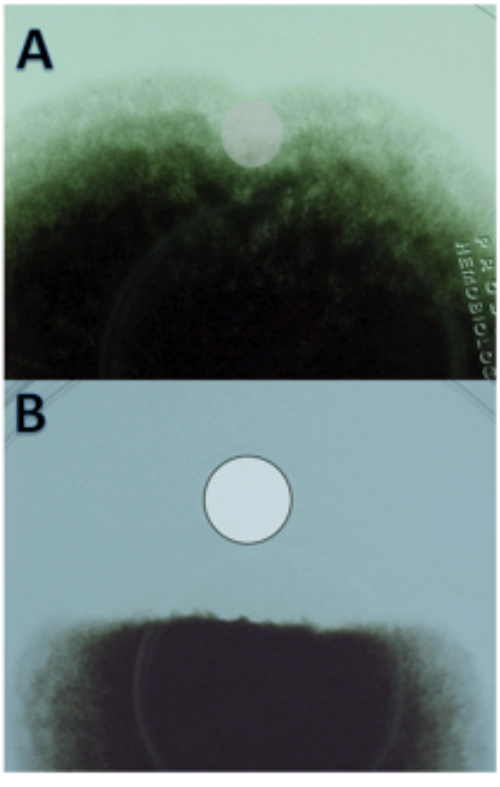

Figure 1. Inhibition of *Alternaria alternata* fungi growing in PDA media by the *Bacillus licheniformis* exudate. A. Control. B. 60 mL of exudate extract. The circle was marked with an image editor to make sure the area where the exudate was collocated was visible.

Effects of the bacterium extract on *in vivo* growth of *A. alternata*

Figure 2 shows the *in vivo* effect of the bacterial extract exudates on tomato fruit during storage at 25°C. Positive control was an inoculated tomato fruit with the suspension of *A. Alternata* (IN treatment) spores. After 19 days, the groups of inoculated tomatoes and treated with bacterial extract exudates (PDB+BE+IN treatment) and (LB+BE+IN treatment), showed 2.06% and 3.87% of damage, respectively. By this time, the positive control treatment (IN) showed a 3.68% damage, whereas the control fruits showed a 0.15% damage index, which is rather close to zero. At the end of the storage time, the damage degree observed for PDB+BE+IN was similar to the IN treatment. However, we recorded lower damage for the LB+BE+IN treatment.

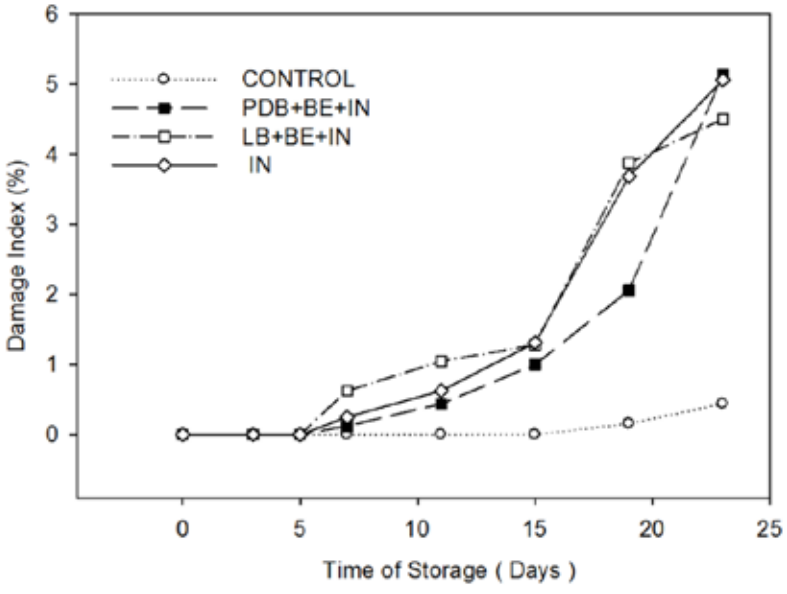

Figure 2. The percentage of damage index was recorded during storage in the different groups of tomato fruit. CONTROL corresponds with the untreated tomatoes, IN corresponds with the inoculated and untreated tomatoes, PDB+BE+IN corresponds with the inoculated tomatoes and treated by spraying with a solution of PDB containing bacterial exudates, and LB+BE+IN corresponds with the inoculated tomatoes and treated by spraying with LB containing bacterial exudates.

Analysis of the postharvest physiology and quality of tomato fruit

No effects were observed in the postharvest physiology or quality characteristics of tomato fruit during postharvest and therefore, the data is not included in the manuscript to save space. Altogether, the results obtained in this study strongly suggest that this bacteria extract could be used as a postharvest treatment to control fungi infections in tomato fruit without adverse effects on the postharvest quality.

Phylogenetic Tree

Figure 3 shows the phylogenetic tree created using the 16S rRNA sequences of Bacillus licheniformis and Escherichia coli ATCC11755 as an outgroup. The results showed a 78% identity with the *Bacillus licheniformis* group. There is a significant similarity between the strains studied in this experiment and several strains of *B. licheniformis* available in the GenBank.

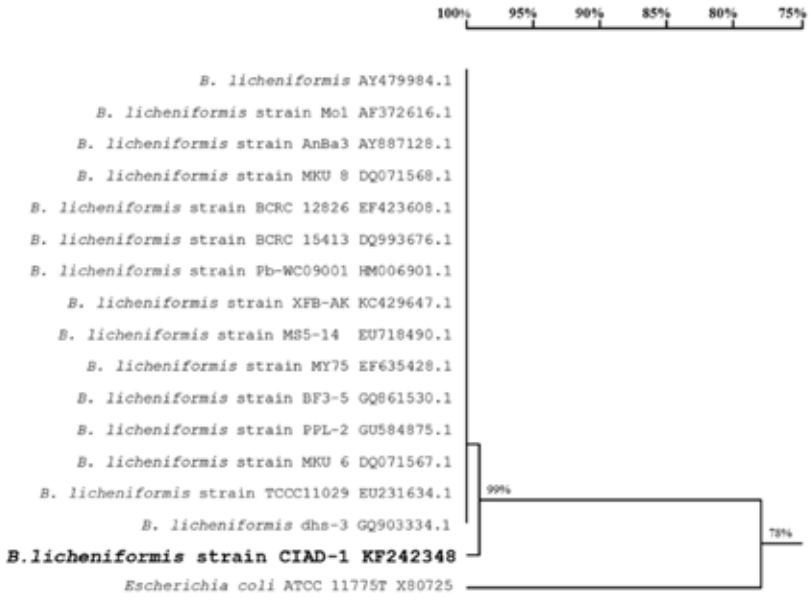

Figure 3. A phylogenetic tree was created using 1413 bases from the 16S rRNA of 15 *Bacillus li* strains published in the GenBank. Numbers in the nodes are percentages indicating the levels of bootstrap support created with the neighbor-joining analyses of at least 1000 resampled data sets. The *B. licheniformis* strain name and the Genbank accession numbers are also included. The strain isolated in the present work is highlighted in bold with the corresponding Genbank accession number.

Discussion

The *Bacillus subtilis* group contains the closely related taxa *Bacillus subtilis* subsp. *subtilis*, *Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus atrophaeus, Bacillus mojavensis, Bacillus vallismortis, Bacillus subtilis* subsp. *spizizenii* and *Bacillus sonorensis*. Most authors have found very few phenotypic or biochemical characteristics that differentiate these species. Also, most of the species of the group share a remarkably high level of 16S rRNA gene sequence identity, which is often 99% or greater (Wang *et al.,* 2007; Rooney *et al.*, 2009).

Bacillus licheniformis is an interesting type of bacteria for humans, mainly because of its exudate antimicrobial properties and potential utilization as a biological control for crops. A 3.4 kDa hydrophilic peptide with antifungal activity, named fungicin M4, produced by *Bacillus licheniformis* M-4 was isolated. Antimicrobial

activity of peptide showed a narrow spectrum, and it is restricted to *Microsporum canis, Mucor mucedo, Mucor plumbeus, Sporothrix schenckii, Bacillus megaterium* and *Corynebacterium glutamicum* (Lebbadi *et al.*, 1994). A protein with antifungal activity of 31.0 kDa was shown to have an inhibitory effect on *Aspergillus niger, Magnaporthe oryzae*, *Rhizoctonia solani*, and *Fusarium oxysporum* (schl.) f. sp. Benincasae, (Cui et al., 2012). Moreover, the potential utilization for biocontrol has been suggested due to its anti-fungal activity. Indeed, it has been found that some strains of *B. licheniformis* producing chitinase enzyme can inhibit the growth of *Fusarium graminearum* (AbdeL-Shakour, 2012), *Aspergillus flavus*, *Aspergillus niger, Aspergillus terreus* and *Pythium sp.* (Gomaa, 2012). Also, it has been shown to inhibit the spore germination of *Fulvia fulva* (Lee *et al.*, 2009). From the results of the *in vitro* assay, we can demonstrate that this particular strain of *B. licheniformis* has a potent inhibitory effect on *Alternaria alternata,* probably due to the production of the chitinase enzyme. Based on the literature reports, it might also be against other species of phytopathogenic fungi, although more experimental evidence is needed to probe further this statement.

The pathosystem Alternaria-tomato is well documented in our lab, during this experiment we observed the classical black spot decay due to the *A. alternata* indicating the active infection of the inoculated tomato fruits in our work. Also, we had already tested this pathosystem with the same strain of *Alternaria alternata* in several experiments evaluating the effect of isothiocyanates (Troncoso-Rojas *et al.*, 2005) or studying the tomato fruit defense responses (Ruelas *et al.,* 2006). The formula of Towsend-Heuberg used in this experiment to analyze the degree of fungi infection had been widely utilized to analyze the degree of damage by fungi attack in plants and fruits (Lozoya-Saldaña & Hernández-Vilchis, 2001; Solís-Aguilar *et al.*, 2001; Juhásová *et al.*, 2005); Further, from the data showed in figure 2, we can observe that the group of control positive (IN) showed the highest level of infection. In contrast, the negative control (CT) did not show significant symptoms of fungi infections during the storage period, which strongly supports the results of this experiment. On the other hand, we could observe that when compared among the different treatments with the positive control (IN), only the fruits of the group LB+BE+IN treatment showed a lower degree of *A. Alternata* infection by the end of the storage time. We cannot directly compare the inhibition percentages of the *in vitro* and *in vivo* experiments because we did not measure the inhibition area in the petri dish. A hedonic scale was used to estimate the percentage of damage area for the tomato surface.

Due to the lack of similar experiments reported in the literature, no comparison can be carried out with the results of this experiment. Finally, it is possible that the bacterium toxin can be utilized to control the *A. alternata* infection in tomato fruit during postharvest shelf life. However, more studies are required to evaluate

the possible effects of this bacteria exudate on human health if it will be utilized as a postharvest treatment in products to be consumed fresh.

Conclusions

Data generated in this study demonstrates that the isolated strain of the bacterium *Bacillus licheniformis* in this work can control the *in vitro* growth of *Alternaria alternata* better than the active principle of bacteria extract on infected tomato fruits. Also, this bacteria extract could be used as an alternative during postharvest storage of tomato fruit to control fungi infections without adverse effects on the respiration physiology and the postharvest fruit quality parameters.

Conflicts of interest

The authors declare that there is no conflict of interest.

Author Contributions:

MJBB is the B.Sci. student who carried out the fruit inoculation, respiration, and ethylene determination and wrote the first draft of the manuscript. AJOC helped in the harvesting of tomato fruit, experimental setup, and fruit quality analysis. ASE isolated and maintained the *A. alternata* strain, multiplied the bacteria in the two different media and discovered the bacteria growing in the fungi petri dish. RTR carried out the fungi spore quantification, supervised the inoculation of tomato fruits, analyzed the data generated during the determination of respiration and ethylene production, and helped in the edition of the manuscript.
EAF carried out the isolation of the DNA, sequencing, identification, and phylogenetic analysis. VAOJ helped in the quantification of fruit quality variables, determination of the damage index, statistical analysis, and creation of different figures. METH designed and supervised the whole experiment, get the financial support, write, and edit the final version of the manuscript.

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