Postharvest dip treatment of Bacillus subtilis for maintaining quality and shelf life of guava cv. Allahabad Safeda

Bharati Killadi, Neelima Garg and Rekha Chaurasia

Abstract
In North India, guava fruits are harvested twice in a year yet maintaining the quality during storage is still a challenge due to its perishable nature. The use of chemicals for extending shelf life of guava should be avoided due to food safety concern since it is consumed along with skin. Recently bio-agents are being used as dip treatment for effective control of storage pathogens like Colletotrichum sp., Pestalotiopsis sp. and Lasiodiplodia sp. in fruits like apple, pear and strawberry. However, no such report is available for guava. In the present study Bacillus subtilis was used as bio-agent for enhancing the shelf life of guava. Guava fruits cv ‘Allhabad Safeda’ were subjected to post harvest dip treatments for 30 minutes with four strains of Bacillus subtilis viz. MTCC 7607, 7606, 7612, and 7605 @ 10⁶ cells/ml dip solution and stored at 18 ± 2 °C and 57±5 % R.H. The fruits were analyzed for physico-chemical parameters as well as microbial population at regular intervals. Among the treatments, fruits treated with Bacillus subtilis strain MTCC 7607 showed highest firmness after 10 days of storage. In general bio-agent treated fruits were glossy in appearance, better in quality and taste with uniform colour development and least disease incidence. Surface microbial analysis reflected the dominance of treated bacterial strain over other microorganisms. Preliminary results indicate that Bacillus subtilis strain MTCC 7607 @ 10⁶ cells/ml could act as a potent bio-agent for shelf-life extension of guava to control postharvest diseases.

Keywords: Bio-agents, shelf-life, ascorbic acid, Bacillus subtilis, post-harvest

Introduction
Control of pathogens during post-harvest storage and handling has been a challenging task for which chemicals are generally used. Recently, bio-agents are considered as preferred tool to replace chemicals because of their safety on human health and the environment. There is a need to look for an effective bio-agent for control of post-harvest pathogens. Bio-agents actively produce antibiotics, whose action partially determines their effectiveness in controlling post harvest pathogens. Commercial products containing Bacillus subtilis (Serenade ad Rhio-plus) are being used for control of powdery mildew, late blight and brown rot on fruits Walton (2002) and Sharma (2009) [31]. The first report about B. subtilis affecting the physiological processes of postharvest of fruits is reduction in ethylene production in melons by Wang et al., (2010) [35]. Use of Bacillus subtilis as a biological antagonist has been reported from fruits such as apricot, avocado, cherry, citrus, litchi, strawberry, apple and pear Pussy and Wilson (1984) [16], Demoz and Korsten (2006) [4], Utkheda and Sholberg (1986) [23], Sing and Daverall (1984) [22], Jiang et al., (2001) [9], Zhao et al., (2007) [26] Qi et al., (2005) [17], Lise and Katze (1992) [11] and Lise and Jager (1995) [12]. The objective of the study was to evaluate strains of Bacillus subtilis as potential bio-agent for maintaining the quality of guava fruits under ambient conditions.

Materials and methods
B. subtilis strains of cow dung origin were obtained from culture collection of Microbiology laboratory in the Division of Post-Harvest Management, ICAR-Central Institute for Subtropical Horticulture, Lucknow. The bacterial culture was maintained on nutrient agar slants. Four bacteria isolated from cow dung were used namely Bacillus subtilis (MTCC 7605), Bacillus subtilis (MTCC 7606), Bacillus subtilis (MTCC 7607), Bacillus subtilis (MTCC 7612) from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh. The anti-pathogenicity was tested against Colletotrichum gloeosporioides, Botryodiplodia theobromae and Pestalotiopsis sp as per standard protocol by dual culture technique (Anjaiah
et al., 1998) [11]. B. subtilis strains were multiplied on nutrient agar plates were incubated at 30±2°C for 3 days. The cells were collected with the help of cell scraper and the final cell number was maintained @10^9 cells/ml in distilled water.

Mature, hard green fruits of guava cv. ‘Allahabad Safeda’ were harvested along with stalk and two leaves during the morning hours from the orchards of ICAR-CISH farm and transported to the Post Harvest Laboratory. The uniform colour break stage and sound fruits were sorted, divided into five lots of equal weight and subjected to bio-agents dip treatments. The treatment included four strains of Bacillus subtilis MTCC7605, MTCC7606, MTCC7607 and MTCC7612 @10^8 cells/ml along with control (in distilled water) for 30 minutes. Subsequently the fruits were air dried, packed in brown paper bags and stored under ambient conditions (18 ±2°C and 57±5 % R.H.).

Fruits were withdrawn at regular intervals of 0, 4, 6, 8 and 10 days from each treatment and cumulative physiological loss in weight (CPLW) percent was recorded. The firmness of fruits were measured with the help of penetrometer (McIntosh, USA, 8mm probe) and expressed as kg/cm². The fruits were thoroughly checked for diseases and number of spoiited fruits divided by the total number of fruits and expressed as percentage spoilage.

For chemical analysis, fruits withdrawn periodically from each treatment in three replications were cut into small pieces to be macerated into uniform pulp and analyzed for total soluble solids with help hand held digital refractometer (Atago, PAL 0-30%) and the reading was noted and adjusted with the correction factor at 20°C temperature and expressed as degree Brix. The titratable acidity and ascorbic acid in fruit pulp was estimated by method as outlined by Ranganna (2000) [18]. Fruits were evaluated subjectively according to McDonald et al. (1998) measuring the rotted area in relation to the skin total area and expressed as percentage. Surface microbial counts were monitored at 2 days intervals as per the method of Collins and Lyne (1984) [2]. All the analysis was carried out in triplicates and the data recorded during the course of investigation were subjected to statistical analysis by SAS 9.3 and CD at 0.05 level.

Results and discussion

Bacillus subtilis is well documented for its antimicrobial potential Krishna et al., (2011) [10]. Based on this antimicrobial property of B. subtilis was screened for its bio-control efficiency in petriplates versus Colletotrichum gloeosporioides, Botryodiplodia theobromae and Pestalotiopsis sp. The results indicated that bacterial isolates had anti-pathogenic potential against the tested pathogens (Table 1 and fig 1).

Cumulative physiological loss in weight per cent (CPLW %) increased with the increase in storage period with least 10.05 per cent in B. subtilis strain MTCC 7607 followed by B. subtilis strain MTCC7606 (10.47) 10 days of the storage period (Fig 2). In control fruits the CPLW percent was highest (15.37 %) on the 10 days of storage. Loss of weight during storage of fruits is one of the criteria used to study shelf life. It is due to evapo-transpiration by the fruit surface during storage. Weight loss throughout storage accelerates cell-breakdown and senescence. Wang et al. (2008) [24] has reported weight loss of watermelon during storage.

Firmness of the fruits expressed as Kg/cm² was found to decrease with the increase in storage period under ambient conditions with the onset of ripening and tissue softening. Significant difference was noticed in the firmness of the fruits among the different strains of B. subtilis (Fig.3.). The maximum firmness (11.87 Kg/cm²) was noted in fruits treated with B. subtilis strain MTCC7607 followed by B. subtilis strain MTCC7606 (9.13 Kg/cm²) and minimum firmness (6.20 Kg/cm²) was exhibited in control fruits on the 6th day of storage thereafter there was a rapid decrease in the texture of fruits. This might be due to the interaction between the bio-agents on the surface of the fruits and storage period.

The total soluble solid content (TSS) of guava fruits variety ‘Allahabad Safeda’ increased and simultaneously the acidity percentage decreased with the increase in storage period. Among the treatments significant difference was studied for the total soluble solid contents. TSS was highest (13.33%Brix) in control fruits on the 6th day of storage while it further decreased on the 8th and 10th day of storage (Fig.4.). Bio-agents treated fruit exhibited minimum TSS of 11.20%Brix in strain MTCC7607 on the 6th day of storage. With the increase in storage period from 6th day to 8th day, the TSS increased from 11 to 13%Brix in B. subtilis strain MTCC7612. The acidity percentage also exhibited an opposite trend to that of TSS in control and B. subtilis treated fruits Fig.5. These findings are in accordance to the findings of Wang et al. (2010) [25] and TSS and acidity of watermelon.

Highest ascorbic acid content was observed on the 6th day of storage period in control fruits expressed as milligram per hundred grams of fruit pulp (248.57 mg/100g) and thereafter it decreased (Fig.6.). The fruits treated with different strains of B. subtilis had ascorbic acid content ranged between 200mg/100g up to 6th day of storage and decreased later on the 10th day of storage. Per cent CPLW is maximum in untreated fruits which are due to evaporation loss of moisture that might have concentrated the ascorbic acid content. These results are in harmony with the findings of Barakat et al., (2012) [14]. Colonization of B. subtilis on the fruit surface is an important and multifaceted process required for the competitive and exclusion of the storage pathogen. Antagonists must have high growth rates under favourable condition of humidity, temperature and nutrients Ippolito and Nigro (2000) [8] and Ippolito et al., (1997b) [7] and guava stored under ambient conditions were conducive for the colonization of bio-agents on the fruit surface.

Anthracnose and stem-end-rot in guava variety Allahabad Safeda was found maximum 14.85 and 19.67 per cent in control fruits followed by B. subtilis strain MTCC7606 i.e., 6.26 and 12.63 % on the 8th and 10th day of storage respectively (Fig.7.) There was no spoilage observed throughout the storage period of 10 days in B. subtilis strains of MTCC7607, MTCC7612 and MTCC7605. Bacillus subtilis produces iturin, a powerful antifungal peptide Gueldner et al., (1988) [6], as well as gramicidin S Edwards and Seddon, (2001) [9]. Bacillus species are the most common types of bacteria and epiphytic and endophytic species of Bacillus have the potential to reduce pathogenicity Omur et al., (2008) [15]. Results are similar to the findings of Lise and Katze (1992) [11] in control of avocado post harvest diseases and mode of action of Bacillus subtilis in avocado post-harvest pathogen Lise and Jager (1995) [12]. Spadero (2004) [3] elucidated the biological control of postharvest fruit diseases thereby enhancing the shelf life of fruits.

The surface microbial load indicated the dominance of bio-agents in treated fruits compared to control with other yeasts, molds and bacteria dominated (Table 2.). Reduction in surface microbes was recorded over the progress of storage period which indicates the surface contaminating microbes might have reduced due to the formation of biofilm by the
bio-agent. Bio-control efficiency of the bio-agent could be activated to the ability of \textit{B. subtilis} to produce acyl-homoserine lactones (HLS) an enzyme which inactivates N-acyl-homoserine (AHL) activity by hydrolyzing the lactone bond of AHL Morikava (2006) \cite{13}. Due to production of iturin, a powerful antifungal peptide Gueldner \textit{et. al.}, (1988) \cite{6}, as well as gramicidin S by \textit{B. subtilis} Edwards and Seddon, (2001) \cite{5}. The influence of competition for nutrition, possible production of antibacterial and antifungal compounds and interaction between the fruit surface and the bio-agent as reported by Castoria \textit{et al.}, (2001) \cite{19}, Samir, \textit{et al.}, (2009) \cite{20} and Sharma \textit{et al.}, (2009) \cite{21}. The present study shows that \textit{Bacillus subtilis} strain MTCC7605, MTCC7607 and MTCC7612 were effective in controlling post harvest diseases of guava viz. anthracnose and end rot. In addition, there is presence of strong bio-control capacity to enhance shelf life of guava. In the present investigation \textit{Bacillus subtilis} strain MTCC7605, MTCC7607 and MTCC7612 for enhancing shelf life of guava cv. Allahabad Safeda as a bio-control organism have a potential for control of postharvest pathogen during storage.

Table 1: Anti-pathogenicity of \textit{B. subtilis} strains for causal pathogens in guava fruits.

<table>
<thead>
<tr>
<th>Causal pathogen</th>
<th>Effective bio-agent</th>
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<tbody>
<tr>
<td>\textit{Phytophthora parasitica}</td>
<td>\textit{Bacillus subtilis} (MTCC 7605), \textit{Bacillus subtilis} (MTCC 7606).</td>
</tr>
<tr>
<td>\textit{Pestalotiopsis sp.}</td>
<td>\textit{Bacillus subtilis} (MTCC 7607); \textit{Bacillus subtilis} (MTCC 7606); \textit{Bacillus subtilis} (MTCC 7612); \textit{Bacillus subtilis} (MTCC 7605).</td>
</tr>
<tr>
<td>\textit{Lasiodiplodia sp.}</td>
<td>\textit{Bacillus subtilis} (MTCC 7607); \textit{Bacillus subtilis} (MTCC 7606); \textit{Bacillus subtilis} (MTCC 7612); \textit{Bacillus subtilis} (MTCC 7605).</td>
</tr>
<tr>
<td>\textit{Colletotrichum sp.}</td>
<td>\textit{Bacillus subtilis} (MTCC 7607); \textit{Bacillus subtilis} (MTCC 7606); \textit{Bacillus subtilis} (MTCC 7612); \textit{Bacillus subtilis} (MTCC 7605).</td>
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Table 2: Surface microbial counts in guava cv. Allahabad Safeda treated with \textit{B. subtilis} strains during storage.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Storage period (days)</th>
<th>Bacteria (cells/g)</th>
<th>Yeast &amp; mould count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
<td>4 day</td>
<td>6 day</td>
</tr>
<tr>
<td>Control</td>
<td>2.25 x 10^3</td>
<td>7.5 x 10^2</td>
<td>2.98 x 10^3</td>
</tr>
<tr>
<td>MTCC 7607</td>
<td>8.960 x 10^4</td>
<td>2.5 x 10^2</td>
<td>1.2 x 10^2</td>
</tr>
<tr>
<td>MTCC 7606</td>
<td>9.600 x 10^4</td>
<td>3.2 x 10^2</td>
<td>1.7 x 10^2</td>
</tr>
<tr>
<td>MTCC 7612</td>
<td>1.500 x 10^4</td>
<td>3.8 x 10^2</td>
<td>3.5 x 10^2</td>
</tr>
<tr>
<td>MTCC 7605</td>
<td>1.125 x 10^4</td>
<td>4.8 x 10^2</td>
<td>4.8 x 10^2</td>
</tr>
</tbody>
</table>

Fig 1: Anti-pathogenicity of \textit{B. subtilis} strains for causal pathogens in guava fruits.
Fig 2: Cumulative physiological loss in weight per cent in guava cv. Allahabad Safeda treated with *B. subtilis* during storage.

Fig 3: Firmness (Kg/cm²) in guava cv. Allahabad Safeda treated with *B. subtilis* during storage.

Fig 4: TSS (degree brix) in guava cv. Allahabad Safeda treated with *B. subtilis* during storage.
Fig 5: Titratable acidity (per cent) in guava cv. Allahabad Safeda treated with *B. subtilis* strains during storage.

Fig 6: Ascorbic acid (mg/100g) in guava cv. Allahabad Safeda treated with *B. subtilis* strains during storage.

Fig 7: Spoilage (per cent) in guava cv. Allahabad Safeda treated with *B. subtilis* strains during storage.
References


