Isolation and identification of bacteria associated with Guava decline in Egypt

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Abstract: Guava (Psidium guajava Linn.) is one of the most popular fruits in Egypt. Guava decline is thought to be a complex disease prevailing in tropical and subtropical regions. Several studies were conducted on the role of fungi and nematode in the deterioration of guava. There is unsatisfactory information on the involvement of bacteria in this disease. In the present work, twelve isolates of bacteria were recovered from diseased guava trees at Rashid territory of El-Behera government in Egypt. The results of 16S rRNA gene sequences compared with the sequences of the Gen Bank DNA database showed that eight of these isolates belong to family Enterobacteriaceae, two isolates belong to family Rhizobiaceae and two isolates belong to family Pseudomonadaceae. The Pectobacterium arioidearum is the only species that has shown a positive result with a hypersensitive reaction (HR) test. Agrobacterium salinitolerans was the only species able to form small tumors in squash fruits. No evidence of formed hyperplastic syndrome on the tomato plants by Agrobacterium salinitolerans or any of these isolated bacteria were recognized. Potato soft rot test confirmed that only Pectobacterium arioidearum have the potential to cause soft rot in potato slices. The results indicate that, despite difference in pathogen propensities, Pectobacterium arioidearum and Agrobacterium salinitolerans can get involved in guava decline syndromes either single or in combination, bearing in mind the role of other pathogens such as fungi and nematodes. Further studies are needed.

[1. Introduction] Guava (Psidium guajava L.) is a fruit tree grown in tropical and subtropical regions, and its fruits have a tasty flavor and high Vitamin C content. India is the main producer of guava in the world along with other countries important in the production of guava such as China, Thailand, Pakistan, Mexico, Indonesia, Brazil, and Bangladesh (Queiroz et al., 2018). Guava is lovely fruits in Egypt. According to the statistics of the Ministry of Agriculture in Egypt, the total cultivated area of guava in 2014 was about 40831 feddans producing about 349626 tons (Atawia et al., 2017). Guava cultivation is concentrated at Lower Egypt especially in Behera, Damietta, Kafr El Sheikh, Alexandria and Qalubiya governorates.

Guava decline is a complex disease distributed throughout the world in tropical and subtropical regions. In Brazil, Guava decline caused by the interaction between Meliodogyne enterolobii and Fusarium solani, it is the major disease affecting guavatrees, and cause losses in productive chain of guava (Gomes et al., 2012, Gomes et al., 2013 and Gomes et al., 2014). No bacteria reported from root tissues collected from guava orchards in Brazil (Gomes et al., 2011). Many fungi were found associated with this complex disease in Pakistan as Botryodiplodia theobromae, Fusarium oxysporum and Colletotrichum gloeosporioides (Ansar et al., 1994 and Safdar et al., 2015). Guava decline caused by Botryodiplodia theobromae, Fusarium oxysporum, and Rhizoctonia solani is one of the major fungal diseases threatening guava production in Egypt (Zaitoun et al., 2015). Several studies were conducted on the role of fungi and nematode in the deterioration of guava. There are limited reports on the involvement of bacteria in this complex disease. Bacterial isolates obtained from galled guava roots from Rosetta (Rashid) and Elkouf farms in Behera Governorate, Egypt were identified as Agrobacterium tumefaciens (Younis et al., 2016).
The purpose of this work was made in examine of other bacterial genera involved in such complex disease in Egypt to determine their role in this disease in Lower Egypt.

2. Materials and Methods

Isolation of the bacteria:

Samples of decline guava roots showing rotten tissues and mini galls (nodules) were collected from different orchards at Rashid region of El-Behera government. The isolation was carried out after shaking the collected roots with hand to dispose of soils. Rotten guava roots tissue with nodules were cut and collected in sterile petri dish. The collected tissue were washed for three times in sterile distilled water and crushed in few drops of sterile distilled water and left for 30-60 min before streaking on King’s B agar. Incubation was made at 28°C for 48hrs. Developing single colony were randomly chosen from plates, picked up on slants of same King’s B media and incubated at 28°C for 72 hrs. Pure isolates were identified by 16S rRNA analysis.

Identification of isolated bacteria by 16S rRNA analysis:

Suspension of two colonies of tested isolate in 100 ul of lysis solution (0.05 M NaOH, 0.25% sodium dodecyl sulphate [SDS]) was incubated for 15 min at 100°C. The suspension was diluted to 20-fold in DNA-free water after centrifuged for 1 min at 14,000 xg (pellet discarded). The PCR amplifications were performed using the two universal eubacteria primers U968-f and U1401-r (Table1) as described by Hiddink et al., 2005. The amplified PCR products were purified using Pure Link TM quick gel extraction kit (Invitrogen, Life Technologies, Löhne, Germany). Twenty ng from each purified PCR product was added to 20 μl PCR Master Mix and amplified according to the diagnostic procedure by ABI Prism® BigDye® Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems, Foster City, CA, USA). The sequencing process was conducted at the Potato Brown Rot Project laboratories (Giza, Egypt) using an 8-capillary Genetic Analyzer (Applied Biosystem). The partial 16S rRNA gene sequences were compared with the sequences of the Gen Bank DNA database using Nucleotide blast. Database of 16S ribosomal RNA sequences (Bacteria and Archaea) was chosen and BLAST algorithm Megablast (Optimize for highly similar sequences) was selected (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for alignments. The distance tree was produced by BLAST pairwise alignments using neighbor joining tree methods.

<table>
<thead>
<tr>
<th>PCR target</th>
<th>Primer name</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial 16s</td>
<td>U968-f</td>
<td>Felske et al., (1996)</td>
</tr>
<tr>
<td>Bacterial 16s</td>
<td>L1401-r</td>
<td>Felske et al., (1996)</td>
</tr>
</tbody>
</table>

Test the pathogenicity of isolates:

Pathogenic potential of was tested by several methods as follows:

Hypersensitive reaction test:

Hypersensitive reaction test was performed using tobacco plants. An amount of 200 μl of bacterial suspensions (10^8 cfu/ml) was injected into lower surface of fully expanded tobacco leaves using syringe with fine needle. Tobacco leaves inoculated with sterile distilled water used as a control. Three tobacco leaves were used for each isolate. Induced necrosis after 24-48h in the tissue infiltrated with bacterial suspensions was recorded as a positive (+) reaction (Vanneste et al.1990).

Tested the ability of isolated bacteria to induce galls in squash fruits:

Pure cultures of isolated bacteria were tested for their ability to induce galls in squash (Cucurbita pepo L.) fruits. Whole mature squash fruits were surface sterilized with 75 % ethanol. Small wells were cut on the surface of the fruit while 50 μl of the bacterial cell suspensions 10^6 cfu/ml of the tested isolate were introduced into the well. Squash fruits inoculated with sterile distilled water used as a control. Three squash fruits were used for each isolate. Inoculated squash fruits were incubated at 28°C in a moist chamber and the progress in the formation of galls was daily examined (Abd-El-Aziz, 2011). The formation of galls was recorded as a positive (+) reaction.

The ability of isolated bacteria to induce galls on tomato plants:

Pure cultures of isolated bacteria were tested for their ability to induce galls on tomato (Solanum lycopersicum L. (plants cv. Castle Rock. Tomato, seedlings (3 weeks old) were grown in pots (20 cm diameter) containing clean sandy clay soil (1: 1 - v/v). All tomato plants wounded at depth 1–2 mm by sterile scalpel at the crown region. Each pot was inoculated with 100 ml bacterial suspension (10^6 cfu/ml) poured on the wounded area. Tomato plants inoculated with 100 ml sterile distilled water were used as a control. Three tomato plants were used for each isolate. Inoculated tomato plants were incubated at 28°C in a high humidity in greenhouse and the progress in the formation of galls or nodules was examined after one month of inoculation.
formation of galls or nodules was recorded as a positive (+) reaction.

**Potato soft rot test:**

Potato soft rot test was achieved as described by Abd El-Ghany *et al.* (2017). Potato slices in sterile petri dishes with filter paper moistened with sterilized water, were inoculated in the center with 50 μl bacterial suspensions (10⁸ CFU/ml) and incubated at 28°C for 72 hrs. Potato slices inoculated with sterile distilled water used as a control. Three replicates were used for each isolate. The rotted potato slice was recorded as a positive (+) reaction.

**3. Results**

**Sample collection and isolation of bacteria:**

Twelve isolates from colonies of bacteria developed on King's B agar media were made. Trial was carried out from rotten guava roots samples showing small galls (Although no galls were observed on the crown region of guava trees) collected from guava trees with symptoms of guava decline at Rashid territory, El-Behera government (Figure 1). These isolates were given the codes 3G, 5G, 7G, 8G, 10G, 13G, 14G, 16G, 18G, 19G, 20G and 21G.

![Figure 1: Rotten guava roots showing small galls (nodules), associated with symptoms of guava decline at Rashid territory of El-Behera government.](image)

**16S rRNA gene sequences analysis:**

The results of 16S rRNA gene sequences showed that, the isolates 3G, 5G and 7G showed 99.74, 99.73 and 98.92% similarity with *Enterobacter asburiae* strain JM-458 and different strains of *Leclercia adecarboxylata*, respectively. The isolates 3G, 5G and 7G showed 99.47, 99.47 and 98.66% similarity with *Enterobacter tabaci* strain YIM Hb-3, different strains of *Cedecea lapagei*, *Enterobacter mori* LMG 25706 strain R18-2 and *Cedecea davisiae* strain DSM 4568, respectively. The isolates 3G, 5G and 7G showed 99.21, 99.20 and 98.39% similarity with *Enterobacter cancerogenus* strain LMG 2693, respectively.

The isolate 8G showed 98.96% similarity with *Salmonella enterica* subsp. *salamae* strain DSM 9220, different strains of *Escherichia fergusonii*, *Salmonella enterica* subsp. *arizonae* strain DSM 9386, *Escherichia albertii* strain Albert 19982, *Salmonella enterica* subsp. *arizonae* strain ATCC 13314, *Shigella flexneri* strain ATCC 29903 and *Escherichia coli* strain U 5/41. The isolate 8G showed 98.70% similarity with *Escherichia marmotae* strain HT073016, and the isolates 10G and 13G showed 98.92 and 99.47% similarity with *Escherichia marmotae* strain HT073016, *Salmonella enterica* subsp.predictica strain Ty2, *Kosakonia oryzendophyticus* strain REICA_082 and *Salmonella enterica* subsp. *salamae* strain DSM 9220, respectively. The isolates 10G and 13G showed 98.65 and 99.20% similarity with different strains of *Escherichia fergusonii*, *Metakosakonia massiliensis* strain JC163, *Atlantibacter hermannii* strain CIP 103176, *Citrobacter youngae* strain GTC 1314 and *Salmonella enterica* subsp. *arizonae* strain DSM 9386, respectively. Moreover, the isolate 14G showed 99.22% similarity with *Serratia nematodiphila* strain DZ0503SBS1 and *Serratia marcescens* subsp. *sakuenis* strain KRED. The isolate 14G showed 98.70% similarity with *Enterobacter tabaci* strain YIM Hb-3, *Cedecea lapagei* strain DSM 4587, different strains of *Serratia marcescens* and *Cedecea davisiae* strain DSM 4568. The isolate 14G showed 98.44% similarity with *Enterobacter soli* ATCC BAA-2102 strain LF7 and *Enterobacter asburiae* strain JM-458.

The isolate 16G showed 99.74% similarity with *Pectobacterium aroidearum* strain SCR 109 and different strains of *Pectobacterium carotovorum* subsp. *odoriferum*. The isolate 16G showed 99.48% similarity with *Providencia vermicola* strain OP1, *Providencia rettgeri* strain DSM 4542, *Serratia ficaria* strain NBRC 102596 and different strains of *Tatumella terresea*. The isolate 16G showed 99.22% similarity with *Providencia rettgeri* strain NCTC 11801 and *Providencia stuartii* strain DSM 4539. Distance trees of isolates belong to family...
Enterobacteriaceae are shown in Figures 2, 3, 4, 5, and 6.

The results of 16S rRNA gene sequences reveal that Unknown isolate 19G gave 99.48% similarity with *Pseudomonas batumici* strain UCM B-321, *Pseudomonas sesami* strain SI-P133, *Pseudomonas baetica* strain a390, *Pseudomonas saponiphila* strain DSM 9751, *Pseudomonas moravensis* strain 1B4 and *Pseudomonas protegens* strain CHA0. The isolate 19G showed 98.22% similarity with different strains of *Pseudomonas tolaasii* and *Pseudomonas corrugate*.

The isolate 20G showed 99.48% similarity with *Pseudomonas prosekii* strain AN/28/1, different strains of *Pseudomonas chlororaphis*, different strains of *Pseudomonas savastanoi*, *Pseudomonas koreensis* strain Ps 9-14, *Pseudomonas cedrina* strain CFML 96-198 and *Pseudomonas kilonensis* strain 520-20. Distance trees of isolates belong to family *Pseudomonadaceae* were showed in Figures 7 and 8.

Also, the results indicated that unknown isolates 18G and 21G showed 99.74% and 98.64% similarity with *Agrobacterium salinitolerans* strain YIC 5082, *Agrobacterium fabrum* strain CS8, *Rhizobium pusense* strain NRCPB10, *Beijerinckia fluminensis* strain UQM 1685, *Rhizobium skiriniwicense* strain Ch11, different strains of *Agrobacterium tumefaciens* and *Rhizobium nepotum* strain 39/7, respectively. The isolates 18G and 21G showed 99.48% and 98.37% similarity with different strains of *Agrobacterium rubi*, respectively. Distance trees of isolates belong to family *Rhizobiaceae* represented in Figure 9.

**Fig. 2:** Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/Bacteria and Archaea _16S_ ribosomal RNA sequences database. cl|Query_216663,171121 and 24465 refers to the bacterial isolates 3G, 5G and 7G isolated from guava plant, respectively.
Fig.3: Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _16S_ ribosomal RNA sequences database. lcl|Query_89675 refers to the bacterial isolates 8G isolated from guava plant.

Fig.4: Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _16S_ ribosomal RNA sequences database. lcl|Query_68203 and 201293 refers to the bacterial isolates 10G and 13G isolated from guava plant, respectively.

Fig.5: Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _16S_ ribosomal RNA sequences database. lcl|Query_88713 refers to the bacterial isolates 14G isolated from guava plant.
Fig. 6: Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _16S_ ribosomal RNA sequences database. lcl|Query_200055 refers to the bacterial isolates 16G isolated from guava plant.

Fig. 7: Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _16S_ ribosomal RNA sequences database. lcl|Query_2611 refers to the bacterial isolates 19G isolated from guava plant.

Fig. 8: Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _16S_ ribosomal RNA sequences database. lcl|Query_46243 refers to the bacterial isolates 20G isolated from guava plant.
The ability of bacterial isolates to induce plant diseases:

All twelve isolates were examined for their ability to induce plant diseases using hypersensitive reaction test, the ability of isolates to induce galls and potato soft rot test (Table 2). The *Pectobacterium aroidearum* is the only species that has shown a positive result with a hypersensitive reaction test using tobacco plants (Figure 10). *Agrobacterium salinitolerans* was the only bacteria able to form small tumors in squash fruits (Figure 11). No evidence of formed hyperplastic syndrome on the tomato plants by *Agrobacterium salinitolerans* or any of these isolated bacteria were recognized (Figure 12). Findings of potato soft rot test confirmed that only *Pectobacterium aroidearum* have the potential to cause soft rot in potato slices after 72 h of incubation of the inoculated potato slices at 28 °C (Figure 13).

**Fig. 9:** Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _16S_ ribosomal RNA sequences database. lcl|Query_241109 and 89313 refers to the bacterial isolates 18G and 21G isolated from guava plant, respectively.
Table 2: Ability of bacterial isolates to induce plant diseases

<table>
<thead>
<tr>
<th>Isolates code</th>
<th>Identification by 16S rRNA sequence Analysis</th>
<th>Hypersensitive reaction on tobacco</th>
<th>Induce galls in squash fruits</th>
<th>Induce galls on tomato plants</th>
<th>Potato soft rot test</th>
</tr>
</thead>
<tbody>
<tr>
<td>3G</td>
<td>Enterobacter asburiae strain JM-458</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5G</td>
<td>Enterobacter asburiae strain JM-458</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7G</td>
<td>Enterobacter asburiae strain JM-458</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8G</td>
<td>Salmonella enterica subsp. salamae strain DSM 9220</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10G</td>
<td>Escherichia marmotae strain HT073016</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13G</td>
<td>Escherichia marmotae strain HT073016</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14G</td>
<td>Serratia nematodiphila strain DZ0503SBS1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16G</td>
<td>Pectobacterium aroidearum strain SCR1 109</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>18G</td>
<td>Agrobacterium salinitolerans strain YIC 5082</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19G</td>
<td>Pseudomonas batumici strain UCM B-321</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20G</td>
<td>Pseudomonas prosekii strain AN28/1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>21G</td>
<td>Agrobacterium salinitolerans strain YIC 5082</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 10: Hypersensitive reaction test on tobacco plants by *Pectobacterium aroidearum*

Fig. 11: Ability of *Agrobacterium salinitolerans* to induce galls in squash fruits. C = Control and Ag= *Agrobacterium salinitolerans* 21G.

Fig. 12: Ability of *Agrobacterium salinitolerans* to induce galls (nodules) in tomato plants. No tumors were formed on the tomato plants by *Agrobacterium salinitolerans* and control.

Fig. 13: Potato slices inoculated with sterile distilled water (Control) and *Pectobacterium aroidearum* (16G).
4. Discussion

The results of 16S rRNA gene sequences classified twelve isolates associated with guava decline in Egypt into seven genera belong to three families. The results showed that eight isolates belong to family Enterobacteriaceae, two isolates belong to family Rhizobiaceae and two isolates belong to family Pseudomonadaceae. Many members of family Enterobacteriaceae mainly from genera Erwinia, Pectobacterium, Dickeya, Pantoea, Enterobacter, and Brenneria reported to be Plant pathogens (Holden et al., 2009). Some Enterobacter species cause diseases for plants or humans, while others play important roles in biological control and as plant growth promoting bacteria (Zhu et al., 2011). Enterobacter asburiae is an opportunistic bacterium that causes human disease and does not pose any significant threat to humans (Koth et al., 2012). The results of 16S rRNA gene sequences showed that, the isolates coded as 3G, 5G and 7G in the present work showed 99.74 similarities with Enterobacter asburiae strain JM-458. Many strains of Enterobacter asburiae found inside many plant species and one of these strains induced early defense-related enzymes against Pectobacterium carotovorum subsp. carotovorum (Jefiyanon and Plianbangchang, 2013). Salmonella enterica subsp. enterica are responsible for 99% of salmonellosis cases in humans and warm-blooded animals. (Chlebicz and Slizewska, 2018). The isolate 8G provided 98.96% similarity with Salmonella enterica subsp. salamae strain DSM 9220. Salmonella usually enters agricultural environments via organic farmyard maturing. Animals can directly contaminate plants or surface water used for irrigation. Research indicates that Salmonella actively colonize plants and moves within plants and causes disease-like symptoms. Some plant pathogens bacteria like soft-rot bacteria promote proliferation of Salmonella in plants. Pseudomonas syringae and Xanthomonas campestris promote growth or survival of Salmonella on plants (Wiedemann et al., 2014). Many species of genus Escherichia have been recognized including Escherichia marmotae which was isolated from feces of the wild marmot (Liu et al., 2015). The isolates 10G and 13G showed 98.92 and 99.47% similarity with Escherichia marmotae strain HT073016. Some Escherichia species are capable to survive on plant surfaces and persist in the soil for long time (Jones et al., 2014). The isolate 14G offer 99.22% similarity with Serratia nematophila strain DZ0503SBS1. Some members of the genus Serratia have clinical importance and some other associated with insects and vertebrates. A root disease complex of alfalfa involving Fusarium sp., Pseudomonas sp., and Serratia marcescens biotype A4a (Grimont and Grimont, 2006). Serratia nematophila was isolated from the intestine of the nematode Heterorhabditidoides chongmingensis (Zhang et al., 2009). The isolate 16G showed 99.74% similarity with Pectobacterium aroidearum strain SCRI 109. Genus Erwinia was split into three genera: Erwinia, Pectobacterium, and Brenneria (Hauben et al., 1998). Members of the genus Pectobacterium cause soft rot disease in dicotyledonous and monocotyledonous plants. Pectobacterium aroidearum mainly cause soft rot diseases of monocotyledonous plants (Nabhan et al., 2013). The results revealed that unknown isolates 18G and 21G showed 99.74% and 98.64% similarity with Agrobacterium salinitolerans strain YIC 5082. Agro bacteria are members of the bacterial family Rhizobiaceae that cause crown gall and hairy root diseases on plant. The strains causing tumor were classified as Agrobacterium tumefaciens, the strains causing hairy root were classified as A. rhizogenes and the nonpathogenic strains were classified as A. radiobacter. Physiological and biochemical analyses revealed that agrobacteria, regardless of their virulence divided into three biovars. Each of the three Agrobacterium biovars could include tumorigenic, rhizogenic or nonpathogenic strains. 16S rDNA sequences analysis suggested transferring all the Agrobacterium spp. members into the Rhizobium spp. molecular-phylogenetic investigations revealed Agrobacterium rhizogenes as a member of Rhizobium spp., while other pathogenic taxa were considered Agrobacterium spp. The availability of complete genome sequencing facilities led to a revised phylogeny of the family Rhizobiaceae. Therefore, several strains were classified as new species i.e. Agrobacterium radiobacter, Agrobacterium rubi, Agrobacterium salinitolerans, Allorhizobium vitis (Agrobacterium vitis), Rhizobium rhizogenes. Taxonomy of “Agrobacterium tumefaciens species complex” (biovar 1) is subject to search due to the inhomogeneous nature of the species and the isolation of novel strains from various environments (Mafakhrieri et al., 2019). Agrobacterium salinitolerans isolated from root nodules of Sesbania cannabina grown in a high-salt and alkaline environment (Yan et al., 2017). The results of 16S rRNA gene sequences indicate that the isolate 19G provide 99.48% similarity with Pseudomonas batumici strain UCM B-321 and the isolate 20G showed 99.48% similarity with Pseudomonas prosekii strain AN/28/1. The genus Pseudomonas includes the microorganisms that occupy a wide range of niches. The genus Pseudomonas is distributed in soil, water and plant roots, and many of which are animal and plant pathogens (Notik et al., 2015). Pseudomonas batumici is antibiotic-producing bacteria isolated from soil of Black Sea coast and Pseudomonas prosekii ispsychrothrophic bacterium isolated from Antarctica (Kiprianova et al., 2011 and Kosina et al., 2013).
Enterobacter cloacae produced soft rot on potato tubers (Ashmawy et al., 2015). Pectobacterium aquoreum induced a hypersensitive reaction in tobacco plants and caused soft rot when inoculated in potato tubers (Moretti et al., 2016). The results in the present work indicated that Pectobacterium aquoreum is the only isolated species from guava trees with symptoms of guava decline that showed a positive result with a hypersensitive reaction test using tobacco plants and cause soft rot in potato slices. Introduction of pathogenic bacteria into plant tissue of non-host plants elicits hypersensitive reaction while the introduction of non-pathogenic bacteria into plant tissue does not result in the appearance of hypersensitive reaction (Willis et al., 1991). Most Agrobacterium strains do not elicit a hypersensitive response (Gohlke and Deeken, 2014). A weak hypersensitive reaction was observed after inoculation of tobacco plants with Enterobacter mori (Zhu et al., 2011). Live bacteria of Salmonella do not produce oxidative burst in tobacco while heat killed bacteria or Salmonella lipopolysaccharide are able to do so (Wiedemann et al., 2014). Inoculation of Serratia proteamuculans, Serratia marcescens and Serratia marinaurubra on tobacco produced a hypersensitivity reaction (Grimont and Grimont, 2006). A. tumefaciens induced tumors on squash fruits (Younis et al., 2016). In the present work, Agrobacterium salinitolerans was the only bacteria able to form small tumors in squash fruits. No evidence of formed hyperplastic syndrome on the tomato plants by Agrobacterium salinitolerans or any of these isolated bacteria were recognized. The results indicate that, despite difference in pathogen propensities, Pectobacterium aquoreum and Agrobacterium salinitolerans can get involved in guava decline syndromes either single or in combination, bearing in mind the role of other pathogens such as fungi and nematodes. Further studies are needed.

References


