Journal of American Science

Websites:
http://www.jofamericanscience.org
http://www.sciencepub.net

Emails:
editor@sciencepub.net
sciencepub@gmail.com

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Isolation and identification of bacteria associated with Guava decline in Egypt

A.F. Abd El-Rahman [1*], Naglaa M. Balabel [1 & 2] and Rabab, M. Abd -El–Aziz [1]

[1] Bacterial Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.
[2]Potato Brown Rot Project, Ministry of Agriculture and Land Reclamation, Dokki, Egypt.
*Corresponding author. A.F. Abd El-Rahman, E-mail address: aabdelrahman2012@gmail.com

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 completed 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 aroidearum* 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 aroidearum* have the potential to cause soft rot in potato slices. The results indicate that, despite difference in pathogen propensities, *Pectobacterium aroidearum* 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.
[A.F. Abd El-Rahman, Naglaa M. Balabel and Rabab, M. Abd -El–Aziz. **Isolation and identification of bacteria associated with Guava decline in Egypt.** *J Am Sci* 2020;16(3):51-62]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 7. doi: 10.7537/marsjas160320.07.

Keywords: Bacteria, 16S rRNA gene sequences, Guava decline, Egypt, *Pectobacterium aroidearum*, *Agrobacterium salinitolerans*.

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 Qaliubiya 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 *Meloidogyne enterolobii* and *Fusarium solani*, it is the major disease affecting guava trees, 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 Edkou 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 by16S 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-foled 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 1: Characteristics of primers used for PCR and sequencing

PCR target	Primer name	Sequence 5′-3′	Primer position	Reference
Bacterial 16s	U968-f	5′-AACGCGAAGAACCTTAC-3′	16S-968	**Felske *et al*., (1996)**
Bacterial 16s	L1401-r	5′-CGGTGTGTACAAGACCC-3′	16S-1401	

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 while50 µl of the bacterial cell suspensions 10^8 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 28ºC 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 (10^8 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 28ºC in a high humidity in greenhouse and the progress in the formation of galls or nodules was examined after one month of inoculation**.** The

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 (10^8 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.

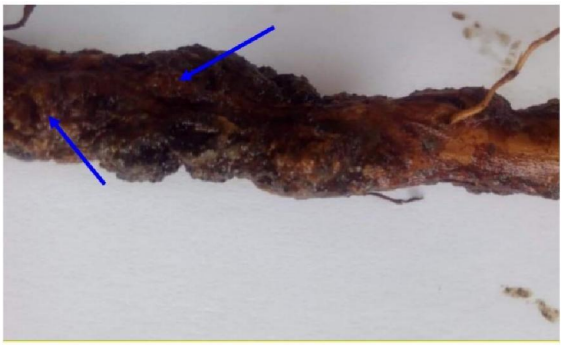

Fig.1: Rotten guava roots showing small galls (nodules), associated with symptoms of guava decline at Rashid territory of El-Behera government.

16S rRNA gene sequences analysis:

The results of 16S rRNA gene sequences of twelve isolates associated with guava decline in Egypt compared with the sequences of the Gen Bank DNA database using Nucleotide blast showed that these isolates were classified into seven genera belong to three families. The results showed that eight isolates belong to family *Enterobacteriaceae* from genera *Enterobacter*, *Salmonella*, *Escherichia*, *Serratia* and *Pectobacterium*. Two isolates these isolates belong to family *Rhizobiaceae* from genus *Agrobacterium* and two isolates belong to family *Pseudomonadaceae* from genus *Pseudomonas*, indicating intensive organic farmyard maturing common at these districts of concern.

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 showed99.47, 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 davisae* 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. *enterica* 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. *sakuensis* 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 davisae* strain DSM 4568. The isolate 14G showed 98.44% similarity with *Enterobacter soli* ATCC BAA-2102 strain LF7and *Enterobacter asburiae* strain JM-458.

The isolate 16G showed 99.74% similarity with *Pectobacterium aroidearum* strain SCRI 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 terrea*. 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, 4,5and 6.

The results of 16S rRNA gene sequences reveal that Unknown isolate 19G give 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 moraviensis* 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 21Gshowed 99.74% and 98.64% similarity with *Agrobacterium salinitolerans* strain YIC 5082, *Agrobacterium fabrum* strain C58, *Rhizobium pusense* strain NRCPB10, *Beijerinckia fluminensis* strain UQM 1685, *Rhizobium skierniewicense* 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*a represented in Figure 9.

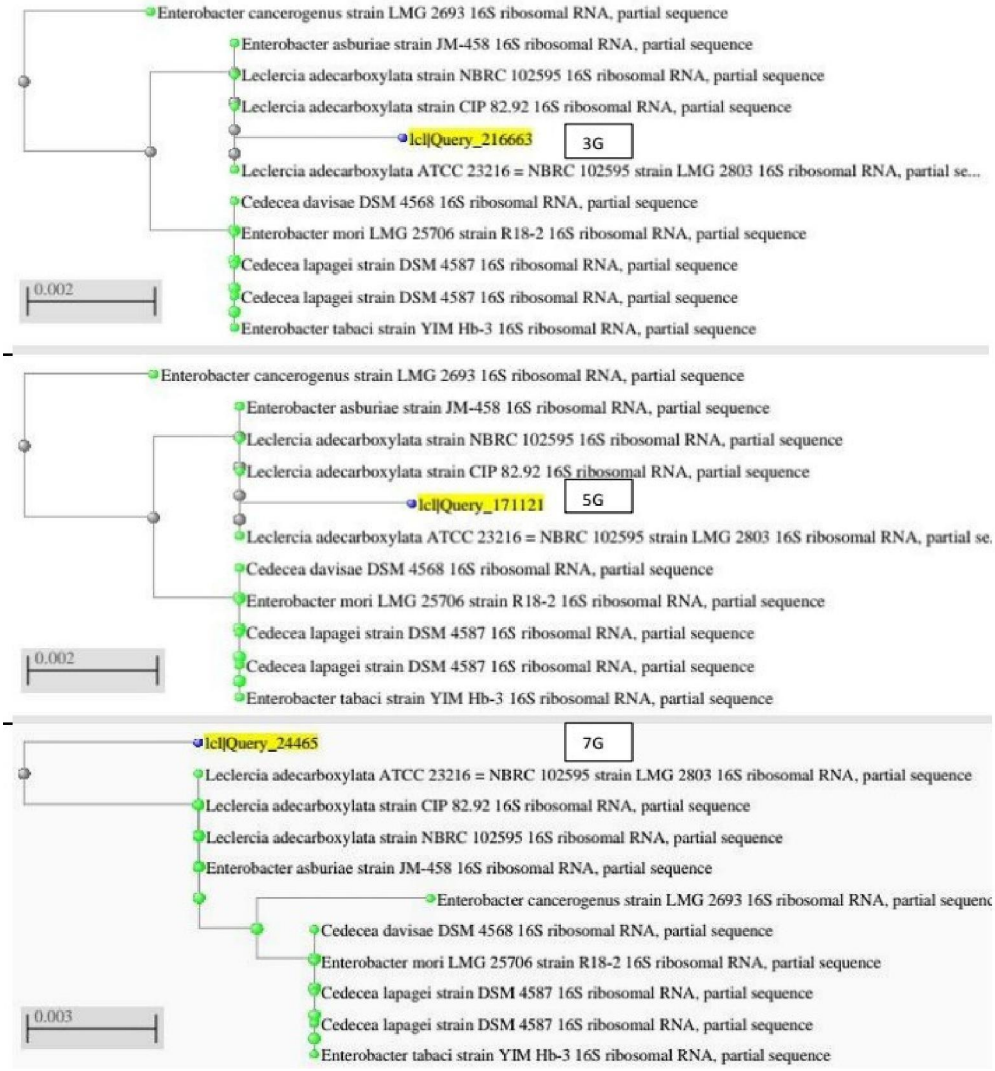

Fig. 2: Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _16S_ ribosomal RNA sequences database. lcl|Query_216663,171121 and 24465 refers to the bacterial isolates 3G, 5G and 7G isolated from guava plant, respectively.

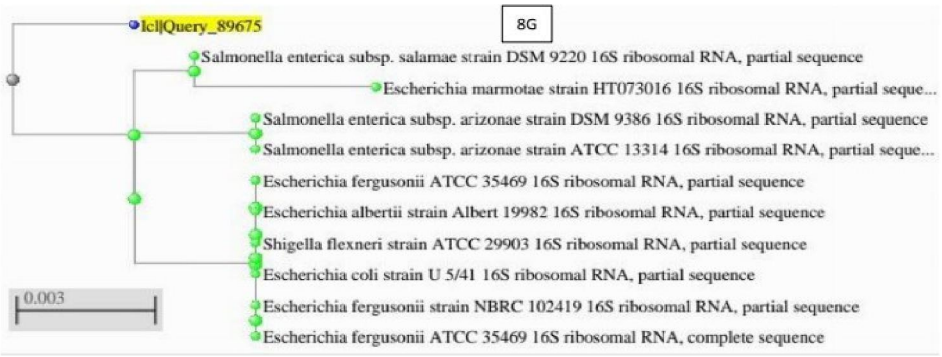

Fig.3: Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _ 16S_ ribosomal RNA sequences database. lcl|Query_89675 refers to the bacterial isolates 8G isolated from guava plant.

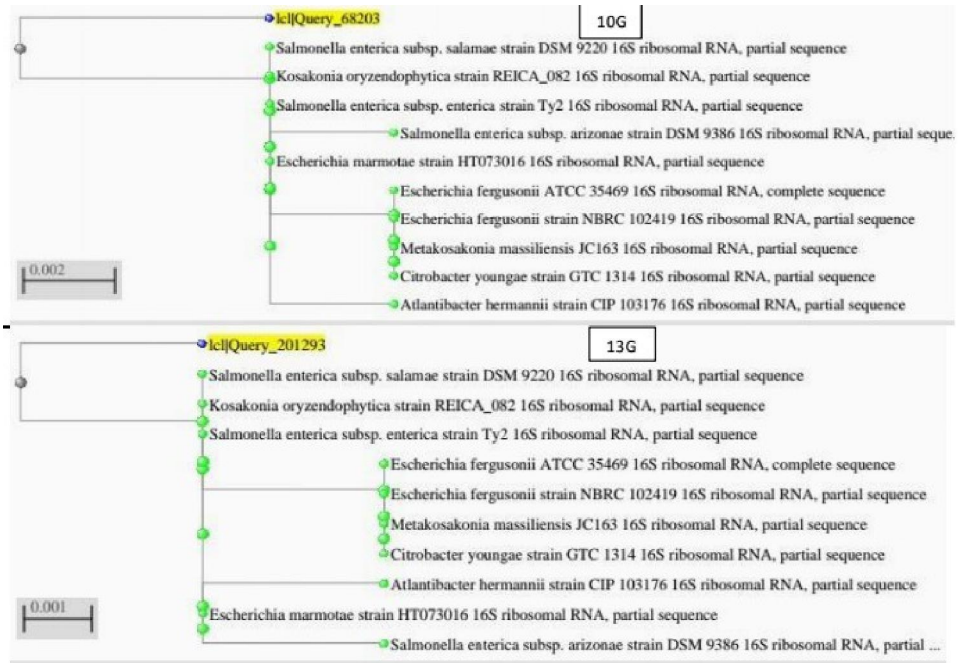

Fig.4: Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _ 16S_ ribosomal RNA sequences database. lcl|Query_68203 and201293refers to the bacterial isolates 10G and 13G isolated from guava plant, respectively.

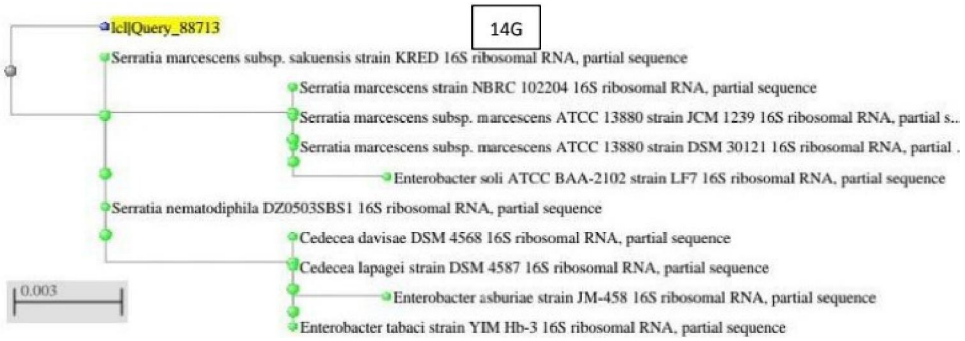

Fig.5: Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _ 16S_ ribosomal RNA sequences database. lcl|Query_88713 refers to the bacterial isolates 14G isolated from guava plant.

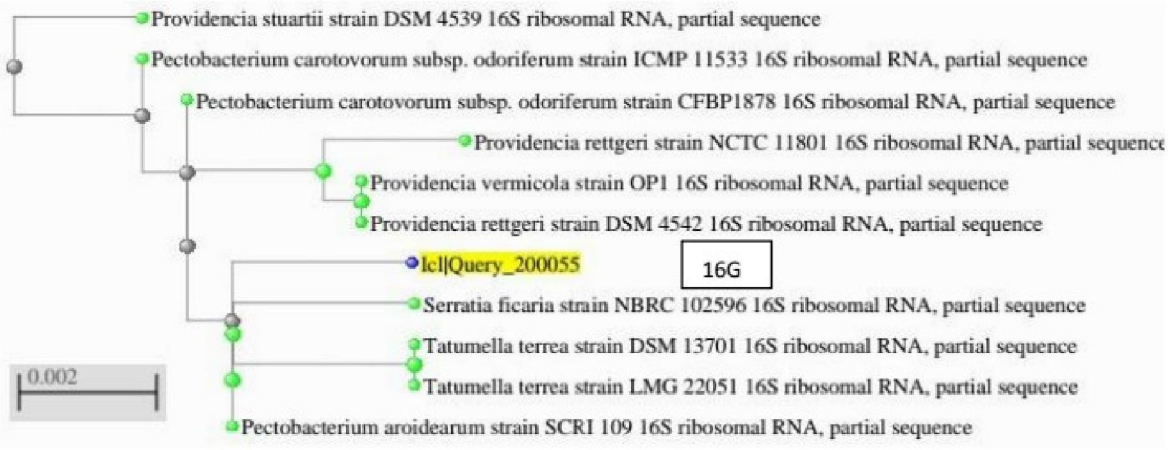

Fig. 6: Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _ 16S_ ribosomal RNA sequences database. lcl|Query_200055 refers to the bacterial isolates 16G isolated from guava plant.

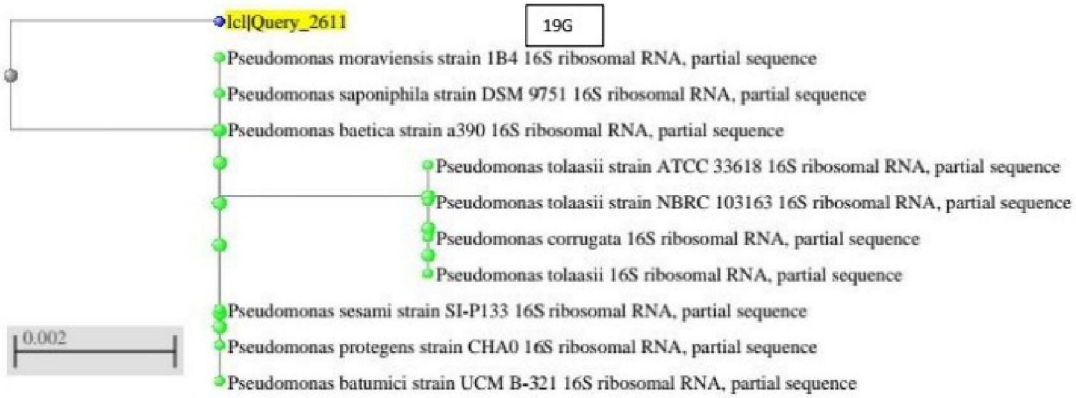

Fig. 7: Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _ 16S_ ribosomal RNA sequences database. lcl|Query_ 2611 refers to the bacterial isolates 19G isolated from guava plant.

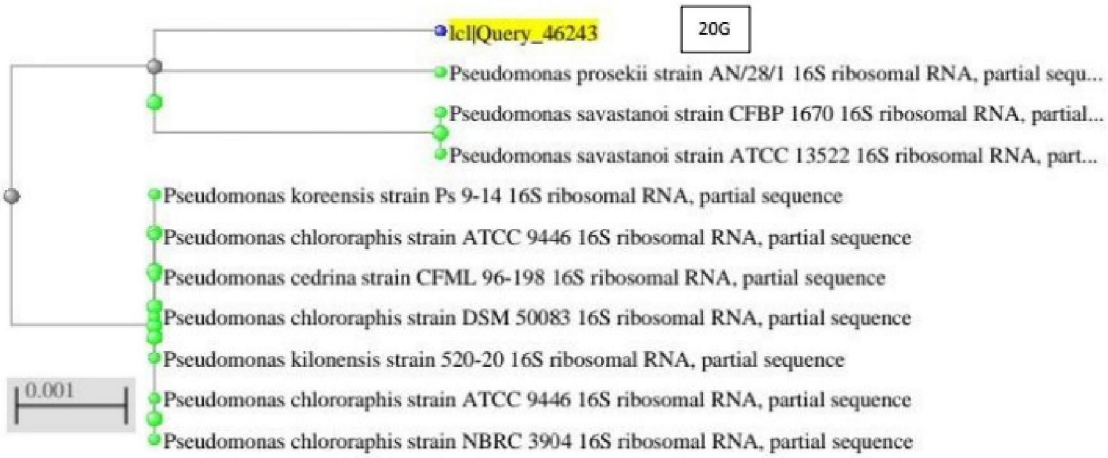

Fig. 8: Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _ 16S_ ribosomal RNA sequences database. lcl|Query_ 46243 refers to the bacterial isolates 20G isolated from guava plant.

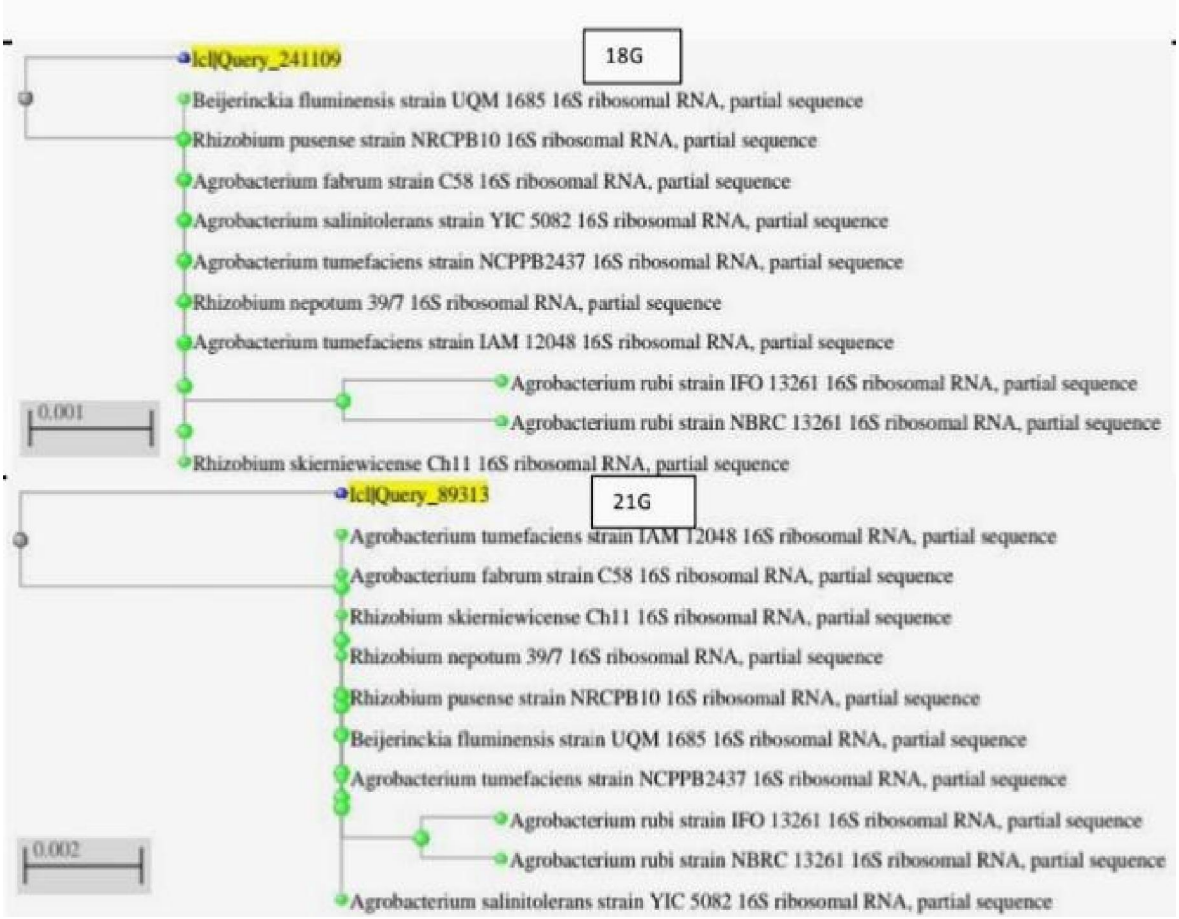

Fig. 9: Distance tree produced by BLAST pair wise alignments using neighbor joining tree methods using rRNA type strains/ Bacteria and Archaea _ 16S_ ribosomal RNA sequences database. lcl|Query_241109 and 89313 refers to the bacterial isolates 18G and 21G isolated from guava plant, respectively.

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).

Table 2: Ability of bacterial isolates to induce plant diseases

Isolates code	Identification by 16S rRNA sequence Analysis	Hypersensitive reaction on tobacco	induce galls in squash fruits	induce galls on tomato plants	Potato soft rot test
3G	*Enterobacter asburiae* strain JM-458	-	-	-	-
5G	*Enterobacter asburiae* strain JM-458	-	-	-	-
7G	*Enterobacter asburiae* strain JM-458	-	-	-	-
8G	*Salmonella enterica* subsp. *salamae* strain DSM 9220	-	-	-	-
10G	*Escherichia marmotae* strain HT073016	-	-	-	-
13G	*Escherichia marmotae* strain HT073016	-	-	-	-
14G	*Serratia nematodiphila* strain DZ0503SBS1	-	-	-	-
16G	*Pectobacterium aroidearum* strain SCRI 109	+	-	-	+
18G	*Agrobacterium salinitolerans* strain YIC 5082	-	+	-	-
19G	*Pseudomonas batumici* strain UCM B-321	-	-	-	-
20G	*Pseudomonas prosekii* strain AN/28/1	-	-	-	-
21G	*Agrobacterium salinitolerans* strain YIC 5082	-	+	-	-

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

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.

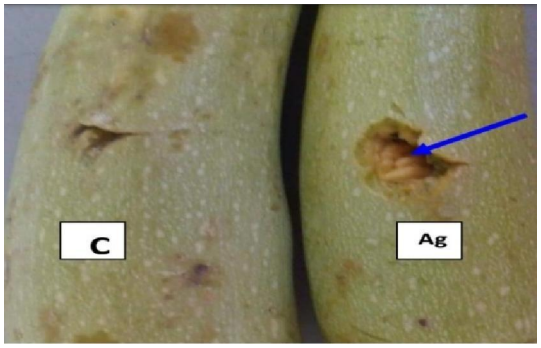

Fig. 11: Ability of *Agrobacterium salinitolerans* to induce galls in squash fruits. C = Control and Ag= *Agrobacterium salinitolerans* 21G.

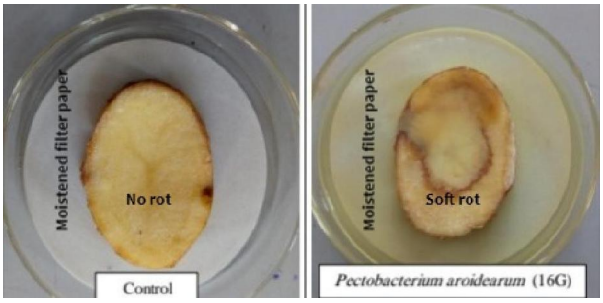

Fig. 13: Potato slices inoculated with sterile distilled water (Control) and *Pectobacterium aroidearum* (16G).

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* **(Jetiyanon 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 nematodiphila* 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 nematodiphila* 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 tore-search due to the inhomogeneous nature of the species and the isolation of novel strains from various environments **(Mafakheri *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 **(Novik *et al*., 2015)**. *Pseudomonas batumici* is antibiotic-producing bacteria isolated from soil of Black Sea coast and *Pseudomonas prosekii* ispsychrotrophic 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 aroidearum* 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 aroidearum* 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 proteamaculans*, *Serratia marcescens* and *Serratia marinorubra* 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 aroidearum* 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

1. Abd- El -Aziz, R. M. (2011). Factors affecting virulence of *Agrobacterim tumefaciens* the causal of crown gall disease. Ph. D. Thesis, Faculty of Agriculture, Cairo University, Egypt.
2. Abd El-Ghany, H., Moussa, Z., Salem, E. A. and Abd El-Rahman, A. F. (2017). Management of Potato Soft Rot by Gamma Irradiation. Arab J. Nucl. Sci. Appl, 50(3):159-173.
3. Ansar, M., Saleem, A. and Iqbal, A. (1994). Cause and control of guava declin the - Punjab (Pakistan). Pak. J Phytopath., 6: 41-44.
4. Ashmawy, N. A., Jadalla, N. M., Shoeib, A. A. and El-Bebany, A. F. (2015). Identification and genetic characterization of *Pectobacterium* spp. and related *Enterobacteriaceae*c a using potato soft rot diseases in Egypt. Journal of Pure and Applied Microbiology, 9:1847–1858.
5. Atawia, A. A. R., El-Gendy, F. M. A., Bakry, Kh. A. I., Abd El-Ghany, N. A. and Singer M. A. A. (2017). Physiological studies on flowering and fruiting of guava trees. Middle East Journal of Agriculture Research, 6 (1), 143-151.
6. Chlebicz, A., and Slizewska, K. (2018). Campylobacteriosis, salmonellosis, yersiniosis, and listeriosis as zoonotic foodborne diseases: a review. International Journal of Environmental Research and Public Health, 15: E863. doi: 10.3390/ijerph15050863
7. Felske, A., Engelen, B., Nubel, U. and Backhaus, H. (1996). Direct ribosome isolation from soil to extract bacterial rRNA for community analysis. Applied and Environmental Microbiology, 62, 4162-4167.
8. Gohlke, J., and Deeken, R. (2014). Plant responses to *Agrobacterium tumefaciens* and crown gall development. Front. Plant Sci., 5:155.
9. Gomes, V. M., Souza, R. M., Almeida, A. M., & Dolinski, C. (2014). Relationships between *M. enterolobii* and *F. solani*: spatial and temporal dynamics in the occurrence of guava decline. Nematoda, 1, e01014,1-5.
10. Gomes, V. M., Souza, R. M., Midorikawa, G., Miller, R., & Almeida, A. M. (2012). Guava decline: evidence of nationwide incidence in Brazil. Nematropica, 42, 153-162.
11. Gomes, V. M.; Souza, R. M.; Silveira, S. F.; Almeida A. M. (2013). Guava Decline: Guava decline: effect of root exudates from *Meloidogyne enterolobii*-parasitized plants on *Fusarium solani in vitro* and on growth and development of guava seedlings under controlled conditions. European Journal of Plant Pathology,137(2), 393-401.
12. Gomes, V. M., Souza, R. M., Mussi-Dias, V., Silveira, S. F., Dolinski, C. (2011). Guava decline: a complex disease involving *Meloidogyne mayaguensis* and *Fusarium solani*. Journal of Phytopathology, 158, 45-50.
13. Grimont, F. and Grimont, P. A. D. (2006). The genus *Serratia*. Prokaryotes,6:219-244.
14. Hauben, L., Moore, E. R., Vauterin, L., Steenackers, M., Mergaert, J., Verdonck, L. and Swings, J. (1998). Phylogenetic Position of Phytopathogens within the *Enterobacteriaceae* Syst. Appl. Microbiol., 21: 384-397.
15. Hiddink, G. A., Termorshuizen, A. J., Raaijmakers, J. M. and van Bruggen, A. H. C. (2005). Effect of mixed and single crops on

disease suppressiveness of soils. Phytopathology, 95,1325-1332.
16. Holden, N., Pritchard, L. and Toth, I. (2009). Colonization out with the colon: plants as an alternative environmental reservoir for human pathogenic enterobacteria. FEMS Microbiol. Rev., 33(4):689-703.
17. Jetiyanon, K. and Plianbangchang, P. (2013). Lipopolysaccharides of *Enterobacter asburiae* strain RS83: a bacterial determinant for induction of early defensive enzymes in *Lactuca sativa* against soft rot disease. Biological Control, 67(3):301–307.
18. Jones, L. A., Worobo, R. W. and Smart, C. D. (2014). Plant-pathogenic oomycetes, *Escherichia coli* strains, and *Salmonella* spp. frequently found in surface water used for irrigation of fruit and vegetable crops in New York State. Appl. Environ. Microbiol.,80 (16): 4814–4820.
19. Kiprianova, E. A., Klochko, V. V., Zelena, L. B., Churkina, L. N. and Avdeeva, L. V. (2011). *Pseudomonas batumici* sp. nov., the antibiotic-producing bacteria isolated from soil of the Caucasus Black Sea coast. Mikrobiol. Z., 73: 3-8.
20. Kosina, M., Bartak, M., Maslanova, I., Vavrova-Pascutti, A., Sedo, O., Lexa, M. and Sedlacek, I. (2013). *Pseudomonas prosekii* sp. nov., a novel psychrotrophic bacterium from Antarctica. Curr. Microbiol., 67(6):637–664.
21. Koth, K., Boniface, J., Chance, E. A. and Hanes, M. C. (2012). *Enterobacter asburiae* and *Aeromonas hydrophila*: soft tissue infection requiring debridement. Orthopedics, 35 (6): e996-e999.
22. Liu, S., Jin, D., Lan, R., Wang, Y., Meng, Q., Dai, H., Lu, S., Hu, S., and Xu, J. (2015). *Escherichia marmotae* sp. nov., isolated from faeces of *Marmota himalayana*. Int. J. Syst. Evol. Microbiol., 65:2130–2134.
23. Mafakheri, H., Taghavi, S. M., Puławska, J., de Lajudie, P., Lassalle, F., and Osdaghi E. (2019). Two Novel Genomospecies in the *Agrobacterium tumefaciens* Species Complex Associated with Rose Crown Gall. Phytopathology. 109: 1859-1868.
24. Moretti, C., Fakhr, R., Cortese, C., De Vos, P., Cerri, M., Geagea, L., Cleenwerck, I., and Buonaurio, R. (2016). *Pectobacterium aroidearum* and *Pectobacterium carotovorum subsp. carotovorum* as causal agents of potato soft rot in Lebanon. Plant Pathology, 144: 205–211.
25. Nabhan, S., De Boer, S. H., Maiss, E., and Wydra, K. (2013). *Pectobacterium aroidearum* sp. nov., a soft rot pathogen with preference for monocotyledonous plants. Int. J. Syst. Evol. Microbiol., 63:2520-2525.
26. Novik, G., Savich, V., and Kiseleva, E. (2015). "An insight into beneficial *Pseudomonas* bacteria," in: Microbiology in Agriculture and Human Health, ed M. M. Shah (InTech), 73–105.
27. Queiroz, D. L., Wrege, M. S., kunast, T. B. S., Garrastazu, M. C. and Burckhardt, D. (2018). Potential distribution of the guava *psyllid Triozoida* limbata (Hemiptera, Psylloidea), today and in global climate change scenarios. Turkish Journal of Zoology, 42 (3), 330-336.
28. Safdar, A., Khan, S. A. and Safdar, M. A. (2015). Pathogenic association and management of *Botryodiplodia theobromae* in guava orchards at Sheikhupura district, Pakistan. Int. J. Agric. Biol., 17:297-304.
29. Vanneste, J. L., Pauln, J.-P. and Expert, D. (1990). Bacteriophage Mu as a genetic tool to study *Erwinia armylovora* pathogenicity and hypersensitive reaction on tobacco. J. Bacteriol. 172:932-941.
30. Wiedemann, A., Virlogeux-Payant, I., Chausse, A. M., Schikora, A., and Velge, P. (2014). Interactions of *Salmonella* with animals and plants. Frontiers in Microbiology, 5:791. doi: 10.3389/fmicb.2014.00791
31. Willis, D. K., Rich, J. J. and Hrabak, E. M. (1991). hrp genes of phytopathogenic bacteria. Mol. Plant Microbe In., 4(2): 132–138.
32. Yan, J., Li, Y., Yan, H., Chen, W. F., Zhang, X., Wang, E. T., Han, X. Z. and Xie, Z. H. (2017). *Agrobacterium salinitolerans* sp. nov., a saline–alkaline-tolerant bacterium isolated from root nodule of *Sesbania cannabina*. Int. J. Syst. Evol. Microbiol., 67:1906–1911.
33. Younis, A. M., Shoeib, A. A., Elsaedy, M. A. and Osman, K. A. (2016). Efficacy of Ozone and Hydrogen Peroxide on Controlling Crown Gall Bacterium and Root Knot Nematode Infected Guava Plants in Egypt. Alex. J. Agric. Sci.,61(6): 517-527.
34. Zaitoun, F. M., Hamad, Y. K., Fahmi, M. M. and Ziyada, S. M. (2015). Role of certain bioagents against Guava decline disease and in enhancement of the growth of guava trees. J. of Phytopathology and Pest Management,2(3):43-54.
35. Zhang, C. X., Yang, S. Y., Xu, M. X., Sun, J., Liu, H., Liu, J. R., Liu, H., Kan. F., Sun, J., Lai, R., and Zhang, K. Y. (2009). *Serratia nematodiphila* sp. nov., associated symbiotically with the entomopathogenic nematode *Heterorhabditidoides chongmingensis* (Rhabditida: Rhabditidae). Int. J. Syst. Evol. Microbiol., 59: 1603-1608.

36. Zhu, B., Lou, M. M., Xie, G. L., Wang, G. F., Zhou, Q., Wang. F., Fang, Y., Su, T., Li, B., and Duan, Y. P. (2011). *Enterobacter mori* sp. nov., associated with bacterial wilt on *Morus alba* L. International Journal of Systematic and Evolutionary Microbiology, 61:2769–2774.

2/26/2020