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Bacterial blight disease caused by *Pseudomonas cichorii* on chrysanthemum in Egypt

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Abstract

Chrysanthemum (*Dendranthema grandiflorum*) is one of the important cut flowers and pot plants which belong to family Asteraceae. The aim of this study is to isolate and identify causal pathogens of Chrysanthemum bacterial blight disease, samples showing typical symptoms of bacterial blight disease on leaves, buds, stems or flowers obtained from different geographical areas of Egypt were used. The most conspicuous symptoms in infected leaves appear as water-soaked spots then become brown and dry. Ten bacterial isolates were isolated from different parts of Chrysanthemum which collected from different localities. In this respect, the bacterial isolates designated as Wl-1 and Wb-2 which were isolated from leaves and buds respectively of chrysanthemum white variety in Qualubia (Moshtohor). Meanwhile, the isolates coded as Ps-3 and Pf-4 were isolated from stems and flowers of chrysanthemum purple variety respectively in the same governorate (El-kanater El-khairia), the bacterial isolates coded as Ws-5 and Wf-6 were isolated from stems and flowers of chrysanthemum white variety planted in Menoufia governorate (Shebeen El-kom). While, the isolates coded as Yl-7 and Yb-8 were isolated from leaves and buds of chrysanthemum yellow variety respectively in Giza governorate (Kirdasah). The isolate Rs- 9 and Rf-10 were isolated from stems and flowers of chrysanthemum red variety from the same governorate (Elmariotia). Identification of isolated bacteria using the traditional techniques according to their inspected morphological, cultural characteristics, biochemical and physiological characteristics, these traditional tests revealed that these isolates may be belong to three genera i.e., Wl-1, Pf-4 and Rs-9 could be identified as *Pseudomonas cichorii*, while, the isolate Yb-8 could be identified as *Pseudomonas fluorescens* while the isolate Wf-6 was identified as *Bacillus subtilis*. The present study examined the level of genetic diversity and its molecular variation of the bacterial blight disease caused by *Pseudomonas cichorii*, three isolates from five geographic regions in Egypt. In addition, the isolates were pathogenic to eight plants from different plant families by artificial inoculations. This bacterium has a wide host range and this work is important for cataloging plant pathogenic bacteria that occur throughout special conditions may become epidemic in Egypt.

Keywords: bacterial blight, *Pseudomonas cichorii*, *Dendranthema grandiflorum*, PCR, identification, host range.

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1. Introduction

The cut flower industry is expanding worldwide and Egypt. Chrysanthemum (*Dendranthema grandiflorum*) is a perennial herb grown well in Egypt. It is highly attractive and charming short-day plant, which behaves both as an annual as well as perennial flowering herb (Arora, 1990). There are about 160 species of chrysanthemum. The inflorescences which call a flower head are greatly required in markets because of its beautiful shape and longevity in vases. The chrysanthemum plants are flowering only under short day conditions. The importance role of chrysanthemum is grown both as potted plants and as cut flowers during the fall months when the other flowers are scarce to supply the flower markets. They are the best keeping flowers for home use, and one of the most adaptable to design work. Chrysanthemum widely used in two types namely standard "Art Queen and White Zambia" (Elnemr, 2018). Chrysanthemum is vegetative propagated flowering plant and is affected by many diseases caused by fungi like vascular wilt diseases caused by *Fusarium oxysporum* f.sp. *chrysanthemi* and *Verticillium dahlia* (Dreistadt, 2001) and bacterial diseases severely reducing the crop yields were bud blight (*Pseudomonas syringae*), bacterial wilt (*Erwinia chrysanthemi*) (Vegh *et.al.*, 2014). The major bacterial diseases severely reducing the crop yields stem necrosis (*Pseudomonas cichorii*) according to (Shamala & Janardhana, 2018). Bacterial blight caused by Pseudomonas species affects a broad range of ornamentals and horticultural crops including Gerbera, Tulip, Cabbage, Coriander *etc.* (Wehlburg, 1963). A sudden appearance of bacterial blight disease in Egypt is of serious concern for Chrysanthemum growers as it directly affects the quality of cut flowers. Stead *et al.* (2013) reported that *Pseudomonas cichorii* have a wide host range affecting high economic importance plants from different family including tomato, lettuce and watermelon. The pathogen was isolated in Florida on chrysanthemum, celery, geranium, and hibiscus recently on *Stevia rubidian* (Strayer *et al.*, 2012) and *Duranta erecta* (Gumtow *et al*,. 2013). The bacterium is widely distributed in worldwide which recent reports from Belgium (Cottyn *et al.* 2009), Greece (Trantas *et al.*, 2013), Italy (Scortichini *et al.*, 2002), Turkey (Aysan *et al.*, 2003), Tanzania (Testen *et al.*, 2015). In recent years, many new marker techniques have been developed in line with the rapid growth of genomic research. Start Codon Targeted (SCoT) polymorphisms are dominant and reproducible markers that are based on the short-conserved region in plant genes surrounding the ATG translation start (or initiation) codon and use a single 18-mer primer in the polymerase chain reaction (PCR) assays and higher annealing temperature (50°C) (Collard & Mackill, 2009). SCoT markers are generally reproducible, and it is suggested that primer length and annealing temperature are not the sole factors determining reproducibility. SCoT markers have been successfully used to assess genetic diversity and structure, identify cultivars, and for quantitative trait loci (QTL) mapping and DNA fingerprinting in different species (Caho *et al.*, 2014; Amirmoradi *et al*., 2012). The level of genetic diversity and its molecular variation of the bacterial blight disease caused by *Pseudomonas cichorii,* three isolations from five geographic regions in Egypt. The purposes of this study were: (a) to assess the genetic diversity and phylogenetic relationship among three *Pseudomonas cichorii* isolates and (b) to examine the effectiveness of the SCoT

markers in *Pseudomonas cichorii* genetic diversity study. These results could facilitate *Pseudomonas cichorii* isolation, conservation and future microbial Identification. In this paper, we reported the pathogenic and genetic diversity found, using traditional and Start Codon Targeted (Scot) technique, among the different bacterial blight pathogen isolates obtained from Chrysanthemum orchards collected from different geographical areas of Egypt.

2. Materials and methods

2.1 Sampling and isolation of causal organism

Diseased samples of Chrysanthemum *i.e.* flowers, leaves, stems and buds with typical bacterial blight symptoms were collected from various locations of Qalubia, Menoufia and Giza governorates, Egypt, in the autumn of 2016 season. Collected samples of different chrysanthemum varieties were transferred to the laboratory in plastic bags, kept in a refrigerator at 7°C, where each sample was kept alone for further studies. Isolation and identification procedures carried out in Plant Pathology Department, Faculty of Agriculture, Benha University, Moshtohor, Egypt. The infected leaves collected were washed with tap water and then air dried. Bacterial lesions were then cut and surface disinfected by first dipping in 95% ethanol for 5 sec., then sodium hypochlorite solution 1.25% for 20 sec. followed by two successive rinses in sterile water. Each lesion was homogenized in 2.0 ml sterilized distilled water. The homogenate was then streaked onto nutrient agar medium. Five colonies from each lesion were selected and retained randomly. A loopful of the resulting suspension was streaked on the surface of nutrient agar (NA) and King's B media. These plates were incubated at 28 ± 2°C for 2-3 days. Observations were daily recorded and any emerged colony was picked up and transferred to nutrient glucose agar slant medium for maintenance till use in subsequent tests. All picked colonies were purified using the single colony technique (Fahy & Persley, 1983).

2.2 Pathogenicity test and hypersensitivity reaction

Bacterial isolates were grown at yeast dextrose carbonate agar (YDC) medium and were scraped with a sterile pin. Chrysanthemum seedlings were inoculated with bacterial inoculum (10^8 cfu/ ml) as foliar spray until it run off. Other plants were untreated as control treatment. The inoculated seedlings were kept moist under greenhouse conditions until appearance of symptoms (Shamala & Janardhana, 2018). Hypersensitivity test was carried out on Seedlings of tobacco (*Nicotiana tabaccum*) which were inoculated with infiltrating bacterial suspension (10^8 cfu/ mL) using a fine syringe into the intercellular spaces of the lower side of the leaves. Characteristic HR symptoms were recorded at 48 h. In order to fulfill Koch's postulates, disease symptoms were observed and the pathogen re-isolated in culture medium.

2.3 Identification of isolated bacteria

2.3.1 Using the traditional techniques

Identification of the bacterial isolates

were conducted on the basis of their morphological, cultural and physiological characteristics according to schemes suggested by Fahy and Persley (1983), Krieg and Holt (1984) and Lelliott and Stead (1987).

2.3.2 Molecular identification using SCoT- PCR technique

2.3.2.1 Bacterial DNA Extraction

DNA was extracted from the three *Pseudomonas cichorii* (Wl-1, Pf-4 and Rs-9) isolates using DNeasy Tissue Mini Kit (QIAGEN). The concentration of DNA was then determined based on a comparison of the DNA samples with standard lambda DNA on 1% (w/v) agarose gel, after which it was adjusted to 5 ng/µl.

2.3.2.2 SCoT-PCR amplification and detection

The identification techniques were done in Molecular biology Laboratory, Agricultural Research Park, Faculty of Agriculture, Benha University, Qalubia, Egypt. Six SCoT primers were randomly selected (Table 1). SCoT-PCR reaction volume was 25 µL, containing 1.5 µL of template DNA (25 ng/µL), 1.0 µL primer at 10 µM, 2.0 µL dNTPs at 10 µM, 0.125 µL Taq DNA polymerase at 5 U/µL, 2.5 µL I0X PCR buffer, and 17.875 µL ddH2O. SCoT-PCR was performed on an TProfessional PCR mechanics (Biometra, Germany). Initial denaturation was carried out at 94 °C for 5 min, followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 2 min, and final extension at 72°C for 5 min. The amplification products were separated in 1.5% agarose gels containing 0.5 µg/mL of ethidium bromide through electrophoresis in IX TBE buffer solution at 5 V/cm and photographed by gel documentation system, Fair Reader - Gel Doc.55+ with Image Lab Software with A 100 bp DNA plus ladder was used as a molecular size standard (El-Shaer *et al.,* 2014).

Table 1: The sequences of the six used SCoT primers.

Primer name	Sequence (5'-3')
SCoT-1	CGACATGGCGACCACGC
SCoT-2	ACCATGGCTACCACCGGC
SCoT-3	CGACATGGCGACCCACA
SCoT-4	ACCATGGCTACCACCGCA
SCoT-5	CAATGGCTACCACTAGCG
SCoT-6	CAATGGCTACCACTACAG

2.3.2.3 Data Analysis

Similarity coefficients were calculated according to dice matrix (Rohlf, 1993; Nei & Li, 1979). Parents were grouped by cluster analysis with the similarity matrix and unweighted pair group method based on arithmetic mean (UPGMA).

2.4 Host range of *Pseudomonas cichorii* bacterium

In this trial, bacterial isolates of *P. cichorii* were tested for their pathogenic reactions on different host plants from different plant families. The isolates were tested for pathogenic reactions on eight different cultivars from four different plant families *i.e.,* Chrysanthemum cv. White Zambla (*Chrysanthemum grandiflorum*) and Lettuce cv. Balady (*Lactuca saliva*) from family *Asteraceae,* cabbage cv. Sabeany *(Brassica oleracea),* from family *Brassicaceae,* Mint cv. Balady (*Mentha viridis*), from family *Lamiaceae* and pepper cv. California wander *(Capsicum annuum),*

tomato cv. super strain b (*Solanum lycopersicum*), Datura cv. Balady (*Datura metel*), eggplant cv. Balady long white (*Solanum melongena*) from family *Solanaceae* under greenhouse conditions by injecting 0.2 ml of the previously prepared bacterial suspension (10^8 cfu/ml) in the tip of growing transplants using a fine hypodermic syringe. All seedlings were covered with plastic sheet for 24 hrs. Disease symptoms were recorded after 15 days post inoculation (Marques *et al.*, 2016).

3. Results

3.1 Disease symptoms

The bacterial blight disease occurs on leaves, stem, buds and flower. The most conspicuous symptoms in infected leaves appear as water-soaked spots then become brown and dry that are often bordered or ringed by yellowing tissue. Moreover, the symptoms on stem appear darkened areas often at the base of buds. In contrast, the disease appears on flowers in blast symptoms**.**

3.2 Sampling and isolation of causal organism

Data in Table (2) showed that ten bacterial isolates were isolated from different parts of Chrysanthemum plants which were collected from different localities of Egypt. Four different isolates from Qualubia governorate (Moshtohor and El-kanater El-khairia) were isolated from white and purple variety (leaf, bud, stem and flower) which designated as Wl-1, Wb-2, Ps-3 and Pf-4. while, two isolates from Menoufia governorate (Shebeen El-kom) were isolated from white variety (stem and flower) which coded as Ws-5 and Wf-6. In this respect, four different isolates from Giza governorate (Kirdasah and Elmariotia) were isolated from yellow and red variety (leaf, bud, stem and flower) which coded as Yl-7, Yb-8, Rs-9 and Rf-10.

Table 2: Source of bacterial isolates which isolated from chrysanthemum plants which collected from different governorates, during growing season 2016.

Governorate	Locality	Variety	Infected sample	Isolate code
Qualubia	Moshtohor	White	leaf	Wl-1
			bud	Wb-2
	El kanater El khairia	Purple	stem	Ps-3
			flower	Pf-4
Menoufia	Shebeen El-kom	White	stem	Ws-5
			flower	Wf-6
Giza	Kirdasah	Yellow	leaf	Yl-7
			bud	Yb-8
	Elmariotia	Red	stem	Rs-9
			flower	Rf-10

3.3 Pathogenicity test and hypersensitivity reaction

In this experiment, ten bacterial isolates were examined for their reaction on different hosts tobacco and chrysanthemum (white, yellow, red and purple, CVs) plants. In this respect, data in Table (3) reveal that Pf-4 and Rs-9 isolates produced HR on tobacco and pathogenic to Chrysanthemum (white, yellow, red and purple, CVs) plants.

Table 3: Hypersensitive reaction and virulence of tested bacterial isolates on differential hosts.

Code of isolate	Reaction				
	Tobacco	Chrysanthemum			
	HR	White	Yellow	Red	Purple
Wl-1	++	++	++	++	++
Wb-2	-	-	-	-	-
Ps-3	-	-	-	-	-
Pf-4	+	+	+	+	+
Ws-5	-	-	-	-	-
Wf-6	+	-	-	-	-
Yl-7	-	-	-	-	-
Yb-8	+	-	-	-	-
Rs-9	+	+	+	+	+
Rf-10	-	-	-	-	-

Meanwhile, Wl-1 isolate was highly virulence on tobacco and highly pathogenic to all Chrysanthemum varieties used. Wf-6 and Yb-8 isolates were pathogenic only on tobacco plants. Meantime, Wb-2, Ps-3, Ws-5, Yl-7 and Rf-10 isolates were not pathogenic on all tested hosts. Symptoms on the tested hosts were recorded clearly on tobacco seedlings, appeared as water-soaking of inoculated tissue with 48 h. then dryness, light-brown localized necrosis with 3 days. Meantime, on chrysanthemum plants, appeared as brown spots in first on inoculated leaves then become blight, small dark lesion at stem and flower blast on all varieties.

3.4 Identification of isolated bacteria

3.4.1 Morphological, physiological and biochemical identification

Data in Table (4) showed that the four isolates *i.e.* Wl-1, Pf-4, Yb-8 and Rs-9 gave negative reaction with gram reaction but, Wf-6 isolate gave positive reaction in the same test. All five isolated bacterial gave positive reaction in KOH (3%) and catalase activity. On the other hand, these isolates gave negative reaction with production of indole and growth at 41°C test. Meantime, they gave negative reaction with starch hydrolysis and gelatin liquefaction except isolate Wf-6 gave positive reaction on those tests. Meanwhile, the three isolates *i.e.* Wl-1, Pf-4 and Rs-9 gave different reaction on methyl red test (M.R.) and production of H_2S. Meantime, the two isolates Wf-6 and Yb-8 gave negative reaction with the same tests. The isolates *i.e.* Wl-1, Pf-4, Yb-8 and Rs-9 gave negative reaction with urease production but, Wf-6 isolate gave positive reaction in the same test. Meantime, these isolates Wl-1, Wf-6, Pf-4, and Rs-9 gave positive reaction with nitrate reduction and oxidase reaction. The isolate Yb-8 gave negative reaction in the same test. The isolate Wf-6 gave negative reaction with levan production test while, the four isolates gave positive reaction in this test. Finally, the aforementioned tests and their result revealed that the three isolates *i.e.* Wl-1, Pf-4 and Rs-9 could be identified as *Pseudomonas cichorii*, while, the isolates *i.e.* Yb-8 could be identified as *Pseudomonas fluorescens* but the isolate Wf-6 was identified as *Bacillus subtilis.*

Table 4: Identification of bacterial isolates which isolated from *Chrysanthemum* plants.

Test	Reaction				
	Wl-1	Wf-6	Pf-4	Yb-8	Rs-9
Gram reaction	-	+	-	-	-
KOH 3%	+	+	+	+	+
Starch hydrolysis	-	+	-	-	-
Gelatin Liquefaction	-	+	-	-	-
Catalase activity	+	+	+	+	+
Methyl red test	d*	-	d	-	d
Production of H_2S	d	-	d	-	d
Production of indole	-	-	-	-	-
Urease production	-	+	-	-	-
Nitrate reduction	+	+	+	-	+
Growth at 41°C	-	-	-	-	-
Levan production	+	-	+	+	+
Oxidase reaction	+	+	+	-	+
Bacterial species	*P. cichorii*	*B. subtilis*	*P. cichorii*	*P. fluorescens*	*P. cichorii*

*d = different reaction

3.4.2 Molecular identification of the bacterial blight disease

Amplification of bacterial blight (*Pseudomonas cichorii*) DNA from three bacterial isolates were done with Six SCoT primers of 115 amplified fragments, 115 were polymorphic (19.17%) / primers and the percentage of polymorphism ranged from 100%in all Scot primers; the fragment sizes were 108bp to 1202bp (Table 5 and Figure 1). The number of bands which obtained by each primer was 28 with Scot- I primer, 26 with the Scot-2 primer, 13 with Scot-3 primer, 12 with both Scot-4 primer and 24 with Scot-5 primer and12 with Scot-6. The Scot-I primer had the higher polymorphism bands; the fragment sizes were 108bp to 1202bp but Scot- 4 and Scot-6 primers gave the lowest polymorphism bands; the fragment sizes were 146bp to 920 bp.

3.4.3 Jaccard's similarity coefficient

The UPGMA cluster analysis based on pairwise genetic similarity coefficient revealed that the similarity between the three-bacterial blight bacterial isolates ranged from 0.848 between Rs-9 and Pf-4 to 0.889 between Pf-4 and Wl-1; the average of similarity among genotypes was 0.867 (Table 6 and Figure 2). The dendrogram constructed from SCoT analysis of *Pseudomonas cichorii* isolates collected from geographically diverse zones of Egypt separated the three isolates into two major SCoT groups (Figure 2).

Table 5: Characteristics of Scot marker banding profiles of *Pseudomonas cichorii* isolates.

Scot Primer	Total number of bands	Monomorphic bands	Specific bands	Polymorphic bands	% of polymorphism	Molecular weight (bp)
Scot-1	28	0.0	0.0	28	100	108-1202
Scot-2	26	0.0	0.0	26	100	120-787
Scot-3	13	0.0	0.0	13	100	282-999
Scot-4	12	0.0	0.0	12	100	217-768
Scot-5	24	0.0	0.0	24	100	126-998
Scot-6	12	0.0	0.0	12	100	146-920
Total	115	0.0	0.0	115	100	--
Average	19.17	0.0	0.0	19.17	100	--

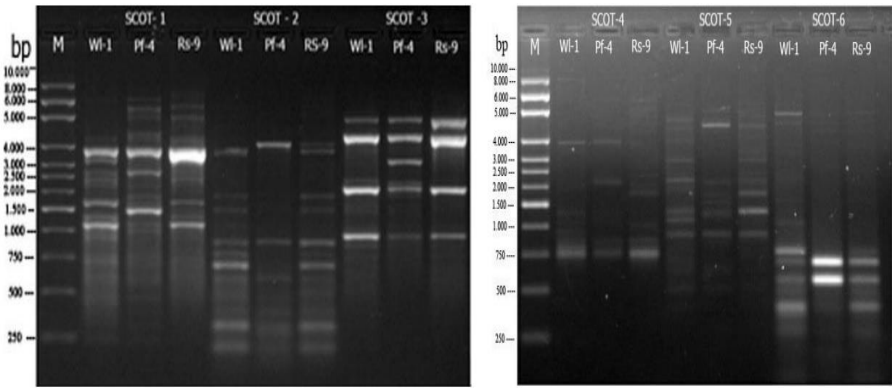

Figure 1: SCoT marker profiles for the three *Pseudomonas cichorii* isolates Wl-1, Pf-4 and Rs-9 with (a): Scot-1, 2 and 3 primers;(b): Scot-4 ,5 and 6 primers and M: 100 bp DNA ladder (Lane 1).

Table 6: Similarity Matrix computed with Dice coefficient.

Isolates	Wl-1	Pf-4	Rs-9
Wl-1	1	0.889	0.865
Pf-4		1	0.848
Rs-9			1

Figure 2: Dendrogram generated using Un-Weighted Pair Group Method with arithmetic average (UPGMA) analysis showing relationships among different *Pseudomonas cichorii isolates* (Wl-1, Pf-4 and Rs-9) from Egyptian, using SCOT.

The first group was formed by **(**Wl-1 and Pf-4); the second group only one isolate (Rs-9) of this group. Finally, found the different within groups, the various bacterial strains diverged slight from each other.

3.5 Host range of *Pseudomonas cichorii* bacterium

From the previous experiments it was clearly shown that three isolates of the identification bacteria isolated from chrysanthemum parts could be identified as *Pseudomonas cichorii i.e.* Wl-1, Pf-4 and Rs-9. But, Wl-1 isolate was highly virulence on pathogenic of all chrysanthemum varieties. Results in (Table 7) clearly showed virulence of *Pseudomonas cichorii* isolate (Wl-1) on eight different host plants from four different plant families. It is clear from the obtained results that Chrysanthemum (*Chrysanthemum grandiflorum*) cv. White Zambla and Lettuce (*Lactuca saliva*) cv. Balady from family Asteraceae were highly susceptible one

among the eight tested hosts to infection with the tested isolate (Wl-1) isolate with injection method of inoculation at 15 days post inoculation. Meanwhile, cabbage (*Brassica oleracea*) cv. Sabeany, from family *Brassicaceae,* Mint (*Mentha viridis*) cv. Balady, from family *Lamiaceae* and pepper (*Capsicum annuum*) cv. California wander, tomato (*Solanum lycopersicum*) cv. super strain b, Datura (*Datura metel*) cv. Balady, eggplant (*Solanum melongena*) cv. Balady long white from family *Solanaceae* were infected with the tested isolate (Wl-1) isolate at 15-day inoculation.

Table 7: Host range reaction and virulence of *Pseudomonas cichorii* isolate (Wl-1) on differential hosts from different plant families.

Common name	Scientific name	Family	Reaction
Chrysanthemum	*Chrysanthemum grandiflorum* cv. White Zambla	*Asteraceae*	++
Lettuce	*Lactuca saliva* cv. Balady	*Asteraceae*	++
Cabbage	*Brassica oleracea* cv. Sabeany	*Brassicaceae*	+
Mint	*Mentha viridis* cv. Balady	*Lamiaceae*	+
Pepper	*Capsicum annuum* cv. California wander	*Solanaceae*	+
Tomato	*Solanum lycopersicum* cv. super strain b	*Solanaceae*	+
Datura	*Datura metel* cv. Balady	*Solanaceae*	+
Eggplant	*Solanum melongena* cv. Balady	*Solanaceae*	+

The results clearly reveal that (Wl-1) isolate was able to infect all tested different host plants from different plant families.

4. Discussion

Chrysanthemum is one of the most important commercial floriculture crops of Egypt. The chrysanthemum is subjected to attack by a number of disease (Cavallini *et al.,* 1992). Chrysanthemum is vegetative propagated flowering plant and is affected by many diseases caused by fungi and bacteria. (Shamala & Janardhana, 2018). Major bacterial blight disease caused by *Pseudomonas cichorii* has become a serious problem in many parts of the world (Timilsina *et al.,* 2017). Shamala and Janardhana (2018) found that the bacterial blight symptoms include dark brown spots and blotches on leaves extending beyond plant leaves with water-soaked lesions on stems, darkening and death of buds and stem terminals. Affected plants showed brown to black decay at the base. As for sampling and isolation of the disease, the bacterial disease occurs on flowers, stems, buds and leaves. Leaf infections appear as water-soaked spots then become brown and dry. Also, ten bacterial isolates were isolated from different parts of Chrysanthemum which collected from different localities of Egypt. In this respect, the bacterial isolates designated as Wl-1 and Wb-2 were isolated from leaf and bud respectively of chrysanthemum white variety in Qualubia (Moshtohor). Meanwhile, the isolates coded as Ps-3 and Pf-4 were isolated from stems and flower of purple variety respectively in the same governorate (El-kanater El-

khairia). On the other hand, the bacterial isolates coded as Ws-5 and Wf-6 were isolated from stems and flower of white variety Menoufia governorate (Shebeen El-kom). while, the isolates coded as Yl-7 and Yb-8 were isolated from leaf and buds of yellow variety respectively in Giza governorate (Kirdasah). The isolate Rs-9 and Rf-10 were isolated from stems and flower of red variety from the same governorate (Elmariotia). Isolation from Chrysanthemum parts are in harmony with those obtained by (Janse, 1981) who identified a bacterium, as *Pseudomonas cichorii*, isolated from Chrysanthemum plants and Jokes *et al*. (1983) described a similar disease, also caused by *P. cichorii*, on field grown chrysanthemum in USA. Moreover, Bolick (1960) and Mcfadden (1961) found that *P. cichorii* had only been the cause of bud blight and leaf spot of chrysanthemum. Horita (1993) found that bacterial disease characterized by *Pseudomonas cichorii* incited Chrysanthemum plants made visible symptoms in infected leaves appear as marginal legion in japan. Isolates were pathogenic to chrysanthemum by spray inoculation. The causal organism produced a fluorescent pigment on King's B medium and was arginine dihydrolase negative, gelatin liquefaction negative and oxidase positive, which identified it as *P. cichorii*. In relation to the identification of isolated bacteria using traditional techniques according to their morphological, biochemical and physio-logyical characteristics, these traditional tests showed that these isolates could be of three isolates *i.e.* Wl-1, Pf-4 and Rs-9 could be identified as *Pseudomonas cichorii*, while, the isolates i.e. Yb-8 could be identified as *Pseudomonas fluorescens* but the isolate Wf-6 was identified as *Bacillus subtilis*. SCoT markers have been successfully used to assess genetic diversity and structure, identify cultivars, and for quantitative trait loci (QTL) mapping and DNA fingerprinting in different species (Caho *et al*., 2014). Stead *et al.* (2013) reported that *Pseudomonas cichorii* have a wide host range of high economic importance including tomato, lettuce and watermelon. Moreover, Marques *et al*. (2016) found that isolates of *Pseudomonas cichorii* were pathogenic to the other 24 plants in artificial inoculations. This bacterium has a wide distribution and range of hosts, and this report is important for cataloging plant-pathogenic bacteria. Timilsina *et al*. (2017) reported that the strains were pathogenic on tomato and were also pathogenic on lettuce. Based on isolation and identification tests, these results show that the cause of bacterial blight of chrysanthemum belongs to *P. cichorii* isolates. In addition, isolates were pathogenic for eight plants from different plant families by artificial inoculations. This bacterium has a wide host range and it is important to list the plant pathogenic bacteria, which can become epidemic in whole Egypt under special circumstances.

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