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PSEUDOMONAS FLORA OF CITRUS-PLANT NURSERIES IN THE JORDAN VALLEY

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Abstract

Pseudomonas species isolated from soils cultivated with citrus plants (204) and plant galls (46) in the Jordan Valley were physiologically and biochemically classified into fluorescent group (74) and non-fluorescent group (176). All plant gall isolates belong to fluorescent group. The soil isolates (145) and the plant gall isolates (46) were pathogenic to tobacco plants. The non-fluorescent group included: *P. maltophilia* (53), *P. cepacia* (41), *P. avenae* (21), *P. solanacearum* (20), *P. cissicola* (18), *P. cattleya* (8), *P. paucimobilis* (4), *P. citrulli* (3), *P. mesophilica* (3), *P. andropogonis* (3) and *P. amygdali* (2) and the fluorescent group *P. syringae* (62), *P. fluorescens* (7) and *P. chlororaphis* (5).

Introduction

Pseudomonas species cause necrotic lesions on fruit stems and leaves, tissue macerations and canker. Most of the phytopathogenic *Pseudomonas* appear to be adapted to survive in soil or in association with citrus plants that represent one of the most important crop in the Jordan Valley. This paper reports on the characteristics and taxonomy of the genus *Pseudomonas* in the Jordan Valley.

Materials and Methods

Area of study: Al Baqureh, one of the largest and oldest plant nurseries of stone fruit and grapevine, 35 km to the west of Irbid in the Jordan Valley was selected. Soil samples were colected from 4 fields: one (F1), two year old (F2), permanent citrus plants (F3), and one field without cultivation used as control (F4).

Culture media: King's medium A & B (King *et al.*, 1954); Yeast extract-malt extract agar and nutrient sucrose agar (Garrett *et al.*, 1966); YDCB medium (Misaghi & Grogan, 1969); and FPA and D4 media (Sand *et al.*, 1980; Kado & Heskett, 1970) were used.

Bacterial cultures: Soil samples were collected and treated as previously described (Almomani & Abussaud, 1990). Soil suspensions were pipetted and spread evenly over the surface of King's medium B agar plate and incubated at 26°C. Suspected *Pseudomonas* colonies were purified by repeated streaks on the same medium and maintained on yeast extract-malt extract agar.

Identification and classification: For identification and classification of the isolates, the following tests were used: Gram-stain reaction; colony morphology on King's medium B and on nutrient sucrose agar medium; accumulation of polybetahydroxybutyrate inclusions (Sand *et al.*, 1980); oxidase test, motility test, hydrolysis of starch and tween 80, reduction of nitrate, denitrification, 3-ketolactose production, and tobacco hypersensitivity test (Fahy & Persley, 1983; Klement, 1963); arginine dihydrolase, levan formation, proteolytic enzymes, and temperature relationships (Sand *et al.*, 1980); gelatin hydrolysis (Sule, 1978); production of diffusible and non-diffusible pig-

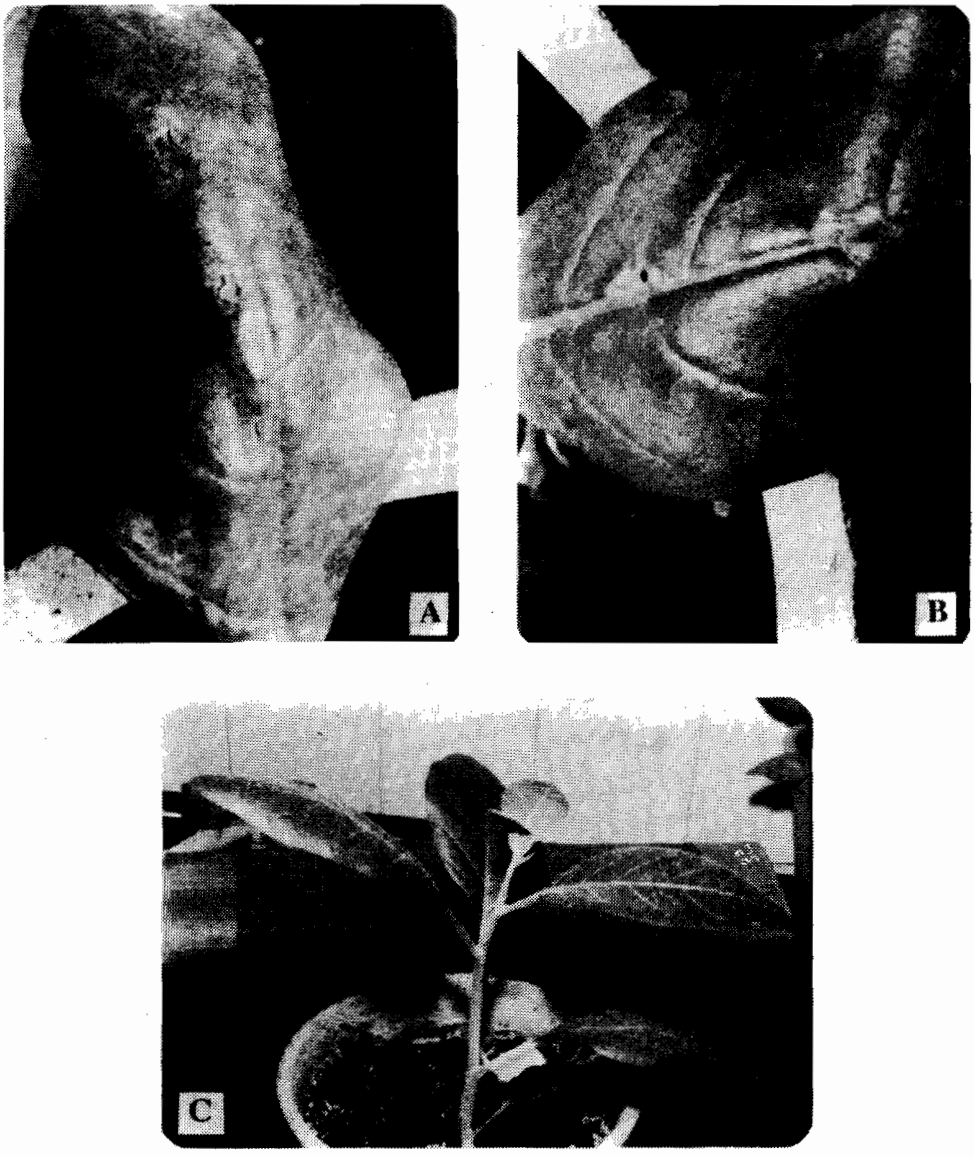

Fig. 1. Plant infected with *Pseudomonas* sp.
A. Soil isolate, B. gall isolate, C. Control

Table 1. Distribution of *Pseudomonas* strains isolated from soils.

Species	Sites of isolation					
	F1	F2	F3	F4	G	Total
P. amygdali	0	0	1	1	-	2
P. andropogonis	1	0	1	1	-	3
P. avenae	3	15	3	0	-	21
P. cattleyae	1	5	2	0	-	8
P. cepacia	16	17	6	2	-	41
P. cissicola	1	14	3	0	-	18
P. maltophilia	11	27	13	2	-	53
P. paucimobilis	1	2	1	0	-	4
P. pseudoalcaligenes subsp. *citrulli*	0	1	2	0	-	3
P. mesophilica	2	0	1	0	-	3
P. solanacearum	4	13	3	0	-	20
Fluorescent strains:						
P. syringae:						
Pv. *syringae*	1	12	8	0	3	24
Pv. *savastoni*	1	3	3	0	22	29
Pv. *antirrhini*	0	0	0	0	9	9
P. fluorescence miscellaneous	0	0	0	0	7	7
P. chlororaphis	0	0	0	0	5	5
Total	42	109	47	6	46	250

Fields planted with citrus plants one year old (F1), two years old (F2) and permanent tree (F3). Unplanted control (F4), Grapevine galls (G).

ments, and production of acid from sucrose (Skinner & Lovelock, 1979); and catalase test (Lelliot *et al.*, 1966). As a sole carbon source the following sugars, organic acids, and amino acids were tested: glucose, sucrose, sorbitol, arabinose, mannitol, inositol, erythritol, lactate, anthranilate, tartrate, trehalose, quinate, trigonelline, alanine, betaine, homoserine and 2-ketogluconate (Sand *et al.*, 1980; Fahy & Persley, 1983; Misaghi & Grogan, 1969).

Result and Discussion

Although King's medium B is not highly selective, it is commonly used for isolating phytopathogenic *Pseudomonas*. Out of 672 suspected isolates isolated from soil 204 isolates were confirmed as *Pseudomonas* species. Other 46 *Pseudomonas* species which were previously isolated from plant galls (Almomani & Abussaud, 1990) were included in this study. Studying the colony morphology of these isolates on King's

Table 2. Percentage of fitness of the examined *Pseudomonas* species in accordance with the classification systems of Fahy & Persley (1983), and Sands *et al.*, (1980), used for classification.

Number of isolates	95-100%	90-94%	85-89%	80-84%
24			*P. syringae pv. syringae*	
29			*P. syringae pv. saastanol*	
9			*P. syringae pv. antirrhini*	
7		*P. fluorescens miscellaneous*		
5				*P. chlororaphis*
2		*P. amygdali*		
3		*P. andropogonis*		
21		*P. avenae*		
8	*P. cattleyae*			
41	*P. cepacia*			
18	*P. cissicola*			
53		*P. maltophilia*		
4	*P. paucimobilis*			
3			*P. pseudoalcaligenes* subsp. citrulli	
3	*P. mesophilica*			
20			*P. solanacearum*	

medium B showed that they were smooth or rough, circular or irregular, entire or curled, raised, white or yellow in colour. On nutrient sucrose agar medium they were circular, entire, glistening, butyrous to slimy, hemispherical and more or less opaque. All isolates were gramnegative, rod shaped, motile catalase positive and gave negative 3-ketolactose test and H_2S test. Inoculation on old leaves of tobacco showed that 145 of the soil isolates and 34 of the plant gall isolates gave positive test (Fig.1). Of all isolates 44 % did accumulate polybetahydroxybutyrate, 26, 11 and 63% of the isolates, respectively showed good, week and no growth on D4 medium.

In the present study a number of biochemical tests as described by Sands *et al.*, (1980); Fahy & Persley (1983) were used to study and classify our *Pseudomonas* isolates. Based on the results of these tests 250 *Pseudomonas* isolates have been divided into fluorescent group containing 74 isolates and a non- fluorescent containing 176 isolates. All plant-gall isolates belonged to fluorescent group. Inoculation of 74 biochemically defined fluorescent *Pseudomonas* isolates on King's medium B, 57 isolates produced pigment that fluoresce under uv-light and 17 did not. Hildebrand & Schroth (1972) found that not all fluorescent species produce fluorescent pigments on King's

medium B but do so on other media. The distribution and species composition of the non-fluorescent *Pseudomonas* isolates isolated from soil from four different fields is shown in Table 1. *P. maltophilia* showed the highest frequency (30%), followed by *P. cepacia* (23%), *P. avenae* (12%), *P. solanacearum* (11%), *P. cissicola* (10%), *P. cattleyae* (4.5%), *P. paucimobilis* (2.3%); and *P. mesophilica, P. citrulli, P. andropogonis* each 1.7 and *P. amygdali* (1%). *P. paucimobilis, P. mesophilica* and *P. maltophilia* were found in association with plants while *P. cepacia, P. avenae, P. solanacearum, P. cissicola, P. cattlegae, P. citrulli* and *P. amygdali* have been reported as plant pathogens (Hayward, 1983).

The species composition of the fluorescent group (Table 1) showed that all 28 fluorescent soil isolates were identified as *P. syringae*, while plant-gall isolates (46) were identified as *P. syringae* (34), *P. fluorescence* (7) and *P. chlororaphis* (5). Most of the fluorescent pseudomonads were classified as one species, *P. syringae* (Skerman *et al.*, 1980) since they differ in their host range specificity and the disease symptoms they form. *P. syringae* contains large number of pathovars (Young *et al.*, 1978). All soil fluorescent *Pseudomonas* strains (28) belong to *P. syringae* pv. *syringae* (21 strains) and *P. syringae* pv. *savastoni* (7 strains). The 46 fluorescent *Pseudomonas* strains isolated from plant galls were identified as: *P. syringae* pv. *savastoni* (22), *P. syringae* pv. *syringae* (3), *P. syringae* pv. *antirrhini* (9), *P. miscellaneous* (7) and *P. chlororaphis* (5).

The results indicate that bacterial canker of stone fruit trees in this area is most probably due to *P. syringae* pv. *syringae* and that *P. syringae* pv. *savastoni* is most probably involved in gall formation on plants, either direct or in association with agrobacteria which needs further study. About 71% of the soil isolates and 74% of the plant-gall isolates can be considered as pathogenic. However, the percentage of the pathogenic non-fluorescent strains in the four fields was not significantly different.

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