

CHARACTERIZATION OF *AGROBACTERIUM TUMEFACIENS* BIOTYPES ISOLATED FROM JORDAN

FOUAD AL-MOMANI* AND MAHMOD ABUSSAUD

*Department of Biology,
Yarmouk University, Irbid, Jordan.*

Abstract

Out of 183 isolates from soil and plant tumors from different locations in Jordan, 12 pathogenic isolates of *Agrobacterium tumefaciens* were obtained from soil, 29 from grapevines, 6 from apple, 2 from pear and 3 from peach. Biotype I was predominant. Eighteen isolates were included in biotype III and 12 isolates in biotype II. Grapevine isolates showed their tumorigenicity mostly on grapevine, some of them were able to transform tobacco and kalanchoe cells. Peach isolates were highly virulent with a wide host range.

Introduction

Agrobacterium is a soil inhabiting, gram negative, aerobic, mesophilic plant pathogen. The oncogenicity of tumor induction by *Agrobacterium* strains is related to the presence of an extra chromosome designated as tumor inducing (Ti) plasmid (Zaene *et al.*, 1974). During the course of infection a defined portion of the Ti-plasmid, the T-DNA, is stably transferred to the plant cell where it is integrated in the nuclear genome (Thomashow *et al.*, 1980). The expression of the integrated portion leads to the formation of neoplastic cells which form crown gall disease. These cells produce unusual amino acids called opines and they can grow on hormone free medium. Crown gall is a world wide distributed disease of economic significance in nurseries, vineyards, and fruit orchards (Siegler, 1940; Keane *et al.*, 1970; New & Kerr, 1972; Panagopoulos & Psallidas, 1973; Schroth & Moller, 1976; Sule, 1978; Ma *et al.*, 1987). On the basis of biochemical activity *Agrobacterium tumefaciens* strains were grouped or clustered into two groups, two clusters or two biotypes (Keane *et al.*, 1970; White, 1972; Kersters *et al.*, 1973; Panagopoulos & Psallidas, 1973). Kerr & Panagopoulos (1977) reported a third biotype isolated mostly from grapevines. Sule (1978) grouped Hungary isolates into three biotypes. China grapevine isolates were grouped into two biotypes (biotype I and III) by Ma *et al.*, (1987). In this study *A. tumefaciens* isolates from plant tumors and soil in Jordan were characterized and biotyped.

Materials and Methods

Soil samples were collected by an Auger holder from the top 20cm after removing the upper 2-3cm layers. The soil was dried at room temperature, passed through 2mm

*Present address: Centre for Advanced Molecular Biology, University of the Punjab, New Campus, Lahore-20, Pakistan.

sieve and 1g sample suspended in 100ml sterile distilled water. After vigorous shaking, serial dilutions were made and 0.1ml of the appropriate dilution was spread by an L-shaped glass rod on the selective medium (Kado & Heskett, 1970; Schroth *et al.*, 1965). The cultures were incubated at 27°C. Tumor samples were randomly collected, washed with tap water, surface sterilized in 10% sodium hypochlorite for 10 minutes and macerated in an electric blender. Serial dilutions were made and 0.1ml of appropriate dilution was cultured as mentioned above.

Identification was made following Bergey's Manual of Determinative Bacteriology (1974) and biotyping was done as reported by Kerr & Panagopoulos (1977). Virulence was tested as reported by Kerr (1969) on tobacco, tomato, kalanchoe and grapevine.

Results

All the isolates were gram negative, motile, produced acids from simple sugars (glucose, lactose, mannitol, xylose, arabinose and sucrose), produced H₂S from cystine. These were catalase and urease positive, failed to utilize gelatin, casein and starch. They were not able to grow at 4°C after 2 days and no pigment was noticed on King's B medium.

Out of 183 isolates 52 were pathogenic at least on one of the tested hosts. Twelve of the pathogenic isolates were from soil, 29 from grapevine, 6 from apple, 2 from pear and 3 from peach (Table 1). Among soil pathogens Walah nursery showed maximum pathogenicity percentage. Jerm grapevine tumors showed the highest pathogenic percentage through grapevine tumors. Biotype I was dominant over biotype II and III. All biotype III isolates were from grapevine tumors except two, which were from Rayyan nursery soil. Other plant tumor isolates belonged either to biotype I or II.

Table 1. Distribution of the *Agrobacterium tumefaciens* and their biotypes.

	Sample No.	Agr. No.	Patho. No.	Percentage	Biotype I	Biotype 2	Biotype 3
Rayyan soil (RR)	11	30	5	16.66	0	3	2
Deiralla soil (D)	11	16	2	12.50	0	2	0
Bagurah sol (B)	11	26	2	7.69	0	2	0
Walah soil (W)	3	5	3	60.00	2	1	0
Rayyan grape tumor (Rgg)	28	15	5	33.33	4	0	1
Deiralla grape tumor (Dgg)	33	15	7	46.66	4	0	3
Baqurah grape tumor (Bgg)	20	12	7	58.33	4	0	3
Ebeen grape tumor	3	10	6	60.00	1	0	5
Jerm grape tumor (Jgg)	4	4	3	75.00	0	0	3
Mean Grape tumor (Mgg)	10	2	1	50.00	0	0	1
Apple	7	23	6	26.08	4	2	0
Pear	5	18	2	11.11	1	1	0
Peach	4	7	3	42.85	2	1	0
Total	150	183	52	28.41	22	12	18

Biochemical and biophysical activities of the isolates are shown in Table 2. All biotypes were 3-ketolactose positive, basic on litmus milk, utilised sodium selenate, tolerated 3% NaCl and 5% ethanol, grew at 38°C and on Schroth *et al.*, (1965) medium. All isolates were mostly citrate, tartarate, malonate negative and oxidase positive; produced acid from melezitose and raffinose, failed to grow on meso-erythritol and mostly formed pellicle on ferric ammonium citrate. All biotype II isolates were malonate, tartarate positive, failed to grow on Schroth medium, failed to form pellicle on ferric ammonium citrate and failed to form acid from melezitose and raffinose. Most of them were 3-Ketolactose negative, citrate positive, grew weakly on 38°C, 3% NaCl and sodium selenite. They could grow on New & Kerr (1972) medium and produced acid from meso-erythritol, mostly reacted basic with litmus milk. All biotype III isolates were tartarate, malonate positive and 3-ketolactose negative. They were able to grow at 38°C, on a medium containing 3% NaCl and tolerated 5% ethanol. They also grew on Brisbani & Kerr (1983) biotype 3 medium, oxidase negative and could utilize selenate but failed to form pellicle on ferric ammonium citrate. They reacted basic with litmus milk.

Peach isolates were highly virulent and varied widely in their host range. Grapevine isolates were mostly pathogenic on the host of their origin. Some of them showed pathogenicity on tobacco and Kalanchoe. Apple isolates gave good response on tobacco and Kalanchoe. Soil isolates gave small proliferation on tobacco.

Discussion

Biotyping of our isolates according to Kerr & Panagopoulos (1977), showed that most of them were either from biotype I or biotype III. This may be due to the selectivity of the media which was reported to be more selective to biotype I and III (Brisbani & Kerr, 1983) or it could be that most of our samples were from grapevine tumors or from soil which had been cultivated with grapevine for few years previously. It had been reported that biotype III is dominant in grapevine isolates (Kerr & Panagopoulos, 1977; Perry & Kado, 1982; Ma *et al.*, 1987). Among infected plants other than grapevine tumors, biotype II was dominant over biotype I. Biotype III was not found. This may be due to the fact that most of them were isolated from stone fruit tumors or pomes and it was found that biotype II was dominant in these isolates as reported by Panagopoulos & Psallidas (1973) and New & Kerr (1972).

In characterization of our pathogenic isolates, we found that they are similar to what has been reported in literature. The similarity of our biotype I to Panagopoulos & Psallidas (1973) and Keane *et al.*, (1970) exceeded 80% and that our biotype II isolates fit their biotype II more than 70%. Twenty-three of our biotype I fit Kerr & Panagopoulos (1977) biotype I by 100%, also 23 of our biotype III fit their biotype by 100%, whereas 6 of our biotype II isolates fit their biotype II in more than 80%. Peach isolates were the most virulent and widest in their host range amongst our isolates. Grapevine isolates showed limited host range as reported by Ma *et al.*, (1987). Some grapevine isolates showed pathogenicity on tobacco.

Biochemical and Biophysical activity of the Pathogenic strains and their biotypes.

Isolate No.	Origin	Citrate	Tartrate	Malonate	3-Ketolactose	Growth at 38°C	Oxidase	Pellicle formation on Ferric Amm. Citrate	Meleziotoze	Meso-erythritol	Raffinose	Limus milk	Selenate	Ethanol	3% NaCl	Biotype	Tomato	Tomato	Kalonchoe	Grape
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	R	+	+	+	-	W	-	-	-	+	+	A	W	W	W	2	+	+	?	?
3	R	+	-	+	-	W	-	-	+	-	+	B	W	W	W	3	+	+	?	+
4	R	+	-	+	-	W	-	-	+	-	+	B	W	W	W	3	-	-	?	+
5	R	+	+	+	-	W	+	-	-	+	-	B	W	W	+	2	+	+	?	-
6	8	+	+	+	-	W	-	-	-	+	-	B	W	W	W	2	+	+	?	+
7	B	-	+	+	-	W	-	-	-	+	+	A	W	W	+	2	++	++	?	+
8	D	+	+	+	-	W	-	-	-	+	+	A	W	+	+	2	+	+	?	+
9	D	+	+	+	+	-	-	-	-	+	-	B	W	W	+	2	?	+	?	+
10	W	+	+	-	+	+	+	+	+	-	+	B	W	W	+	1	+	+	?	+
11	W	+	+	+	-	W	+	-	-	+	+	B	W	W	+	2	+	+	?	?
12	W	-	+	+	+	+	+	+	+	-	+	B	W	W	+	1	+	+	?	?
13	R _{gg}	+	+	-	+	+	+	+	+	-	+	B	+	+	+	1	+	+	?	?
14	R _{gg}	-	-	-	+	+	+	+	+	-	+	B	W	W	+	1	+	+	?	+
15	R _{gg}	+	+	+	-	+	-	-	+	-	+	B	+	+	+	3	++	+++	?	?
16	R _{gg}	-	-	-	+	+	+	+	-	-	+	B	+	+	+	1	++	+++	?	+
17	R _{gg}	-	+	-	+	+	+	+	+	-	+	B	+	+	+	1	++	+	?	+++
18	D _{gg}	-	+	-	+	+	+	+	+	-	+	B	+	W	+	1	+	++	?	++
19	D _{gg}	+	+	-	+	+	+	+	+	-	+	B	+	+	+	1	++	?	?	+
20	D _{gg}	+	+	-	-	-	-	-	+	-	+	B	+	+	+	3	+	?	?	+
21	D _{gg}	-	-	-	+	+	+	+	+	-	+	B	W	W	+	+	+	?	?	+++
22	D _{gg}	+	+	+	-	+	+	-	+	-	-	B	W	W	+	3	+	+	?	++

AGROBACTERIUM TUMEFACIENS BIOTYPES FROM JORDAN

(Table 2. Contd.)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
23	Dgg	-	+	+	-	+	+	+	+	+	-	+	B	W	+	+	1	++	+++	?	?
24	Dgg	+	+	+	+	+	+	-	-	+	-	+	B	+	+	+	3	+	+	?	+
25	Bgg	-	+	+	-	+	+	+	-	+	-	+	B	+	+	+	1	+	+++	?	++
26	Bgg	+	+	+	-	+	+	+	-	-	-	+	B	+	W	+	1	++	++	?	+
27	Bgg	-	+	+	+	+	+	+	+	-	-	+	B	+	+	+	1	+	+	+	+
28	Bgg	-	+	+	-	+	+	+	-	+	-	+	B	+	+	+	1	+	+	?	+
29	Bgg	+	+	+	+	-	+	+	-	+	-	-	B	-	-	+	3	+	++	?	+
30	Bgg	-	+	+	+	-	W	+	-	+	-	-	B	W	W	W	3	+	++	?	?
31	Bgg	+	+	+	+	-	+	-	-	+	-	-	B	+	+	W	3	+	+	?	+
32	Egg	+	+	+	+	-	W	-	-	+	+	+	B	W	W	+	3	+	+	?	+
34	Egg	+	+	+	+	-	-	-	-	+	-	+	B	W	W	+	3	+	+	?	+
35	Egg	-	+	+	-	+	+	+	+	+	-	+	B	+	W	+	1	+	+	+	++
36	Egg	+	+	+	+	-	-	-	-	+	-	+	B	W	+	+	3	+	+	?	+
37	Egg	+	+	+	+	-	-	-	-	+	-	-	B	W	W	+	3	+	+	?	+
38	Jgg	+	+	+	+	-	+	+	-	+	-	-	B	W	W	+	3	+	+	?	+
39	Jgg	+	+	+	+	-	+	+	-	+	-	-	B	W	W	+	3	+	+	?	+
40	Jgg	+	+	+	+	-	+	+	-	+	-	-	B	W	W	W	3	+	?	?	+
1	Mgg	+	+	+	+	-	+	-	-	+	-	-	B	W	+	+	3	+	+	?	+
42	Apple	-	+	+	-	+	+	-	+	+	+	+	B	W	+	+	1	+	++	+	+
42	Apple	+	+	+	+	-	W	-	-	-	+	+	B	W	W	+	2	+	++	?	+
44	Apple	-	+	+	-	-	W	-	-	-	-	-	A	+	+	W	2	+	++	?	?
45	Apple	+	+	+	-	+	+	+	+	+	-	+	B	+	+	+	1	+	++	?	+
46	Apple	+	+	+	-	+	+	+	+	+	+	-	B	+	+	+	1	+	++	?	+
47	Apple	+	+	+	-	+	+	+	+	+	-	+	B	+	+	+	1	+	++	?	?
48	Pear	+	+	+	+	-	W	+	-	+	+	-	B	W	+	+	2	++	++	?	?
49	Pear	-	+	+	-	+	+	+	+	+	-	-	B	+	W	+	1	+	+	?	?
50	Peach	-	+	+	-	+	+	+	+	+	-	+	B	+	+	+	1	+++	+++	?	?
51	Peach	-	+	+	-	+	+	+	+	+	-	+	B	+	+	+	1	+++	+++	?	?
52	Peach	+	+	+	+	-	W	-	-	-	-	-	B	W	W	W	2	+++	+++	?	?

Notes: + = Positive; - = Negative; W = Weak growth; A = Acidic; B = Basic; / = Not tested.

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