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Phenotypical characterization of Iranian isolates of *Agrobacterium vitis*, the causal agent of crown gall disease of grapevine

by

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Summary: From Karaj and Takestan (Iran) vineyards bacterial colonies typical of *Agrobacterium* were isolated from soil, plant sap and young galls on a selective medium during early spring 1996. Bacterial isolates that were gram-negative and oxidase- and catalase-positive were cultured on King's B medium in order to be distinguishable from fluorescing pseudomonads. Thirty-two *Agrobacterium* isolates were inoculated on test plants such as *Datura*, *Nicotiana* and *Lycopersicon*. Pathogenic isolates were inoculated on 10 different Iranian grape varieties for gall formation. A biovar differentiation study showed that 7 pathogenic strains and 15 non-pathogenic strains belong to *A. vitis*, whereas 6 non-pathogenic strains belong to biovar 1 and 4 non-pathogenic strains to biovar 2 of *Agrobacterium* spp. Pathogenic strains of *A. vitis* were characterized on the basis of phenotypic tests, protein and plasmid profiles and an antibiotic sensitivity test. Electrophoretic studies revealed that *A. vitis* strains were different with regard to the protein profile but shared a common high molecular weight plasmid DNA band in the agarose gel. It is concluded that the Iranian strains of *A. vitis* are phenotypically quite heterogeneous and distinguishable.

Key words: Agrobacterium vitis, grapevine, crown gall disease, bacterial pathogenicity, Ti plasmid, PAGE (polyacrylamide gel electrophoresis), SDS (sodium dodecyl sulfate).

Introduction

The crown gall disease, incited by *Agrobacterium vitis*, has been reported to occur in many vine-growing areas of Iran (Amani 1966; Al-e-Yasin and Bani-Hashemi 1993). For Takistan and Ghazvin vineyards, it is thought that the crown gall disease induced by an *A. vitis* infection is initiated as a result of the feeding injuries caused by cicada (*Cicadatra ochreata*) larva on crown and root tissues.

DNA fingerprinting analysis and protein profile have shown that the genus *Agrobacterium* falls into three taxonomic groups: *A. tumefaciens* (biovar 1), *A. rhizogenes* (biovar 2) and biovar 3 (Moore *et al.* 1988). DNA homology and serological studies revealed that biovar 3 strains isolated from grapes belong to a new species named *A. vitis* (Ophel and Kerr 1990). DNA fingerprinting studies by Gillings and Ophel-Keller (1995) show that *A. vitis* is intraspecifically heterogeneous and thus it is possible to differentiate *A. vitis* strains from various hosts and geographical origins. The use of ELISA with a monoclonal antibody has been very effective in detecting *A. vitis* in plant materials and in discriminating it from other biovars (Bishop *et al.* 1989). More recently, Eastwell *et al.* (1995) were able to identify *A. vitis* strains in grapevine cuttings using polymerase chain reaction (PCR) with *peh*A-specific primers.

A. vitis strains are able to produce acid from L(+)arabinose, D(-)arabinose, L-arginine, L-cysteine, D(-)galactose, D(+)galactose, D(+)glucose, glycogen, isoleucine, maltose, D(-)mannitol, L-methionine, L(+)rhamnose, sucrose, L-valine and D(+)xylose (Kerr 1992; Bouzar *et al.* 1993). Acid production from ascorbate, *meso*-erythritol, L-histidine and nicotinamide is variable. *A. vitis* does not produce alkali from citric acid or propionic acid (Burr and Katz 1983). It tolerates 2 % NaCl but does not grow at 37 °C. It is indole-negative but catalase-, levan-, oxidase- and urease-positive. The bacterium is unable to hydrolyze caesin, gelatin and Tween 80. *A. vitis* produces neither H_2S nor reducing compounds from sucrose. Aesculine and arginine are hydrolyzed. L-Tyrosine is not utilized but ferric ammonium citrate is. The bacterium grows on Roy-Sasser selective medium at 28 °C and forms small circular colonies with reddish centers and white margins (Moore *et al.* 1988; Ophel and Kerr 1990; Kerr 1992; Bouzar *et al.* 1993).

DNA hybridization studies show 78-92 % homology among *A. vitis* strains and 7-42 % homology with strains from other species (Ophel and Kerr 1990). The type strain of *A. vitis* is K309 (NCPPB3554) which was originally isolated from grapevines in South Australia in 1977. This strain induced octopine-type gall formation on grape, sunflower, tomato and carrot root disks (Ophel and Kerr 1990).

In addition to tumor formation, *A. vitis* causes root decay on grapevines (Burr *et al.* 1987 and Rodriguez-Palenzuela *et al.* 1991). This is due to the production of pectic enzyme hydrolases by both pathogenic and non-pathogenic strains of *A. vitis*.

This study was undertaken to investigate the possible occurrence of crown gall disease on grapevines in the Karaj region (Iran) as well as to characterize *A. vitis* strains phenotypically.

Material and Methods

Bacterial isolation from tumor: Young and actively growing white tumors were collected from vineyards of the Karaj and Takestan region and brought into the

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lab. After a brief washing in sterile, distilled water, tumors were surface-sterilized in 1 % (w/v) sodium hypochlorite for 10-20 min. In order to be sure that the disinfectant would not penetrate into the tissue, tumor samples without treatment were also included. Tumorous tissue was cut into 1-2 mm^3 sections and soaked in 2 ml sterile water for 30 min to discharge the bacterial cells into the medium (MOORE *et al.* 1988). Bacterial suspension was plated out on a non-selective YE medium and incubated at 28 °C for 3-4 d.

Bacterial isolation from soil: Soil samples (20 cm deep) were collected from the infected soil around the trunks showing typical gall symptoms. Samples (about 100 g each) were obtained from 5 grapevines in a row and 1 g of soil was suspended in 10 ml of sterile, distilled water and shaken for 15 min. Following clarification, 100 µl of the suspension was plated out on a nutrient agar medium and the plates were incubated at 28 °C (MOORE *et al.* 1988).

Bacterial isolation from plant sap: During early spring, plant sap was collected from infected vines when the soil temperature around the root system was 5 °C and swollen buds were still closed. Small quantities of crude sap were collected in sterile vials after surface-sterilizing the tissue 2-3 cm above the tumor with 95 % ethanol and then stabbing it 1 cm deep. Samples (100 µl) were plated out on a selective Roy-Sasser medium containing D-cycloserine, trimethoprim and cyclohexamide. Typical colonies of *Agrobacterium* with deep red centers and white margins appeared after 4 d at 28 °C and were streaked out on YM plates. Colonies that were gram-negative and nonfluorescent on King's B medium were used in a pathogenicity test.

Pathogenicity tests: Indicator plants used in the pathogenicity test of *Agrobacterium* isolates included *Lycopersicon esculentum* (var. Red Cloud and Early Urbana), *Datura tatula*, *D. straminna*, *Nicotiana glauca* and *N. turkish*. Stem tissue was surface-sterilized with 96 % ethanol and then wound-inoculated with a loopful of *Agrobacterium* (5×10^8 cells·ml^{-1}) grown in NAG culture medium. The inoculation site was covered with a sterile moist cotton and parafilm for 2-4 d to prevent desiccation (MOORE *et al.* 1988).

Iranian grapevine varieties used in the subsequent infection test were Asgari, Black Shâhâni, Kondori, Mehdikhâni, Red Seedless, Red Yâghooti, Samarghandi, White Fakhri and White Shâhâni. Disease-free vine cuttings in triplicate were transplanted in pasteurized soil in pots in a greenhouse and wound inoculation was carried out on young stems. Controls included sterile water- or *A. radiobacter*-inoculated plants.

Callus formation was also tested on carrot root tissue. Fresh carrot root disks (0.5 cm thick) were surface-sterilized in 2 % (w/v) sodium hypochlorite for 10 min and washed twice in sterile, distilled water. Disks were inoculated with 0.5 cm^3 bacterial suspension with OD_{600} = 1.0 on a moist filter paper in a Petri plate (LIAO and HEBERLEIN 1978).

Phenotypical characterization tests: Phenotypical, physiological and biochemical tests for the identification and differentiation of *A. vitis* from other species and strains were carried out on all 32 *Agrobacterium* isolates as described by MOORE *et al.* (1988).

Antibiotic sensitivity test: Antibiotic sensitivity tests were carried out using pretreated antibiotic filter disks. In this study, 100 µl bacterial suspension (5×10^8 cells·ml^{-1}) was plated out on a nutrient agar medium containing 1 % glucose. Antibiotic disks were then placed on each plate in duplicate. The plates were incubated at 28 °C for 24-48 h. The results were expressed as the diameter of inhibition zone in mm. (Tab. 4 shows the antibiotics used in this study).

SDS-PAGE: SDS-polyacrylamide gel electrophoresis of soluble proteins was carried out according to LAEMMLI (1970) using a Sigma vertical slab gel unit (16.5 x 28 cm). Bacterial isolates were cultured on a nutrient agar medium containing 1 % glucose and suspended in 1 ml sterile, distilled water at OD_{600} = 1.0 in Eppendorf tubes. Samples were then centrifuged at 13,000 *g* for 5 min, washed in saline buffer twice, resuspended in 5x sample buffer and placed in a boiling water bath for 3-5 min. Each well was loaded with a 50 µl sample. Protein samples were electrophoresed in 12 % resolving gel and 6 % stacking gel at a constant voltage of 150 V. Following electrophoresis, the gel was stained in 0.1 % (w/v) Coomassie brilliant blue G250 and destained in a mixture of methanol:water:acetic acid 5:5:1 (v:v:v).

Agarose gel electrophoresis: Plasmid DNA isolation, purification and electrophoresis was performed using the SDS-Alkaline lysis method as described by MANIATIS *et al.* (1982). DNA samples were eletrophoresed in 0.7 % (w/v) agarose in Tris-borate-EDTA buffer (pH 8.3) at a constant voltage of 50 V. DNA bands were stained with ethidium bromide, visualized on a UVP transilluminator and photographed.

Results

Typical *Agrobacterium* colonies from tumors, soil and plant sap were isolated on selective (RS) as well as NA and YM general media; they appeared to be convex, smooth with distinct edges and were morphologically distinct from other bacteria (BURR and REID 1994). Bacterial isolates that were gram-negative and catalase- and oxidase-positive were cultured on King's B medium in order to exclude fluorescing pseudomonads. A total of 32 *Agrobacterium* isolates was identified that belonged to *A. vitis* and *Agrobacterium* biovars 1 and 2. Twenty-two isolates were found to be *A. vitis* among which 7 isolates were pathogenic and 15 isolates non-pathogenic. Six isolates were identified as biovar 1 and 4 isolates belonged to biovar 2. Tab. 1 lists the sources and locations of the agrobacteria used in this study.

Table 1

Agrobacterium isolates used in this study

Number of isolate	Source	Location
1, 2, 3, 6	Grapevine sap	The University farm, Karaj
4, 5, 7	" "	Takestan vineyards
8-22	Soil and galls	Takestan vineyards
23-28	" "	The University farm, Karaj
29-32	" "	The University farm, Karaj

Tab. 2 shows the phenotypical characteristics differentiating *A. vitis* from *Agrobacterium* biovars 1 and 2. All 7 pathogenic strains (1-7) of *A. vitis* were capable of utilizing acetate, adonitol, aesculine, arabinose, arginine, cellobiose, cysteine, fructose, galactose, glucose, isoleucine, lactate, lactose, malate, malonate, maltose, mannitol, mannose, rhamnose, saline, sucrose, tartrate, valine and xylose. These strains were urease- and levan-positive but were indole-negative and unable to reduce nitrate, to induce HR on tobacco, to utilize citrate, dulcitol, erythritol, ferric ammonium citrate, L-leucine, melezitose, propionate, sorbitol and L(-)xylose, to hydrolyze caesin, starch, Tween 80 or to produce 3-ketolactose. They showed variable results with regard to ascorbate, *meso*-erythritol, ethanol, histidine, methionine, nicotinamide and tyrosine utilization, litmus milk activity, H_2S production and gelatin hydrolysis (Tab. 3).

Fig. 1 shows gall formation on grapevines in the Karaj region. Pathogenicity tests revealed that *A. vitis* strains induced tumor formation on *Datura*, *Nicotiana* and *Lycopersicon* plants 2-4 weeks post-inoculation (Fig. 2). Control plants showed a typical wound healing response. In grapevines, small tumors appeared on stems 4 weeks after the inoculation (Fig. 3). The variety Asgari showed tumors 3 weeks after inoculation which became relatively larger than those of the other varieties. All 7 *A. vitis* strains caused tumor formation on their main host plant, *i.e.* grapevine. Carrot root tissue inoculated with *A. vitis* often showed pectolytic maceration after 2 d. However, in a few cases, small whitish calli were observed around the macerated areas on the disks.

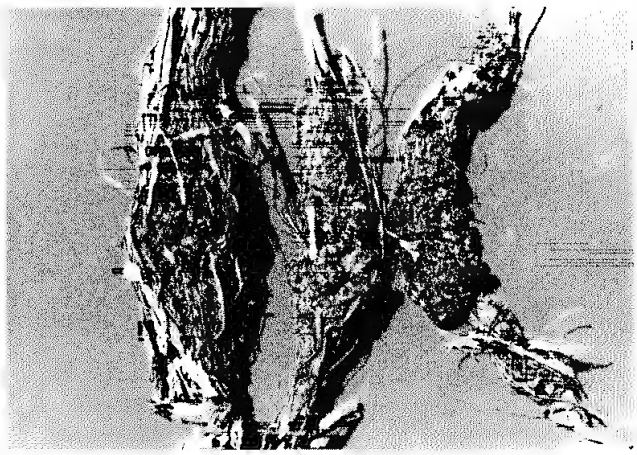

Fig. 1: Tumor formation on a grapevine in the vineyards of Karaj.

Antibiogram test: All pathogenic strains of *A. vitis* exhibited a complete resistance to amoxicillin, cephalexin, clindamycin, glucose acillin, oxacillin, penicillin and vancomycin. This was true for cephalotin and sefradin, except the fact that strain 6 was partially susceptible to these antibiotics. *A. vitis* strains were partially resistant to ampicillin, carbanicillin, colistin sulfate, furasolidon, gentamycin and neomycin but susceptible to the remaining antibiotics (Tab. 4).

SDS-PAGE analysis: SDS-polyacrylamide gel electrophoresis showed that *A. vitis* pathogenic strains 1, 2 and 4 have similar protein profiles that are somewhat different from those of strains 3, 5, 6 and 7 (Fig. 4). Strain 3 seems to be different from all other *A. vitis* strains.

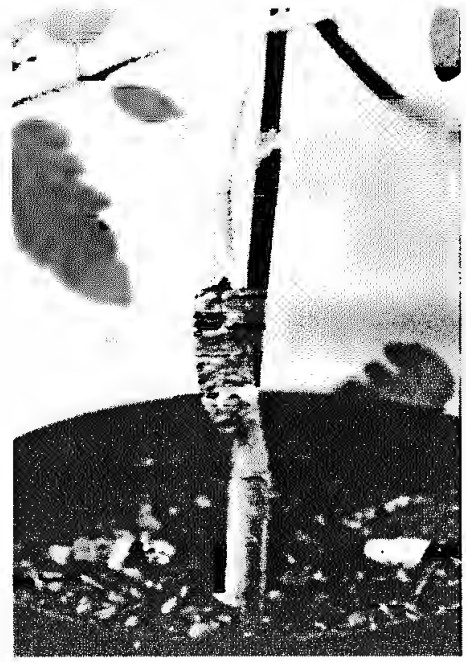

Fig. 2: Tumor formation on a tomato plant induced by *A. vitis* strain 1.

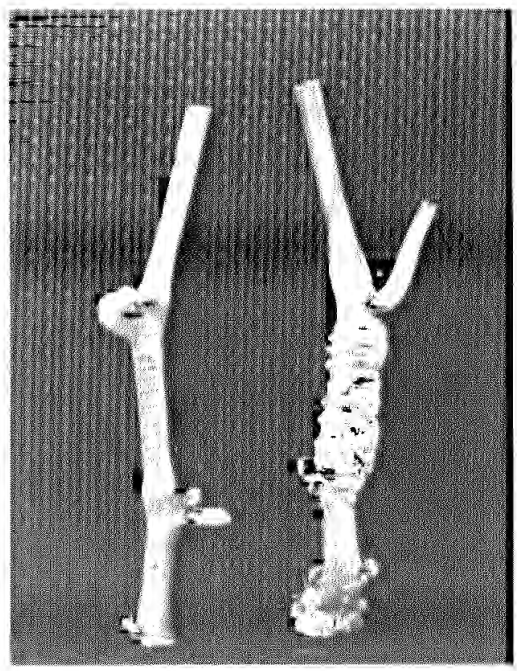

Fig. 3: Tumor formation on grapevine (var. Asgari) induced by *A. vitis* strain 2 (right). Water-inoculated control (left).

Plasmid DNA profile (Fig. 5): Agarose gel electrophoresis analysis revealed a common plasmid DNA band in all pathogenic as well as non-pathogenic strains of *A. vitis* with the exception of strain 10. A similar DNA band was also observed in *A. tumefaciens* and *A. radiobacter*.

Discussion

Although non-pathogenic strains of *Agrobacterium* were found in tumor and soil samples, pathogenic strains of *A. vitis* were isolated from xylem sap of vines on the selective RS medium during early spring when buds were swollen. Bauer *et al.* (1994) have shown that the population

Table 2

Differentiating phenotypical characteristics of *A. vitis* and *Agrobacterium* biovars 1 and 2

Isolate	3-Ketolactose production	Growth on 2 % NaCl	Growth at 37 °C	Litmus milk activity		Ferric ammonium citrate	Acid from			Oxidase test	Alkali from			L-Tyrosine	Nitrate utilization
				Alkaline	Acid		Sucrose	Erythritol	Melezitose		Malonic acid	L(-)-Tartaric acid	Propionic acid		
1	-	+	-	+	-	-	+	-	-	+*	+	+	-	-	+
2	-	+	-	+	-	-	+	-	-	+*	+	+	-	-	+
3	-	+	-	-	+	-	+	-	-	+	+	+	-	+	+
4	-	+	-	+	-	-	+	-	-	+	+	+	-	-	+
5	-	+	-	-	+	-	+	-	-	+*	+	+	-	+	+
6	-	+	-	-	+	-	+	-	-	+	+	+	-	-	+
7	-	+	-	-	+	-	+	-	-	+	+	+	-	+	+
8	-	+	-	-	+	-	+	-	-	+	+	+	-	+	+
9	-	+	-	+	-	-	+	-	-	+	+	+	-	-	+
10	-	+	-	+	-	-	+	-	-	+	+	+	-	-	+
11	-	+	-	+	-	-	+	-	-	+*	+	+	-	+	+
12	-	+	-	+	-	-	+	-	-	+	+	+	-	-	+
13	-	+	-	+	-	-	+	-	-	+	+	+	-	-	+
14	-	+	-	+	-	-	+	-	-	+	+	+	-	-	+
15	-	+	-	+	-	-	+	-	-	+	+	+	-	-	+
16	-	+	-	+	-	-	+	-	-	+	+	+	-	-	+
17	-	+	-	+	-	-	+	-	-	+	+	+	-	-	+
18	-	+	-	+	-	-	+	-	-	+	+	+	-	-	+
19	-	+	-	+	-	-	+	-	-	+	+	+	-	-	+
20	-	+	-	+	-	-	+	-	-	+	+	+	-	-	+
21	-	+	-	+	-	-	+	-	-	+	+	+	-	-	+
22	-	+	-	+	-	-	+	-	+	+	+	+	-	+	+
23	+	+	+	+	-	-	+	-	+	+	-	-	-	-	+
24	+	+	+	+	-	+	+	-	+	+	-	-	-	-	+
25	+	+	+	+	-	+	+	-	+	+	-	-	-	-	+
26	+	+	+	+	-	+	+	-	+	+	-	-	-	-	+
27	+	+	+	+	-	+	+	-	+	+	-	-	-	-	+
28	+	+	+	+	-	+	+	-	+	+	-	-	-	-	+
29	-	-	-	-	+	-	-	-	-	+	+	+	-	+	+
30	-	-	-	-	+	-	-	+	-	+	+	+	-	+	+
31	-	-	-	-	+	-	-	+	-	+	+	+	-	+	+
32	-	-	-	-	+	-	-	+	-	+	+	+	-	+	+

* Delayed oxidase reaction. 1-7: pathogenic, 8-22: non-pathogenic strains of *A. vitis*; 23-28 and 29-32: non-pathogenic strains of *Agrobacterium* biovar 1 and biovar 2, resp.

Table 3

Bacteriological properties of seven pathogenic strains of *Agrobacterium vitis* isolated from grapevines

Characteristic	1	2	3	4	5	6	7
Gram reaction	-	-	-	-	-	-	-
O/F test	o	o	o	o	o	o	o
Aesculin hydrolysis	+	+	+	+	+	+	+
Arginine dihydrolase	+	+	+	+	+	+	+
Caesin hydrolysis	-	-	-	-	-	-	-
Fluorescent pigment	-	-	-	-	-	-	-
Gelatin hydrolysis	+	-	+	-	-	+	-
H_2S from peptone	+	+	-	+	-	+	-
HR on Tobacco	-	-	-	-	-	-	-
Indole formation	-	-	-	-	-	-	-
Levan production	+	+	+	+	+	+	+
Nitrate reduction	-	-	-	-	-	-	-
Reducing compound	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	-
Tween 80 hydrolysis	-	-	-	-	-	-	-
Urease	+	+	+	+	+	+	+
Utilization of:							
Acetate	+	+	+	+	+	+	+
Adonitol	+	+	+	+	+	+	+
L(-)Arabinose	-	-	-	-	-	-	-
L(+)Arabinose	+	+	+	+	+	+	+
L-Arginine	+	+	+	+	+	+	+
L-Ascorbic acid	+	+	-	+	-	+	-
D(+)Cellubiose	+	+	+	+	+	+	+
Citrate	-	-	-	-	-	-	-
L-Cysteine	+	+	+	+	+	+	+
Dulcitol	-	-	-	-	-	-	-
meso -Erythritol	+	+	-	-	+	+	+
Ethanol	-	-	+	-	-	+	+
D(-)Fructose	+	+	+	+	+	+	+
D(+)Galactose	+	+	+	+	+	+	+
D(-)Galactose	+	+	+	+	+	+	+
D(+)Glucose	+	+	+	+	+	+	+
L-Histidine	+	+	-	+	+	+	+
Isoleucine	+	+	+	+	+	+	+
Lactate	+	+	+	+	+	+	+
α-Lactose	+	+	+	+	+	+	+
L-Leucine	-	-	-	-	-	-	-
Malate	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+
D(-)Mannitol	+	+	+	+	+	+	+
D(-)Mannose	+	+	+	+	+	+	+
D(+)Mannose	+	+	+	+	+	+	+
L-Methionine	+	+	+	+	+	-	+
Nicotinamide	+	+	-	+	-	+	-
L(+)Rhamnose	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+
Starch	-	-	-	-	-	-	-
D-Sorbitol	-	-	-	-	-	-	-
L(+)Tartrate	+	+	+	+	+	+	+
L-Valine	+	+	+	+	+	+	+
L(-)Xylose	-	-	-	-	-	-	-
D(+)Xylose	+	+	+	+	+	+	+

Table 4

Antibiotic sensitivity of *Agrobacterium vitis* strains and *A. radiobacter*. The size of the inhibition zone is presented in mm

Antibiotic	µg/disk*	Strain 1	2	3	4	5	6	7	*A. radiobacter*
Amicacin	30	3	4	9	4	5	9	11	10
Amoxicillin	25	0	0	0	0	0	0	0	0
Ampicillin	10	0	0	4	0	0	0	4	0
Carbanicillin	100	4	3	4	5	3	4	5	4
Cephalexin	3	0	0	0	0	0	0	0	0
Cephalotin	30	0	0	0	0	0	5	0	0
Cephazolin	30	2	2	7	2	5	8	5	0
Clindamycin	2	0	0	0	0	0	0	0	0
Chloramphenicol	30	9	9	7	8	13	0	6	2
Colistin sulfate	10	2	2	3	3	5	6	2	2
Erythromycin	15	0	0	5	0	7	5	0	0
Furasolidon	15	2	2	4	3	4	4	5	0
Gentamycin	10	4	3	4	3	3	4	5	3
Glucose acillin	5	0	0	0	0	0	0	0	0
Kanamycin	30	7	8	8	9	8	8	7	0
Lincomycin	2	4	6	7	4	11	10	6	4
Nalidixic acid	30	8	7	6	8	11	6	9	5
Neomycin	30	2	2	3	2	2	5	6	0
Oxacillin	1	0	0	0	0	0	0	0	0
D-oxy-cycline	30	12	12	11	10	4	15	4	5
Oxy-tetracycline	30	9	10	10	9	13	15	5	0
Penicillin	10	0	0	0	0	0	0	0	0
Rifampicin	30	5	5	6	5	6	10	4	6
Sefradin	30	0	0	0	0	0	5	0	0
Seftysorkims	30	6	5	10	6	13	0	5	0
Streptomycin	10	4	3	7	4	11	10	6	4
Tobramycin	10	3	2	8	3	7	8	2	0
Trimethoprim-sulfaxasol	-	0	0	7	0	5	0	7	4
Vancomycin	30	0	0	0	0	0	0	0	2

* Unit/disk used for amoxycillin, ampicillin and penicillin.
Resistant, no inhibition zone (0 mm); partially resistant, inhibition zone diameter < 6 mm; sensitive, inhibition zone diameter ≥ 6 mm.

density of *A. vitis* in infected grapevines depends upon annual physiological changes. *A. vitis* populations are lowest in summer and reach a peak in winter.

A. vitis strains 3, 5 and 7 differed with regard to ascorbic acid, nicotinamide and tyrosine utilization and H_2S production from other pathogenic strains and those reported in the literature (MOORE *et al.* 1988). Since these strains caused tumorigenesis on grapevines, they were characterized as *A. vitis*. THIES *et al.* (1991) also observed that pathogenic strains of *A. vitis* were different in some phenotypic characteristics from a typical *A. vitis* and were thus named biovar 2 or 3. In antibiotic sensitivity tests, *A. vitis* pathogenic strains 1, 2 and 4 exhibited a similar pattern that is somehow different from other strains and there are sensitivity differences among the remaining strains. Among 10 different Iranian grapevine varieties tested for tumor formation by *A. vitis* cv. Asgari was the most sensitive and produced relatively large tumors. Formerly AMANI (1966) has characterized Asgari as the most sensitive and Chefteh as a relatively resistant grape variety in Iran. Neither *A. tumefaciens* nor *A. radiobacter* induced tumor production on grapevines. *A. tumefaciens* caused tumorigenesis on tomatoes but not on grapevine. These results are consistent with those reported by TARBAH and GOODMAN (1987) in that pathogenic strains of *Agrobacterium* biovars 1 and 2 were non-tumorigenic on grapes but induced tumors on tomato and castor bean. *A. tumefaciens* type strain NCPPB 2437 caused tumor formation on sunflower and boysenberry but not on grapevines (OPHEL and KERR 1990). On the other hand, several reports have indicated tumor induction on grapevines by *Agrobacterium* biovars 1 and 2 (PERRY and KADO 1982; IRELAN and MEREDITH 1996). In this study, non-pathogenic strains of *A. vitis* and *Agrobacterium* biovars 1 and 2 caused no tumor formation on

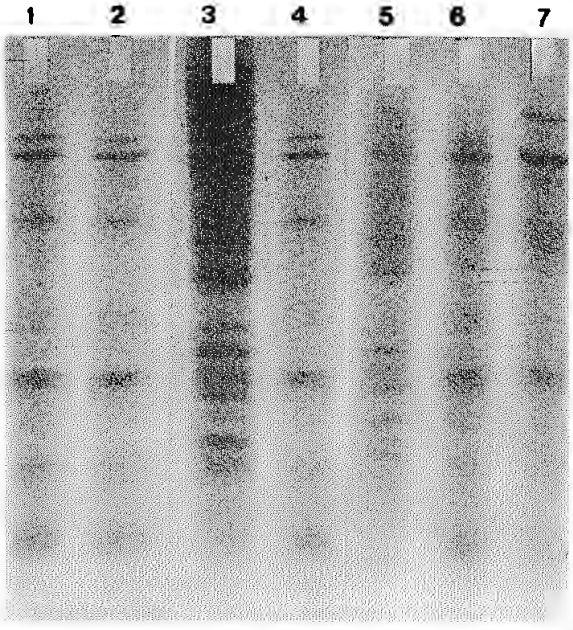

Fig. 4: SDS-PAGE analysis of the protein profile from pathogenic strains of *A. vitis*.

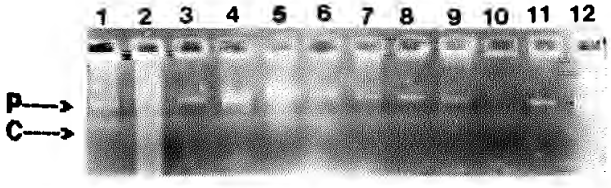

Fig. 5: Agarose gel electrophoresis of Ti plasmid profile from pathogenic strains of *A. vitis* (1-7), non-pathogenic strains of *A. vitis* (8-10), *A. tumefaciens* (11) and *A. radiobacter* (12). P, plasmid; C, chromosome.

test plants. *A. vitis* strains, when applied at a low concentration, also induced small tumors on carrot root disks along with some tissue maceration. OPHEL and KERR (1990) also observed *A. vitis* tumor formation on carrot tissue. SDS-PAGE analysis revealed that *A. vitis* pathogenic strains 1, 2 and 4 are very similar in protein profile but different from other strains. This is consistent with some pheno-typic characteristics such as litmus milk activity, ethanol and L-tyrosine utilization, hydrogen sulfide production and antibiotic sensitivity test which these strains share. DNA fingerprinting performed by GILLINGS and OPHEL-KELLER (1995) showed that a significant intraspe-cific heterogeneity exists in *A. vitis* and thus the population structure of *A. vitis* is composed of several clonal lines that are limited to specific groups of hosts or geographical regions. Based on the results of this study indicating that strains 1, 2 and 4 belong to one group and strains 3, 5, 6 and 7 fall into another group, it can be concluded that *A. vitis* isolates from Iran are heterogeneous. Our recent (unpubl.) results confirm these differences among *A. vitis* strains, particularly with an acidic litmus test which is considered to be a unique property of *Agrobacterium* biovar 2. Work is in progress to identify and differentiate *A. vitis* strains from other *Agrobacterium* biovars by PCR using *peh*A-specific primer pair (EASTWELL *et al.* 1995).

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