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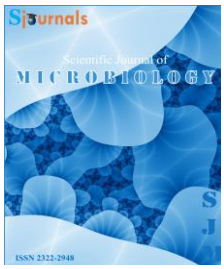

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Original article

Evaluation of Erythromycin–resistant and Chloramphenicol-sensitivity on *Agrobacterium vitis* in Iran

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ABSTRACT

The purpose of this study was to determine the prevalence of Erythromycin and Chloramphenicol resistant/sensitivity of *Agrobacterium vitis* that isolated from environmental samples in Iran vineyards. From May to June 2014, 20 samples isolated from Vineyards of five grape cultures from west of Iran. After identification of *Agrobacterium vitis* by biochemical, microbiological and molecular methods, antibiotic susceptibility testing was performed by Kirby-Bauer disk diffusion test for these antibiotics. The lowest and highest resistance was seen for Chloramphenicol (n=1) and Erythromycin(n=10). The results showed that the frequency of Erythromycin resistance is very high in *Agrobacterium vitis* strains isolated from vineyards that had crown Gall in west of Iran. Thus, urgent interventions are needed to keep the emergence and transmission of these isolates to a minimum. The lowest resistance on Chloramphenicol in *Agrobacterium vitis* strains showed that we can use it for urgent occasions.

1. Introduction

Agrobacterium vitis is a gram negative bacteria and III biovar of *Agrobacterium tumefacien* that occurs in the soil naturally and cause diseases in adaptable hosts. Pathogenicity of these bacteria is related to its tumor inducer

plasmid. *Agrobacterium* could transfer its plasmid into the plant genome and in this way will be starting the secretion to make some growth hormones like Opens and made Crown Gall of grape. Crown gall of grapevine can cause extensive losses in whole world Vineyards, particularly on cold-sensitive cultures (Bazzi et al., 1987). Galls usually occur on the trunk just above the soil have been observed. Reports from Greece (Burr et al., 1984, 1988), Hungary (Clinical and Laboratory Standards Institute, 2011), and preliminary reports from the United States (Bazzi, et al., 1987; Burr et al., 1993) have identified *Agrobacterium tumefacien* biovar 3 (AT 3) as the predominant biovar on grapevines. In some parts of Iran spatially in the west of Iran crown gall is accession; unfortunately farmers used Erythromycin to the contrast of Galls, but in recent years this antibiotic did not show any effects on galls of vineyards. This present study aimed to determine the antimicrobial susceptibility extension of *A.vitis* isolates from grapevine vineyards in west of Iran with emphasis to the possible presence of Erythromycin resistance. Also, we try to contrast Chloramphenicol and Erythromycin antibiotics on these bacteria to appraise their bacteriostatic/ bactericidal effects on A.vitis bacteria.

2. Materials and methods

Isolations were made from fresh galls on naturally infected vines collected from May to June 2014. Galls were washed thoroughly in running tap water and blotted dry. A total number of 20 samples from 5 grapevine vineyards collected. Small sections of surface tissues were removed with a sterile scalpel, put in sterile physiological serum. Then the samples on in vitro condition mixed for 45 minutes (Moore, 1988; Ataee et al., 2011) and filtered fluids by Watman filter paper to isolating impurities from them. After that, cultured from these fluids on yeast mannitol agar (Himedia, Germany) and incubated for 48 hours on 28C degrees. Just one kind of the colonies that growth on plates, selected as *Agrobacterium*. 10 plates of isolation *Agrobacterium vitis,* were selected after identification by standard biochemical and microbiological tests, including Gram staining, Oxidase, catalase, motility, SIM, TSI and growth on 2% of NaCl tests (table 1).

Table 1
Results of biochemical, microbiological and pathogenicity tests.

Bacteria Tests	**Agrobacterium Vitis**	**Agrobacterium Tumefacien**	**Studied strain**
Aerobic	+	+	+
Gram	N	N	N
Oxidase	−/+	−/+	−/+
Catalase	+	+	+
Simon citrate	+	N	+
NaCl 2%	+	+	+
Growth on 35 C	+	+	+
Urea	+	+	+
TSI	G+ glucose+ H2S-	G+ -/+ glucose H2S-	G+ glucose+ H2S-
M.R	+	+	+
SIM	+	+	+
3ketolactose	N	+	N
Movement on PH=7	N	+	N
Tomato	−	+	−
Grape	+	+	+
geranium	NT	NT	+
CARROT	+	+	+

NT. Not tested

To confirm the strains, PCR amplification of 16srRNA gen was performed for all isolates with these primers (table 2) (Szegedi et al., 2002); the result of PCR is mentioned on figure1.

Table2
The primers that used for PCR.

Name	Primer	Sequence	Size
Agrobacterium tumefacien A348	Forward	5'-ATCATTTGTAGCGACT-3'	730 bp
	Reward	5'-AGCTCAAACCTGCTTC-3'	
Agrobacterium vitis F2/5	Forward	5'-GGGGCAGGATGCGTTTTTGAG-3'	466 bp
	Reward	5'-GACGGCACTGGGGCTAAGAT-3'	

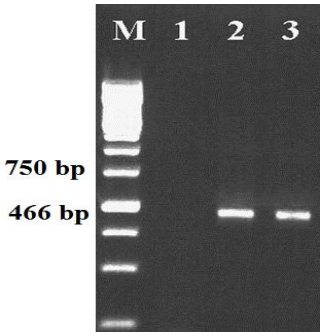

Fig. 1. PCR results. First column is negative control , second column is primers for *Agrobacterium vitis F2/5* and third column for studied strain with both primers. As it showed our studied strain is *Agrobacterium vitis* with 466 bp.

Also inoculations were made on 4 hosts: tomato (Lycopersiconesculentum Mill), grapevine (Vitis vinifera L.) (Burr et al., 1987), geranium (Geraniaceae Geranium L.), and carrot slices (Daucuscarota L.) (Burr et al., 1982). The stems of all hosts were repeatedly punctured through the inoculums with a fine, sterile needle smeary with bacteria. Carrot disks were placed in petri dishes with moist filter paper and heavy inoculums suspensions were inoculums like plants. The carrot slices incubated for 48 hours in 28C degree; Results are detailed on table 1. Disc agar diffusion (DAD) test was carried out using Kirby-Bauer method according to CLSI procedure with the following discs, which are all from Pad tan company, Iran: Chloramphenicol (15 mcg/disc) and Erythromycin (15 mcn/disc). All the petri dishes incubated for 48 hours on 28C degree.

3. Results and discussion

In this study, 10 samples of Agrobacterium vitis isolated from grapevines of five vineyards of west of Iran. The biochemical and microbiological tests results are detailed in table 1. Amplification of 16srRNA gene of *Agrobacterium vitis* strain produced distinct bands corresponding to their respective molecular sizes that were easily recognizable (fig.1). *Agrobacterium vitis* showed highest resistance to Erythromycin in all 10 plates and lowest resistance to Chloramphenicol in 9 plates after incubated in 28 C degree for 48 hours (table 3 and figures 2,3).

Table 3
Antibiotics non growth halo rates on *Agrobacterium vitis cultures* base on mile meter.

Antimicrobial non growth halo rate	Plates									
	1	2	3	4	5	6	7	8	9	10
Erythromycin	0	0	0	0	0	0	0	2	0	0
Chloramphenicol	22	25	23	24	24	25	25	24	23	24

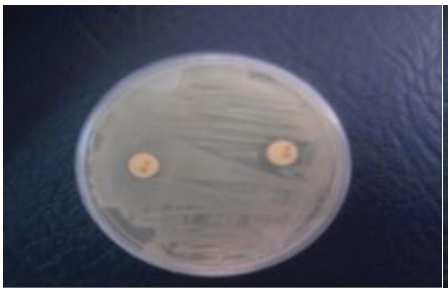

Fig. 2. One sample of Agrobacterium vitis culture and Erythromycin discs.

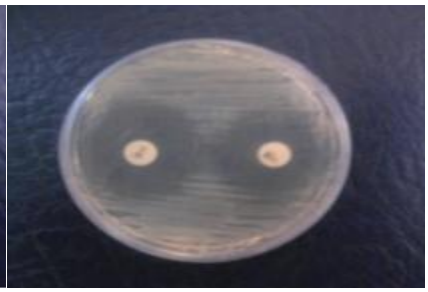

Fig. 3. One sample of Agrobacterium vitis culture and Chloramphenicol discs.

The prospects for controlling grape crown gall in commercial vineyards look very promising. Biological control of grape crown gall appears to be very effective and may prove to be a reliable treatment for vines that are to be planted on sites that are contaminated with the pathogen. Selecting planting sites that are not conducive to freeze injury and using rootstocks and scion cultivars that are relatively resistant to crown gall remain important management considerations. However, in spite of many significant research accomplishments, several important questions related to pathogen biology and disease control remain. Although methods for detection of *A.vitis* are reliable, their sensitivities are largely unknown. This paper showed that *A.vitis* strain in Iran has high frequency of resistance for Erythromycin and lowest resistance for Chloramphenicol. This result comes from antimicrobial disc agar diffusion of Erythromycin and Chloramphenicol after 48 hours. Approximately there was any sensitivity halo around the discs on all the plates that tested with Erythromycin. Erythromycin in high concentrations inhibits the growth of bacteria, but its mechanism is not completely understood. It has been reported that erythromycin suppresses protein synthesis and subsequent structure and function processes which are important for life (Fraschini et al., 1986). It can attach on 23srRNA of the 50s subunit of bacterial ribosome and interact with transition and ripen of beginning complexes of protein synthesize. But the sensitivity halos around the Chloramphenicol discs were about 24mm. Then all the discs existed from plates, and put all of them again on incubator for 48 hours to understand that this antibiotic made bactericidal/bacteriostate effects; zero clones growth on these halo parts after 48 hours. These results showed that these bacteria were sensitive in contrast of Chloramphenicol antibiotic. This antibiotic targeted the 23sr RNA of the 50s subunit of bacterial ribosomes, too, and inhibit at or near the peptidyl transferase region. Chloramphenicol and its analogues bind to the peptidyl-transferase centre (PTC). The *cfr* gene, whose product, the Cfr methyltransferase, modifies a 23S rRNA residue in the PTC, confers resistance to chloramphenicol. Chloramphenicol has potent activity against a broad spectrum of pathogens and is well distributed intracellularly, but its use is limited. The gene of resistance to Chloramphenicol is on the bacterial plasmid.

Thus, urgent interventions are needed to keep the emergence and not let these bacteria, to have resistance factor of antimicrobial agents and transferred these genes to other strains of *Agrobacterium*; because it can make *Agrobacteria* stronger in comparison of antibiotics and then controlling crown gall can be impossible.

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