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The use of *Spiroplasma melliferum* as a model organism to study the antagonistic activity of grapevine endophytes against phytoplasma

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Abstract

The objective of the research was to isolate from grapevines endophytes with antagonistic activity against phytoplasmas. In order to overcome the inability of grow phytoplasmas *in vitro*, the antagonistic activities of endophytes isolated from various grapevines on *Spiroplasma melliferum,* a phylogenetic close and cultivable Mollicute as a model organism was tested. No correlation was found so far between the inhibitory activity and the different plant sources, i.e. healthy, recovered or infected vines.

Key words: *phytoplasmas, Spiroplasma melliferum, endophytes, recovery*.

Introduction

The recovery phenomenon and the various degrees of susceptibility of grapevine cultivars to yellows disease may indicate a possible involvement of endophytes in the mechanism of plant resistance.

The objective of the research project was to isolate from grapevines endophytes with antagonistic activity against phytoplasmas. However, the inability to cultivate phytoplasmas on artificial medium prevent from a direct trial. In order to overcome this problem, the antagonistic activities of endophytes isolated from various grapevines on *Spiroplasma melliferum*, a phylogenetic close and cultivable Mollicute as a model organism was tested.

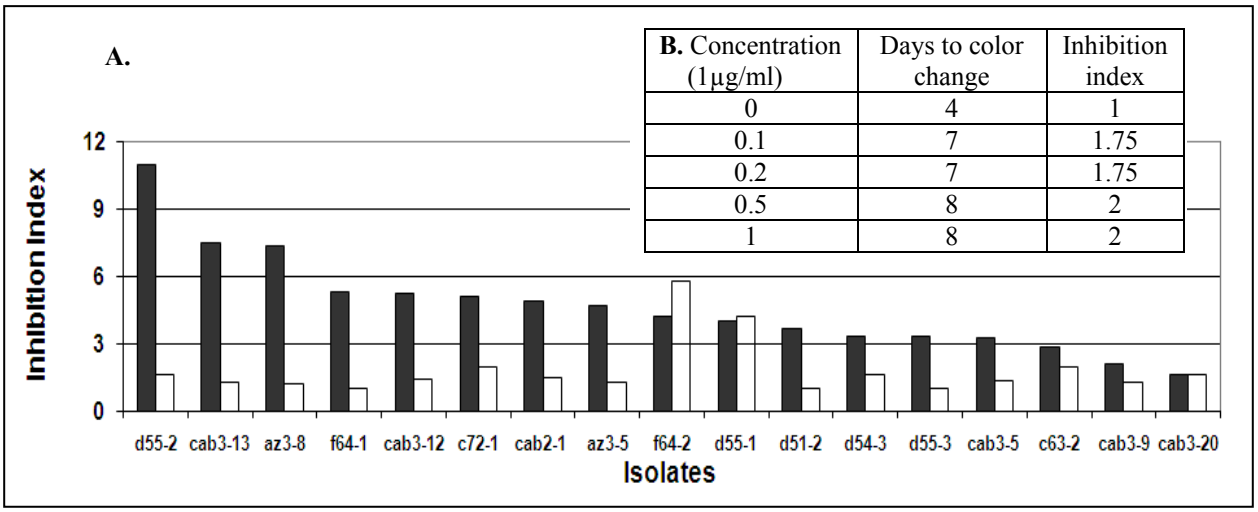
Materials and methods

Isolates from infected, recovered and healthy Cabernet-Sauvignon vines and from deserted vines were isolated in the early and late summer by placing sterilized discs of canes on nutrient agar and on PDA. The percentage of discs from which fungi or bacteria developed was recorded (table 1).

To test the antagonistic activity of the isolated endophytes, the different isolates were grown in a modified spiroplasma broth for 10 days (Trachtenberg and Gilad, 2001). After centrifugation (4,000 rpm; 30 min) and filtration, spiroplasma cells were added to either 50% diluted or 100% of the supernatant to make an initial concentration of ca. 10^6 cells/ml and incubated at 29°C for five days. Spiroplasma growing in fresh modified broth served as a positive control and the inhibitory effect of 0.5 µg/ml oxy-tetracycline was used as a reference. To monitor spiroplasma development in the filtrates we inoculated fresh spiroplasma medium with 1 µl of the incubated filtrate. The fresh medium was supplemented with phenol red as a color marker for cells growth (Trachtenberg and Gilad, 2001). Spiroplasma cell growth causes a pH decrease that changes the color of the medium from red to yellow. The time required to color change is correlated with the initial spiroplasma concentration and was therefore used as a quantitative parameter for the inhibitory effect of the filtrates. Inhibition index was defined as the ratio between the number of days to color change in the filtrate and the time required in the positive control. The inhibition index of 0.5 µg/ml oxy-tetracycline was 2. Thus, an isolate was considered inhibitory if the inhibition index of its filtrate was >=2 (figure 1A and B).

Table 1. Percent of discs with endophytic fungi or bacteria from four collection dates. Discs were cut from deserted grapevines and from healthy, recovered 1y, recovered 2y and infected Cabernet-Sauvignon grapevines.

Date of isolation	Deserted		Healthy		Recovered 2y		Recovered 1y		Infected	
	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria
5.2009	1.7	45.6	0.0	6.0	1.9	20.0	2.9	20.9	0.8	10.0
7.2009	35.8	7.8	10.4	2.7	4.9	2.8	5.9	0.8	9.5	1.7
5.2010	22	0.6	1.2	0.7	2.5	6.5	2	0.8	1.0	4.0
8.2010	69.3	3.7	8.0	2.0	6.3	9.3	16.7	13.3	24.7	3.3
Average	34.5	6.9	4.9	2.87	3.9	9.7	6.9	9.5	9.0	4.8



B. Concentration (1µg/ml)	Days to color change	Inhibition index
0	4	1
0.1	7	1.75
0.2	7	1.75
0.5	8	2
1	8	2

Figure 1. Inhibition index for spiroplasma of different isolates (Mean of 2-15 replications).
A. In: ■ -100% filtrate;□ -50% diluted filtrate.
B. Inhibition index of oxy-tetracycline.

Results and discussion

In general, the highest number of populated discs was found in deserted vines (34.5% fungi, 6.9 bacteria) in contrast to healthy vines with the lowest percentage of isolates (avg. 4%). Endophytes developed from 4.8-9.7% of the discs cut from canes of recovered and infected vines. In the first date of collection we isolated more bacteria than fungi while in the other three dates the number of fungal isolates was higher.

Using this method, one fungus and several bacteria (out of 300 tested isolates) showed a relatively high inhibition activity against spiroplasma (figure 1). Growing in 100% filtrates caused 2-10 folds inhibition of spiroplasma cell growth relative to the positive control and was similar or higher compared to inhibition activity of 0.5 µg/ml oxy-tetracycline. However, the inhibitory effect of the diluted (50%) filtrate was much lower. No correlation was found so far between the inhibitory activity and the different plant sources, i.e. healthy, recovered or infected vines. This study shows that spiroplasmas can serve as an initial model system to test the effect of different compounds on phytoplasma development. In further studies the filtrates will be tested on phytoplasma in nurse culture *in vitro*.

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References

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