

STUDIO DI UN CASO

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2 vigneti nel comune di Gaiole in Chianti

Vitis vinifera Sangiovese



Clematis vitalba





- ✓ Diagnosi di FD
- ✓ Confronto tra gli isolati di FDp nei due ospiti presenti nello stesso areale

Diagnosi e caratterizzazione di FDp: polimorfismo nucleotidico del gene 16Sr

1) Identificazione del fitoplasma associato a FDp (protocollo di qPCR): Flavescenza Dorata

2) Caratterizzazione del sottogruppo (protocollo PCR diretta e nested):

16S(V)-C (isolato rif. FD70, FD2000): più diffuso, caratterizzato in Francia

16S(V)-D (isolato rif. FD92, FD88): meno diffuso, caratterizzato in Spagna

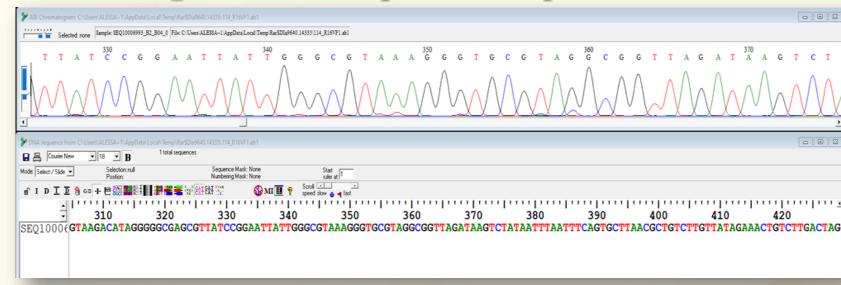
2020: diagnosi condotta sui campioni prelevati dal vigneto di Gaiole:

- ✓ 12 campioni di *V. vinifera* (11 infetti)
- ✓ 6 campioni di *C. Vitalba* (tutti infetti)
- ✓ 17 sequenze

Amplificazione di una parte del gene 16S (1300bp)



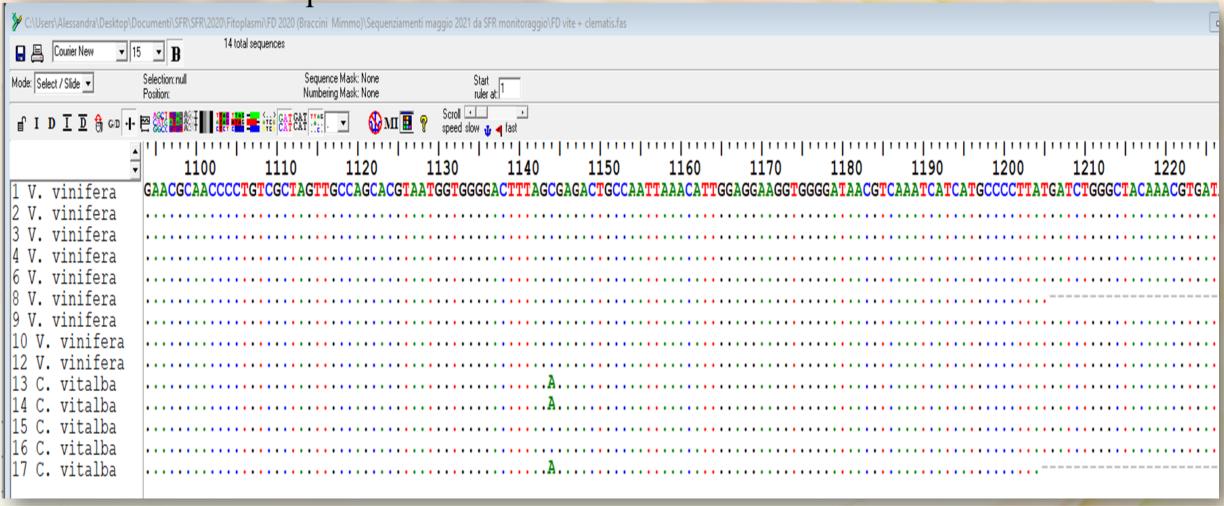
Cromatogramma e sequenza amplicone



Sequenza errata



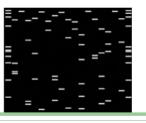
Allineamento delle sequenze in BioEdit





*i*PhyClassifier

- Taxonomic assignment
- Group/subgroup classification
- Virtual gel analysis
- Pattern similarity coefficients



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46 sfr The query 16S rDNA sequence is identical to that of "Candidatus Phytoplasma vitis" reference strain (GenBank accession: AF176319). The phytoplasma under study is either the "Candidatus Phytoplasma vitis" reference strain of a cross sy related strain that cannot be distinguished by 16S rDNA sequence alone.

https://plantpathology.ba.ars.usda.gov/tmp/ip...

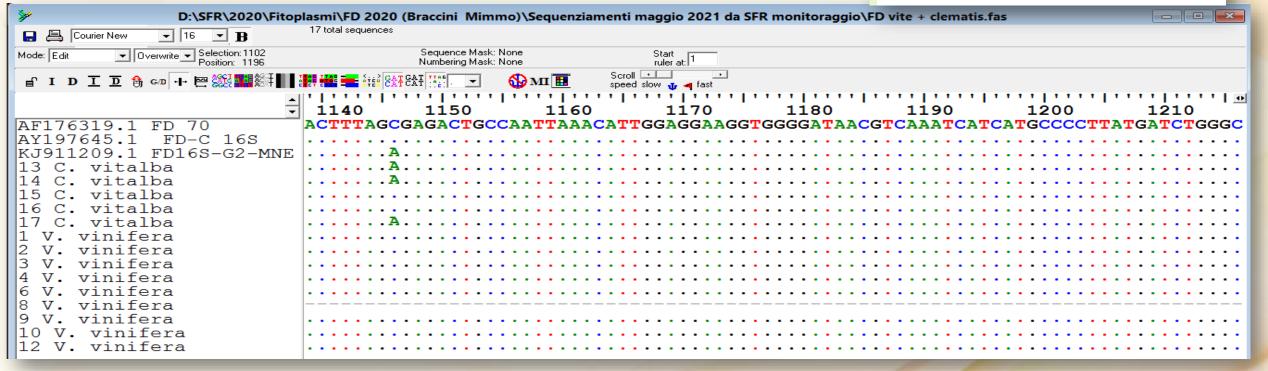
https://plantpathology.ba.ars.usda.gov/tmp/ip...

16S group/subgroup classification

46 sfr Flavescence d--The virtual RFLP pattern derived from the query 16S rDNA F2nR2 fragment is identical (similarity officient 1.00) to the reference pattern of 16Sr group V, subgroup C (GenBank accession: AY197642). The phytoplasma under study is a member of 16SrV-C.

updated and running Feb 22nd, 2022. Please email us for any problems that might occur. Paste query sequence in FASTA format here □ 'Candidatus Phytoplasma' species assignment □ 16Sr group/subgroup classification based on similarity coefficient ► □ RFLP similarity coefficient table □ Deviation allowed □ →

Allineamento con sequenze di riferimento in BioEdit



GEHDAHK *

'Clematis vitalba'/'Dictyophara europaea' flavescence doree phytoplasma genotype 16S-G2-MNE 16S ribosomal RNA gene, partial sequence

```
GenBank: KJ911209.1
```

```
FASTA Graphics PopSet
Go to: ♥
LOCUS
            KJ911209
                                    1446 bp
                                                       linear BCT 31-DEC-2017
DEFINITION 'Clematis vitalba'/'Dictyophara europaea' flavescence doree
            phytoplasma genotype 16S-G2-MNE 16S ribosomal RNA gene, partial
            sequence.
ACCESSION
            KJ911209
VERSION
            KJ911209.1
KEYWORDS
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            phytoplasma
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            phytoplasma
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            Acholeplasmataceae; Candidatus Phytoplasma; 16SrV (Elm yellows
            group).
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                     /country="Montenegro"
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                     genotype: 16S-G2-MNE"
                     <1...>1446
    rRNA
```

/product="16S ribosomal RNA"

23 corrispondenze (100%) con FD16S-G2-MNE in NCBI

RM29

4621

Bosnia

Germania

Alnus glutinosa

Ulmus glabra

			102 sequences selected 😲				
				Description	Score	E value	Accession
	1			Candidatus Phytoplasma sp. [mycoplasmas]	▼ Next ▲	Previous	s ≪ First
Ospite	Origine	Isolato	Acc. numbe	Phytoglasma sp. isolate 4621 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic spacer, partial sequence	1855	0.0	MN394842
Ospite	origine			Phytoglasma sp. isolate 2625 16S ribosomal RNA gene and 16S-23S ribosomal RNA intergenic spacer_partial sequence	1855	0.0	MN394841
				'Clematis vitalba'/Dictyophara europaea' flavescence doree phytoplasma [mycoplasmas]	▼ Next ▲	Previous	s ≪First
			'Clematis v	talba//Dictyophara europaea' flavescence doree phytoplasma genotype 16S-G1-MNE 16S ribosomal RNA gene, partial sequence	1855	0.0	KJ911208
Dictyophara europea	Montenegro	FD16SG2MNE	'Clematis v	talba'/Dictyophara europaea' flavescence doree phytoplasma genotype 16S-G1-SRB 16S ribosomal RNA gene. partial sequence	1855	0.0	KJ911207
			'Clematis v	talba/flictyophara europaea' flavescence doree phytoplasma genotype 16S-G2-MNE 16S ribosomal RNA gene. partial sequence	1849	0.0	KJ911209
			KJ911208	Candidatus Phytoplasma vitis [mycoplasmas]	▼ Next ▲	Previous	s ≪ First
			NJ9112UQ _{idatus}	Phytoglasma vitis isolate Ls1MS 16S ribosomal RNA gene. partial sequence	1855	0.0	MT629815
			Candidatus	.Phytorlasma.vitis isolate.SjRag 16S.ribosomal RNA.gene, partial sequence	1855	0.0	MT629811
			Candidatus	Phytorlasma vitis isolate.Sj2MS 16S ribosomal RNA gene. partial sequence	1855	0.0	MT629806
		l	Candidatus	<u>Phytorlasma vitis isolate SjMilo 16S ribosomal RNA gene, partial sequence</u>	1855	0.0	MT629804
Spartium junceum	Italia	sjRag	MT629811	Phytoslasma vitis 16S rRNA gene (partial). IRNA-lle gene and 16S-23S IGS (partial). isolate FD92	1855	0.0	FN562932
Span traint junte cant	100110	5J-246	Candidatus Ph	<u>Phytorlasma vitis strain Portugal1 16S ribosomal RNA gene. partial sequence</u>	1855	0.0	FJ611961
			Candidatos	Fbyter lasma vitis isolate 221417 16S ribosomal RNA gene. partial sequence	1855	0.0	FJ423158
Wide winifers	manturas li	TTC110C Candidatus	Phytorlasma vitis isolate 121206 16S ribosomal RNA gene. partial sequence	1855	0.0	FJ423157	
Vitis vinifera	Portogallo	portugali	FJ61196 Indidatus	<u>Phytorlasma vitis strain FD57 16S ribosomal RNA gene, partial sequence</u>	1855	0.0	EF581166
J		•	Flavescend	e dores phytopiasma strain FD-C 16S ribosomal RNA gene. partial sequence	1855	0.0	AY197645
			Flavescend	<u>e dores, phytoplasma ribosomal RNA operon. partial sequence</u>	1855	0.0	AF176319
Vitis vinifera Macabeg	Spagna	LSV91	AJ54878	e dores phytoplasma partial 16S rRNA gene and IGS, strain FD1487	1855	0.0	AJ548787
				e dores phytoplasma partial 16S rRNA gene. strain FD91	1855	0.0	AJ548791
J J	1 0		Flavescend	e dores phytoplasma partial 16S rRNA gene_strain FD947	1855	0.0	AJ548792
			Flavescend	e Cores phytoplasma partial 16S rRNA gene _strain FD1	1855	0.0	AJ548788
Grapevine	Serbia	FD57	EF581166 scene	e dores phytoplasma partial 16S rRNA gene _strain FD951	1855	0.0	AJ548793
Grupevine	Serbia	TD31		e doreg phytoplasma partial 16S rRNA gene, strain FD952	1851	0.0	AJ548794
			Flavescene		1851	0.0	AJ548789
~ 1 11	-		Candidatus	Phytoglasma vitis clone RM29 16S ribosomal RNA gene, partial sequence	1849	0.0	MN662623
Scaphoideus titanus	Francia	FD92	FN562912				
~ Corp. To the order to the corp.							
Witin winiform large	Doutegalle	EDD1	E1422150				
Vitis vinifera loureiro	Portogallo	FDD1	FJ423158				
U							

MN662623

MN394842

Organism Taxonomy

Standard Nucleotide BLAST

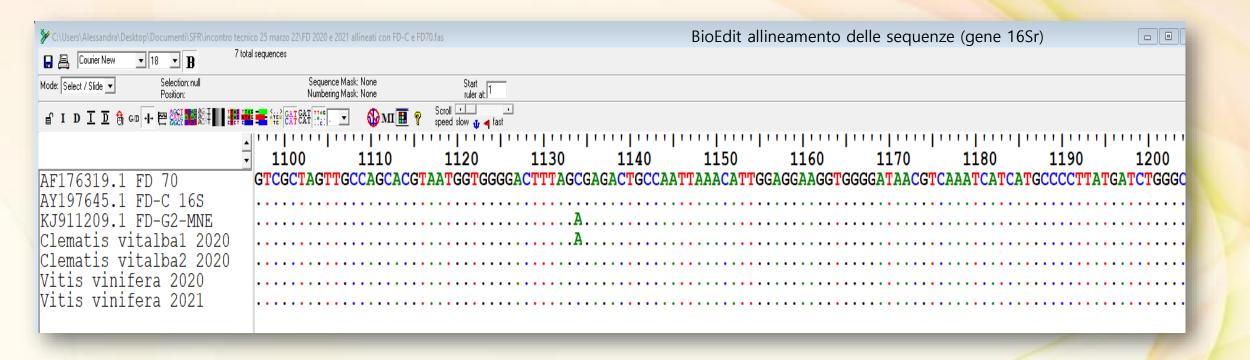
Riassumiamo

Ospite	Sequenza di riferimento (% similitudine)	Accession Number		
V. vinifera (tutte) 11 piante	FD-C (100%)	AY197645.1 AF176319.1		
C. vitalba (2 sequenze) 1 pianta	FD70 (100%)			
C. vitalba (3 sequenze) 2 piante	FD16S-G2-MNE (100%)	KJ911209.1		

2021 caratterizzazione di più campioni provenienti da vigneti dei comuni di Gaiole, Radda, Castelnuovo Berardenga, Castellina:

- ✓ 123 campioni di *V. vinifera*, 38 infetti
- ✓ 30 sequenze

Allineamento delle sequenze di FDp in BioEdit

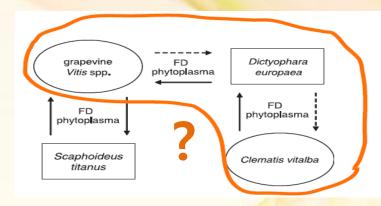


Risultati delle caratterizzazione dei campioni toscani ad oggi

Ospite	Caratterizzazione	Area		
V. vinifera 2019	46 piante FD-C (71,9%) 18 piante FD-D (28,1%)	Toscana (64 infetti)		
V. vinifera 2020	FD-C (100%)			
C. Vitalba 2020	2 piante FD16S-G2-MNE 1 pianta FD-C	Gaiole (9 infetti)		
V. vinifera 2021	FD-C (100%)	Chianti Classico (38 infetti)		
V. vinifera 2022	lavori in corso	Toscana (400 infetti)		

Considerazioni

- ✓ Presenza di V. vinifera infetta con FD-C e S. titanus: ciclo epidemiologico noto
- ✓ Presenza di 1 pianta *C. vitalba* infetta da FD-C
- ✓ Presenza di 2 piante *C. vitalba* infette da FD-16S-G2-MNE
- ✓ Assenza di FD-16S-G2-MNE su *V. vinifera*



C. vitalba: pianta ospite (serbatoio) di genotipi diversi di FDp (FD-C)

Dictyophara europea: cicalina ospite di FDp

Possibile presenza di diversi attori nel ciclo epidemico di FDp

Prossimi obiettivi

- ✓ Verifica della presenza di FD16S-G2-MNE su *C. vitalba e V. vinifera*
- ✓ Indagini su *Dictyophara europea*
- ✓ Indagini su Scaphoideus titanus
- ✓ Caratterizzazione con markers molecolari extra-ribosomali