Title:

Identification and characterization of *Neofusicoccum stellenboschiana* in branch and twig dieback-affected olive trees in Italy and comparative pathogenicity with *N. mediterraneum*

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Text Table S1 Methods for DNA extraction, PCR amplifications and sequencing of DNA regions of *Neofusicoccum stellenboschiana* isolated from olive trees in Apulia, Latium and Tuscany (Italy). In Table A the DNA regions amplified and primers used are reported. In Table B the thermal cycling for each DNA region is described. Below sequences generated in this study and their accession numbers are also listed.

Genomic DNA (gDNA) of fungal isolates was obtained from 100 mg mycelium sampled from axenic cultures actively growing on sterile cellophane discs (BIO-RAD, Hercules, CA, USA) layered on PDA plates and collected by scraping with a sterile pipette tip. Mycelium samples were powdered with liquid nitrogen, then gDNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

PCR amplifications were carried out on a PCR T100™ Thermal Cycler (BIO-RAD). Reaction assembly was in 50µl volume and contained each primer at 0.2 µM, 2.5 U of High Fidelity Platinum Taq DNA Polymerase (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), 0.2 mM (ITS) or 0.4 mM (TUB2 e TEF1-α) of each dNTP, in the buffer supplied by the Taq polymerase manufacturer.

Primer sequences and citations are reported in table A. Thermal cycling is reported in table B.

The PCR products were analysed by agarose gel electrophoresis, excised from gel, and eluted with ISOLATE II PCR and gel kit (Meridian Bioscience, River Hills Drive, Cincinnati, OH, USA) following the manufacturer's instructions. Then amplicons were directly sequenced in both directions by Sanger technology (Bio-Fab research s.r.l. Rome Italy).

Table A. DNA regions amplified and primers used

DNA region	Forward primer	Sequence	Reverse primer	Sequence	Citation
ITS	ITS5	5′-GGAAGTAAAAGTCGTAACAAGG-3′	ITS4	5′-TCCTCCGCTTATTGATATGC-3′	[25]
TEF1-α	EF688F	5′-CGGTCACTTGATCTACAAGTG C-3′	EF1251R	5′-CCTCGAACTCACCAGTACCG-3′	[26]
TUB2	T1-β tubulin[1]	5′-AACATGCGTGAGATTGTAAGT-3′	Bt2b[2]	5′-ACCCTCAGTGTAGTGACCCTTGGC-3′	[1] [27] [2] [28]
RPB2	fRPB2-6F	5′-TGGGGKWTGGTYTGYCCTGC-3′	fRPB2-7cR	5′-CCCATRGCTTGYTTRCCCAT-3′	[29]

Table B. Thermal cycling (35 cycles) to amplify DNA regions used for fungal identification (Blast and phylogeny)

Loci DNA	Initial denaturation	Denaturation for each cycle	Annealing	Extension	Final extension
ITS	94°C, 3min	94°C, 30s	50°C, 30s	68°C, 1min 15s	68°C, 10min
TEF1-α	94°C, 3min	94°C, 30s	55°C, 45s	68°C, 1min 30s	68°C, 10min
TUB2	94°C, 3min	94°C, 30s	50°C, 30s	68°C, 1min	68°C, 10min
RPB2	94°C, 3min	94°C, 40s	55°C, 30s	68°C, 1min	68°C, 10min

References

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Sequences

>accession Nos_fungal isolate code DNA locus:

ITS

>OP893662_CREA-DC TPR OL.60 ITS
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