Protocol for the detection of a G143A mutation in *Alternaria alternata*-isolates

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adopted from Ma Z, Felts D, Michailides TJ, 2003: Resistance to azoxystrobin in *Alternaria* isolates from pistachio in California. Pesticide Biochemistry and Physiology 77, 66-74.

- DNA is isolated from small amounts of mycelium scraped off from fully colonized agar plants of single spore isolates.
- DNeasy Plant Mini Kit (Qiagen) was used, according to the manufacturer's protocol, preceded by a bead-beating step with 10-20 bashing beads (2 mm diam.), for 3 min in a Retsch mill. A little water (ca. 50 µl) will be needed for this in the tube (but not buffer 1 from the Qiagen kit, as this will result in too much foam!)
- (a quick-prep direct boiling extraction protocol is described by Ma et al., but Qiagen – or other kits – extraction will be safer, e.g. a chelex quick-prep method did not work in our lab)
- PCR with primers from Ma et al.: AF (5'-ACACTGCTTCAGCATTTTTCTTCATAG-3') and AR (5'-TTGTCCAATTCATGGTATAGCACTCA-3')
- reaction mix, final concentration: 1x PCR buffer, 1.5mM $MgCl_2$, 0.2µm each primer, 0.2mM dNTPs, 0.04 U/ µl Taq polymerase, 1µl DNA extract
- PCR conditions, according to Ma et al.: initial pre-heat for 3 min at 95 °C, 35 cycles of denaturation at 94 °C for 40 s, annealing at 68 °C for 40 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min
- PCR results checked on gel
- PCR products purified (e.g. Zymo Research DNA Clean & Concentrator™-5 Kit), and sequenced. Often, sequencing with commercial services will also work with unpurified PCR products, but with purification we got 100% successful sequencing reactions.
- sensitive isolates (w/o G143A): .....TGTCATTATGA**GGT**GCAACAGT.....
- resistant isolates (with G143A): .....TGTCATTATGA**GCT**GCAACAGT.....
- as an alternative, a PCR-RFLP protocol is given by Ma et al. for the identification of the mutation, but sequencing is quicker, safer and more straightforward (and probably not even much more expensive): "after PCR amplification, a 25-µl aliquot of PCR products was digested by adding 1.5U of the restriction endonuclease Fnu4HI (New England BioLabs, Beverly, MA) according to the manufacturer's protocol. Digests were analyzed on 3% agarose gels in TAE buffer. (...) The restriction endonuclease Fnu4HI recognized the sequence GCTGC at codon 143 and 144 of the cyt b gene from resistant isolates only. Fnu4HI cut the 226-bp PCR product at the position 177 from resistant isolates but not from sensitive isolates"