

## Publication

### Molecular characterization of cellulose synthase (CesA) genes and impact of mutations on fungicide resistance in oomycetes

#### Thesis (Dissertationen, Habilitationen)

**ID** 1536952

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**Year** 2012

**Title** Molecular characterization of cellulose synthase (CesA) genes and impact of mutations on fungicide resistance in oomycetes

**Pages** 198

**Type of Thesis** Dissertation;

**Start of thesis** 01.08.2008

**End of thesis** 21.03.2012

**Name of University** Universität Basel

**Name of Faculty** Philosophisch-Naturwissenschaftliche Fakultät;

**Supervisor(s) / Fachvertreter/in** Gisi, Ulrich ; Boller, Thomas ;

Many oomycetes are important pathogens of plants (e.g. arable crops, fruit and forest trees), animals or microbes and, upon attacking their hosts, cause considerable economic damage mainly in agriculture and aquaculture. To minimize yield losses efficient disease control, primarily relying on the application of anti-oomycete compounds with a single-site mode of action, is very important. Some anti-oomycete compounds specifically inhibit mitochondrial respiration, RNA synthesis, microtubule organization or cell wall synthesis. The oomycete cell wall is mainly composed of  $\beta$ -1,3-,  $\beta$ -1,6 glucans and cellulose that provide rigidity to the cell. The synthesis of cellulose was recently shown in *Phytophthora infestans* to be the target for mandipropamid (MPD), which belongs to the oomycete specific carboxylic acid amide (CAA) fungicides controlling members of the Peronosporales. However, there are several oomycetes that the CAAs cannot control (e.g. the entire genus *Pythium*), even though cellulose is an important cell wall component of all oomycetes. Furthermore, the mode of action of CAA fungicides is highly specific, thus the resistance risk is assumed to be moderate to high. Consequently, resistant individuals were recovered in *Plasmopara viticola* and *Pseudoperonospora cubensis* populations, but the underlying mechanism of resistance remained unknown. This thesis aims to identify and characterize the cellulose synthase (CesA) genes of various oomycete species and thereby to investigate the mechanism(s) of resistance in sensitive species, i.e. species normally affected by CAAs, and tolerance in insensitive species, i.e. species unaffected by CAA fungicides. A family of four cellulose synthase genes was identified and fully sequenced in the downy mildew pathogens *P. viticola* and *P. cubensis*. Phylogenetic analyses of the four genes revealed their close relatedness to cellulose synthase genes of *Phytophthora* spp. and the red algae *Porphyra yezoensis*. Sequencing of the CesA genes in CAA-resistant and -sensitive field isolates of *P. viticola* and *P. cubensis* uncovered single nucleotide polymorphisms (SNPs) affecting the amino acid structure of CesA proteins. Inheritance of resistance in *P. viticola* was confirmed to be correlated with one recessive SNP located in the CesA3 gene. This SNP led to an exchange from a highly conserved glycine (encoded by GGC) to serine (AGC) at position 1105 (G1105S), whereas in *P. cubensis*, mutations causing amino acid substitutions from glycine (GGG) to valine (GTG) or tryptophane (TGG) (G1105V, G1105W) occurred in the same codon. The results demonstrate for the first time that SNPs in codon 1105, when present in both alleles, lead to amino acid exchanges in the CesA3 enzyme causing inheritable and stable resistance to all CAA fungicides.

**Digital Object Identifier DOI** 10.5451/unibas-005942804

**URL** [http://edoc.unibas.ch/diss/DissB\\_9891](http://edoc.unibas.ch/diss/DissB_9891)

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