MOLECULAR IDENTIFICATION AND PHYLOGENETIC RELATIONSHIPS OF *COLLETOTRICHUM* ISOLATES PATHOGENIC ON CULTIVATED PLANTS IN BULGARIA

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Introduction

Fungi belonging to the genus *Colletotrichum* are causal agents of plant diseases with constantly growing economic importance. Accurate species identification and characterization of *Colletotrichum* pathogens is compulsory for effective disease control and requires the utilization of a combined marker approach using DNA and morphological markers coupled with ecological and geographical data. No universal DNA barcode or marker combination is available for species discrimination in all groups of the genus *Colletotrichum* as some barcodes may work well for particular species complexes. This study applies classical phenotypic characteristics and contemporary DNA barcoding approach for detailed species characterization and phylogenetic analysis of selected pathogenic *Colletotrichum* isolates.

Aim of the study

Phenotypic and molecular identification of *Colletotrichum* isolates from cultivated plants in Bulgaria

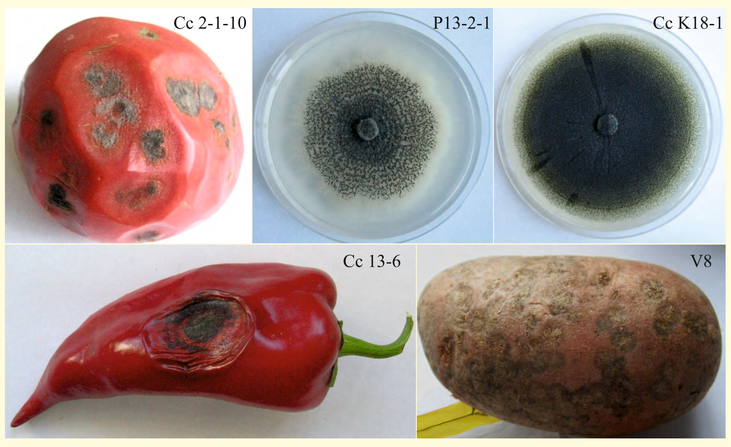
Materials and Methods

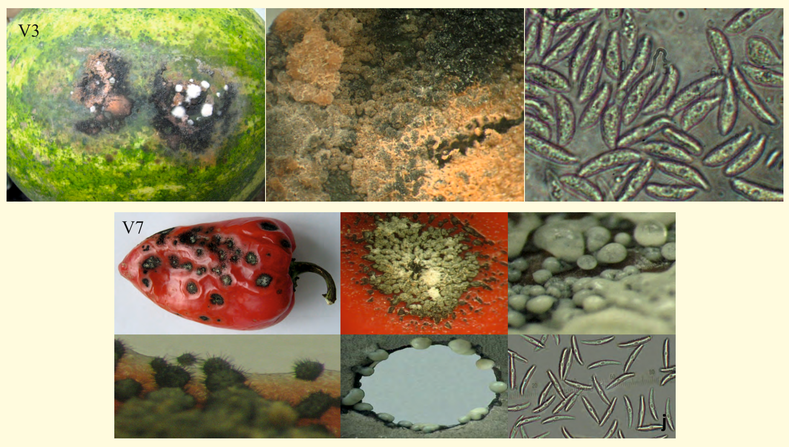
- Thirty five *Colletotrichum* isolates obtained from plant hosts belonging to five botanical families
- Description of disease symptoms and pathogen isolation from infected plant material
- Phenotypic characterization of *Colletotrichum* isolates based on their macroscopic and microscopic characteristics
- Production of fungal biomass on PDA for 10 days at 22ºC
- DNA extraction from the fungal mycelium of selected isolates
- PCR amplification with specific primers targeting four gene regions: *Internal transcribed spacers* (*ITS*), *Actin* (*ACT*), *Elongation factor - 1a* (*EF-1*α), *beta tubulin (TUB2)*
- Agarose gel electrophoresis and imaging, Sanger sequencing, Sequence editing and alignment
- Phylogenetic analysis with MEGA 11 software

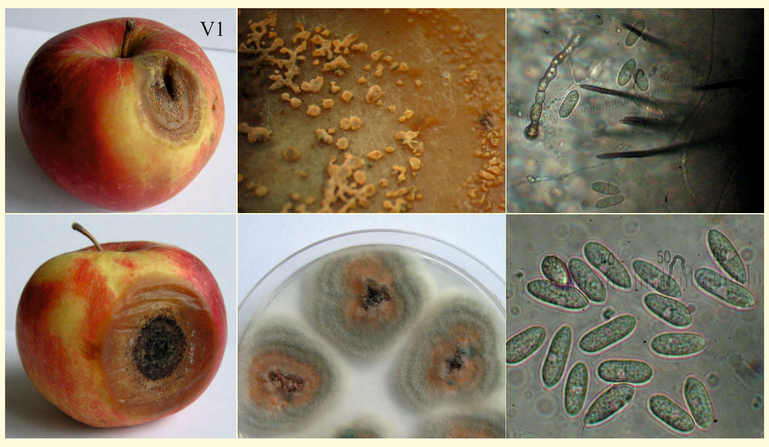
Host/substrate	Isolate designation
Solanaceae	
Capsicum annuum, fruit	Ca13-1; CgB1-1; CgB27-1; B8-1; Cc7-1; Cc7-2; V2; V7; CcV10; Cc26-1; Cc26-2; Cc13-6
Capsicum annuum, roots	B8-2; CcB12-9; CcB12-13; CcK18-1
Solanum lycopersicum, fruit	CaD13-3; CgD6-1; CgD6-2; Ca44-1; Cc2-1-10; CBS 490.92
Solanum lycopersicum, roots	CBS 527.77; V9
Solanum melongenae, fruit	Cc40-1
Solanum tuberosum, tuber	P5-4; V8
Solanum tuberosum, root	P13-1
Solanum tuberosum, stolon	P13-2-1
S. tuberosum, stem basis	P13-2-3
Rosaceae	
Malus domestica, fruit	V1
Fragaria X ananassa, fruit	V6
Cucurbitaceae	
Citrulus lanatus, fruit	V3
Caryophyllaceae	
Dianthus caryophyllus, leaf	V4
Musaceae	
Musa sp., fruit	Cm13

Results and Discussion

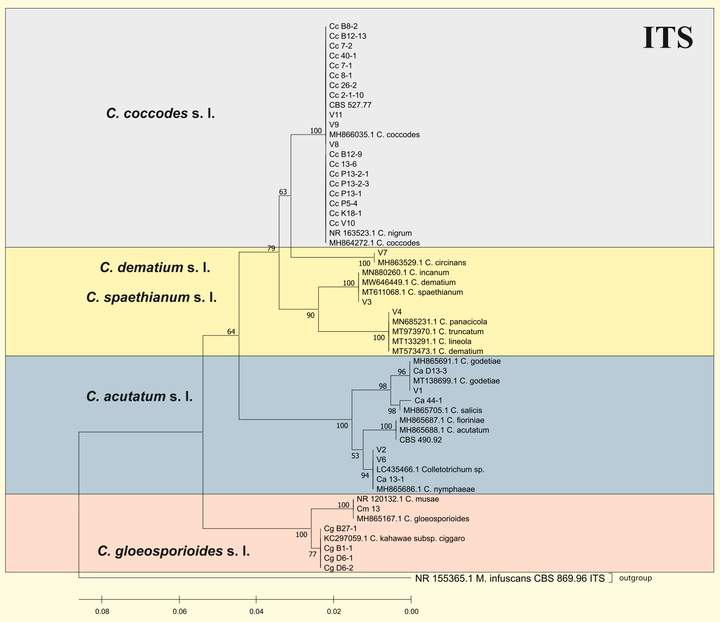
Phenotypic characterization of *Colletotrichum* isolates

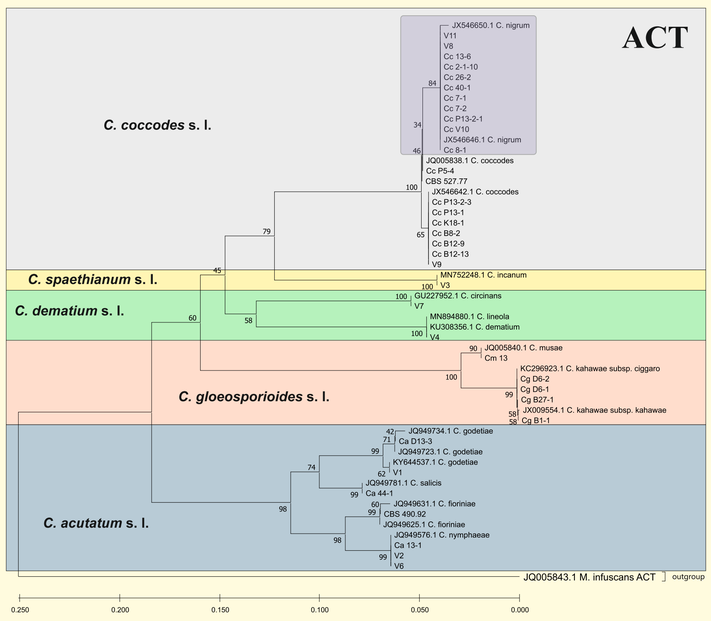


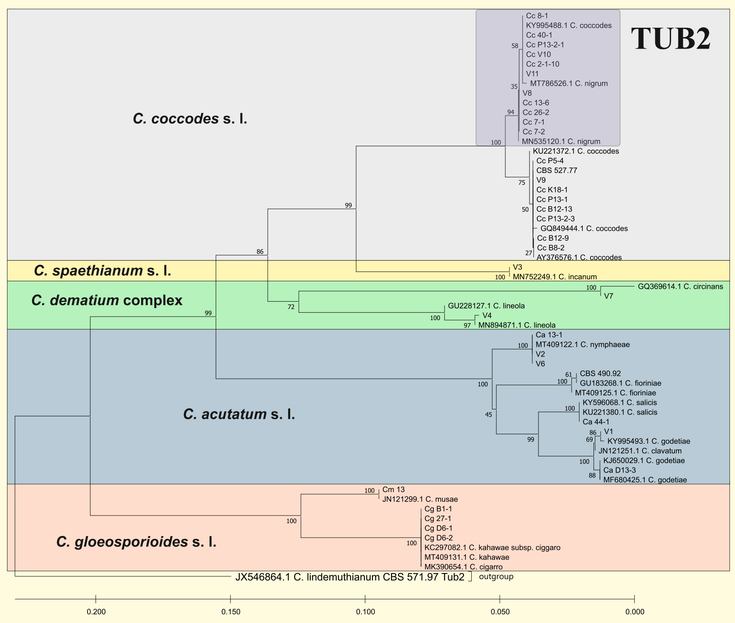




Phylogenetic trees based on DNA barcodes



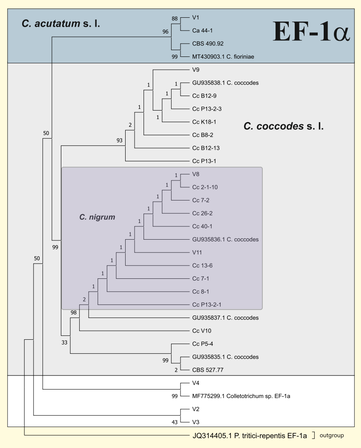




- The resolution power of ITS barcode is not sufficient to discriminate inter-species variations within *C. coccodes*, *C. dematium* and *C. spaethianum* complexes confirming the requirement for secondary gene regions in order to resolve the genetic variability of *Colletotrichum* isolates.

- BLAST analyses identify eleven *Colletotrichum* species assigned to five different complexes – *C. coccodes, C. acutatum, C. gloeosporioides, C. dematium* and *C. spaethianum.*

- DNA barcoding data reveal higher species variation among the isolates from pepper (*Capsicum annuum*).



- ITS, ACT and TUB2 barcodes display complete success rate of PCR amplification and sequencing. EF-1α fails to amplify in the *C. gloeosporioides* group and shows significant variation in the length of the amplified fragment in some of the *C. acutatum* isolates.

- Secondary barcodes possess greater potential for inter and intra-species discrimination within *C. coccodes* group. TUB2 specifically detects the variations between *C. nigrum* isolates.

- Three DNA barcodes identify *C. circinans* as a causal agent of anthracnose disease on *Capsicum annuum*.

Conclusions

The combination of classical and molecular methods allows precise identification of *Colletotrichum* isolates, especially regarding species not previously reported as pathogens in Bulgaria (*C. incanum*, *C. circinans*, *C. godetiae*, *C. cigarro*, *C. sallicis*, etc.) as well as discrimination of fungi belonging to the morphologically similar complexes *C. acutatum* and *C. gloeosporioides*. DNA barcoding analysis is crucial for the correct taxonomic affiliation of the isolate V7 (*C. circinans*), obtained from pepper fruit, initially determined as *C. truncatum* based on its morphological characteristics. According to our knowledge, this is the first report associating *C. circinans* with pepper anthracnose. Data obtained enhance our understanding of the phylogenetic relationships between different species of the genus *Colletotrichum* and highlight the genetic diversity among the *Colletotrichum* population pathogenic on cultivated plants in Bulgaria.

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