

Colletotrichum scovillei: a new record of pepper anthracnose in Europe and evaluation of *Actinomyces* as potential biocontrol microorganisms

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Introduction

Pepper (*Capsicum annuum* L.) is one of the most important vegetables cultivated in Balkans countries, especially in Kosovo with more than 3,108 hectares (MAFRD, 2020). In September 2022, two commercial fields in the municipalities of Peja and Rahovec (Kosovo), showed 40% of pepper fruits with dark lesions, surrounded by brown haloes (Fig. 1). In this study the morphological and molecular identification were performed, and Koch's postulates were fulfilled. Moreover, four *Actinomyces* spp. isolates were tested to evaluate their effectiveness in reducing the mycelial growth.



Materials and Methods



Fig. 1: Typical symptoms of pepper anthracnose in the field

Morphological and molecular identification

After fungus isolation onto PDA, colonies and single spores' morphology was carried out under microscopy. For molecular identification, three housekeeping genes, *ITS*, *GAPDH* and *TUB2* were used.

Pathogenicity test

Healthy pepper fruits, cv. Somborka, were inoculated with a conidial suspension (10⁶ conidia/mL) and visual symptoms were assessed 10 days after inoculation.

Antimicrobial activity in vitro

Four *Streptomyces* spp., DLS1, DLS2, DLS3 and DLS4 isolated from tomato stems (Ferrari, 2017), were used in this study. Their direct antagonistic effect on mycelial growth of *C. scovillei* were test in **dual plate assay**. For the evaluation of VOCs, **double plate assays** were performed. Furthermore, fungal growth was observed on PDA medium supplemented with the **culture filtrate** of the four *Streptomyces* spp. isolates.

Results

Occurrence of *Colletotrichum scovillei* in Europe confirmed

Colony and single spore's observation of the isolates revealed similarity with the characterization of *Colletotrichum scovillei*



Fig. 3: Pepper fruits inoculated with the isolated *C. scovillei*

described by Damm et al. (2012). Sequencing of *ITS*, *GAPDH* and *TUB2* genes confirmed that both isolates were identical to *C. scovillei* reference strain CBS 126529 (Damm et al., 2012) (Fig. 2).

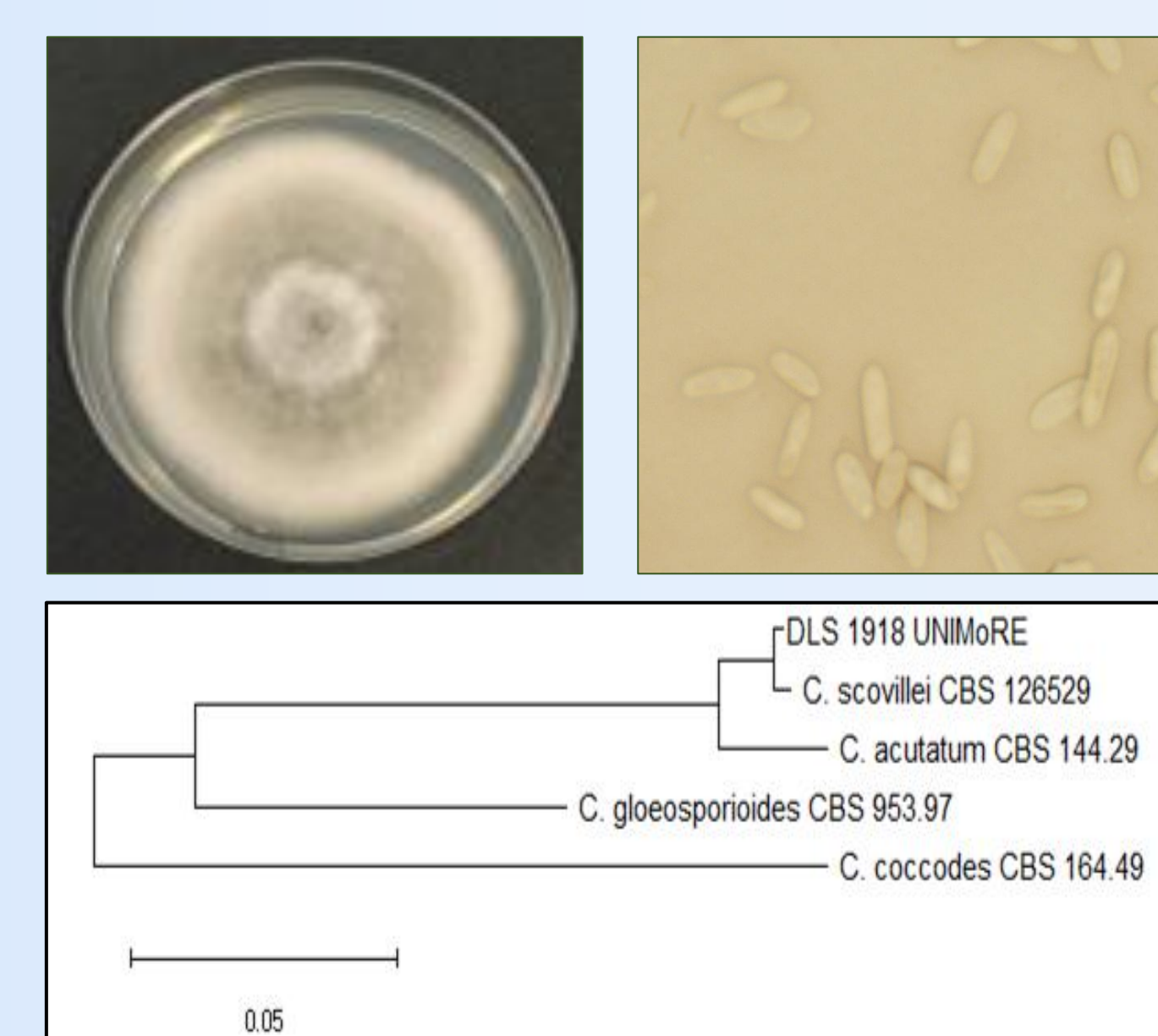


Fig. 2: Morphological and molecular aspect of *C. scovillei*

Pepper fruits inoculated with the 2 isolates showed **typical anthracnose symptoms** (Fig. 3), similar to those observed in the two fields.

Streptomyces spp. strains significantly inhibit *Colletotrichum scovillei* growth in vitro

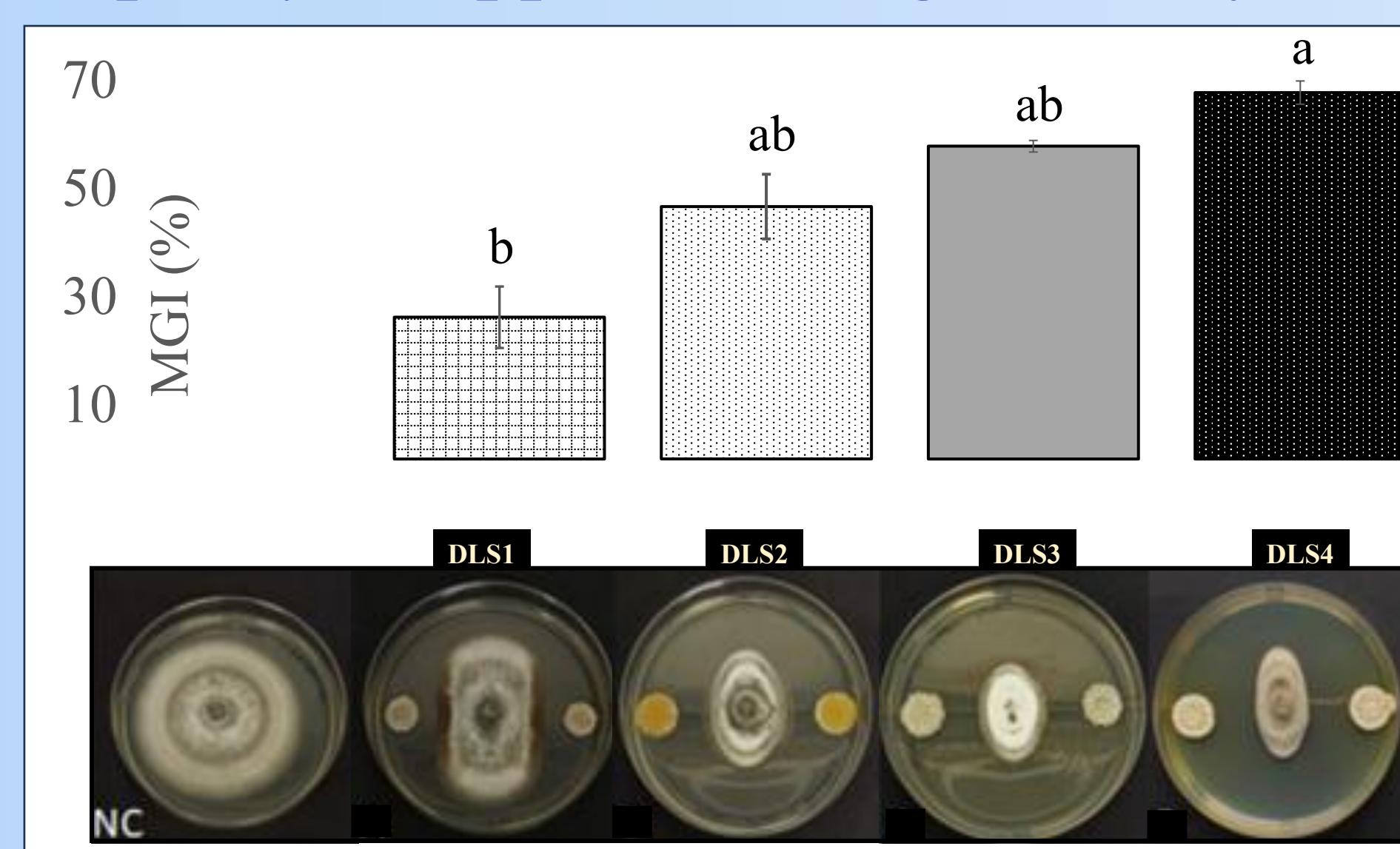


Fig. 4: Dual plate assay against *C. scovillei*

A high antagonistic effect of *Streptomyces* spp. strains on *C. scovillei* mycelial growth was observed with a **reduction** of about **50%**, **60%** and **70%**, for **DLS2**, **DLS3** and **DLS4**, respectively (Fig. 4).

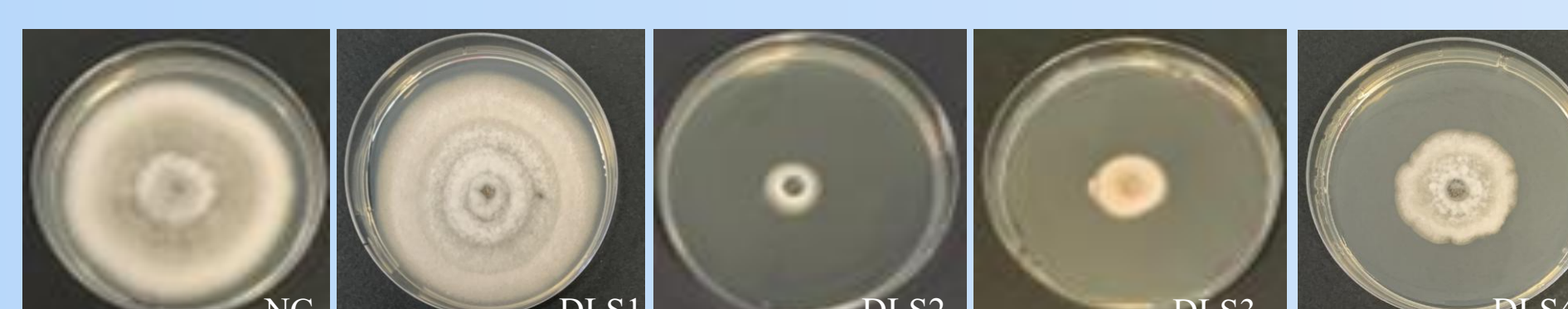


Fig. 6: Culture filtrate effect on *C. scovillei* growth

Indirect effect of VOCs emitted by *Streptomyces* spp. strains **inhibited** *C. scovillei* mycelial growth of about **5%**, **25%**, **26%** and **30%** for **DLS1**, **DLS2**, **DLS3** and **DLS4**, respectively (Fig. 5).

The culture filtrate assay highlighted the **reduction** of *C. scovillei* mycelial growth of about **75%**, **60%** and **50%** for **DLS2**, **DLS3** and **DLS4**, respectively. **DLS1** did not show inhibition (Fig. 6).

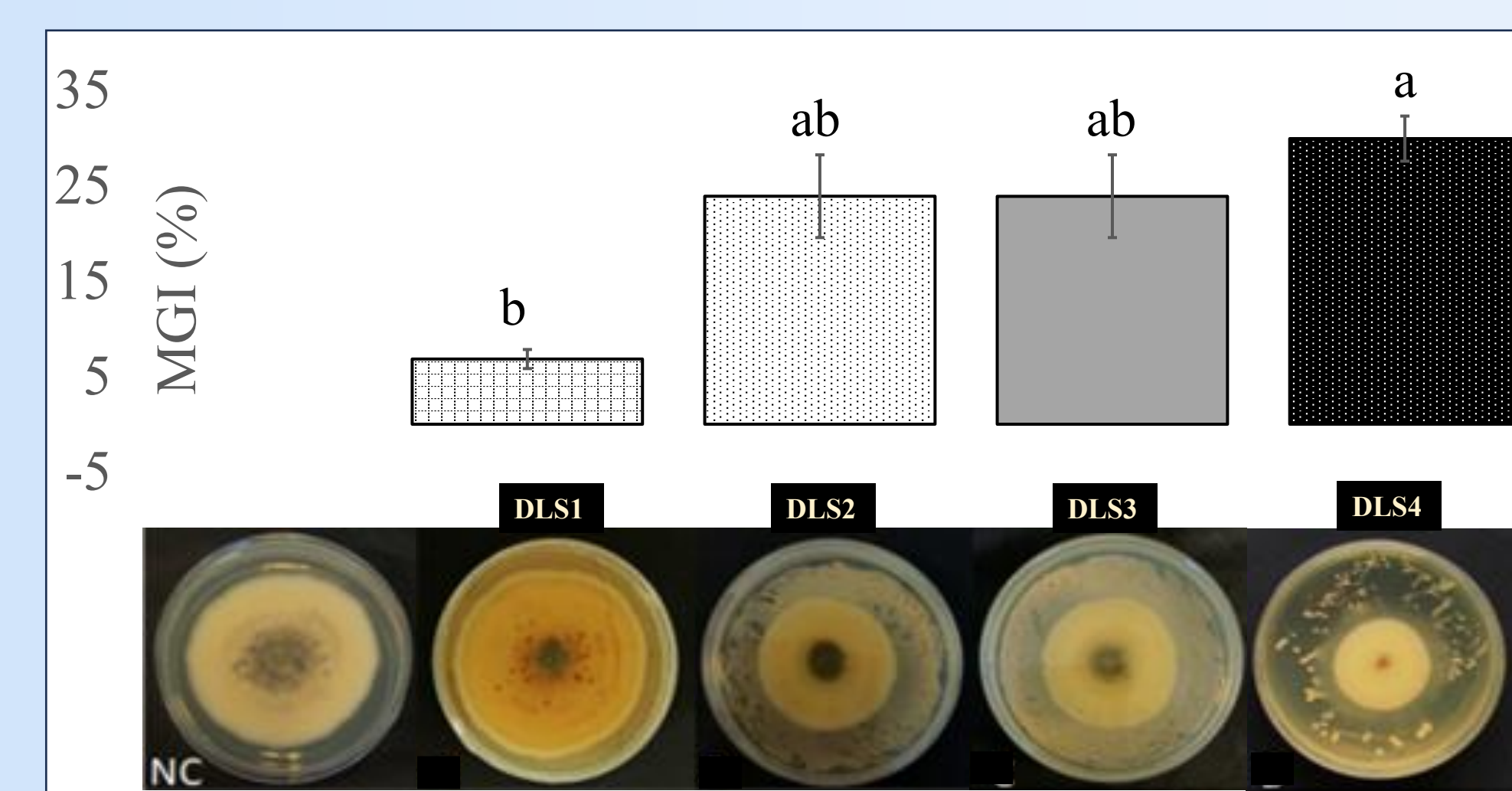


Fig. 5: VOCs effect on *C. scovillei* growth

Conclusion

To the best of our knowledge, this is the first report of anthracnose caused by *C. scovillei* in Europe. Given the importance of the crop and the yield losses observed, measures are urgently needed aiming to pathogen eradication or, alternately, to ensure an efficient disease control to avoid pathogen spread to other geographical areas. Finally, four microorganisms from the UNIMORE bacterial collection showed their effectiveness in reducing mycelial growth. Studies are currently ongoing on detached fruits under controlled conditions.

Reference

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