Screening of soil-borne microorganisms as potential biological control agent against Fusarium wilt of banana in Eastern DR Congo

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Introduction

Fusarium wilt of banana, caused by fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (FOC), is one of the most destructive banana diseases worldwide. The disease was first reported in the Democratic Republic of Congo (RDC) in 2000 (Karangwa *et al*., 2016). A recent survey in the Kivu highlands highlights the high incidence of the disease in the region, ranging from 0 to 63% depending on the cultivars (Figure 1). The absence of wilt on the Cavendish subgroup of bananas indicates that the tropical race 4 (TR4) is not occurring in this region, but the non-adoption of Cavendish cultivars in the region and the use of susceptible cultivars to other races have not favoured the control of the disease. The survival of the causal agent by the production of chlamydospores and the evolution of FOC races overcoming cultivar resistance make the disease difficult to control. In order to develop control strategies adapted to the populations from Kivu region, a molecular characterization of FOC populations occurring is now under progress and the biocontrol potential of indigene bacterial and fungal strains against FOC is investigated.

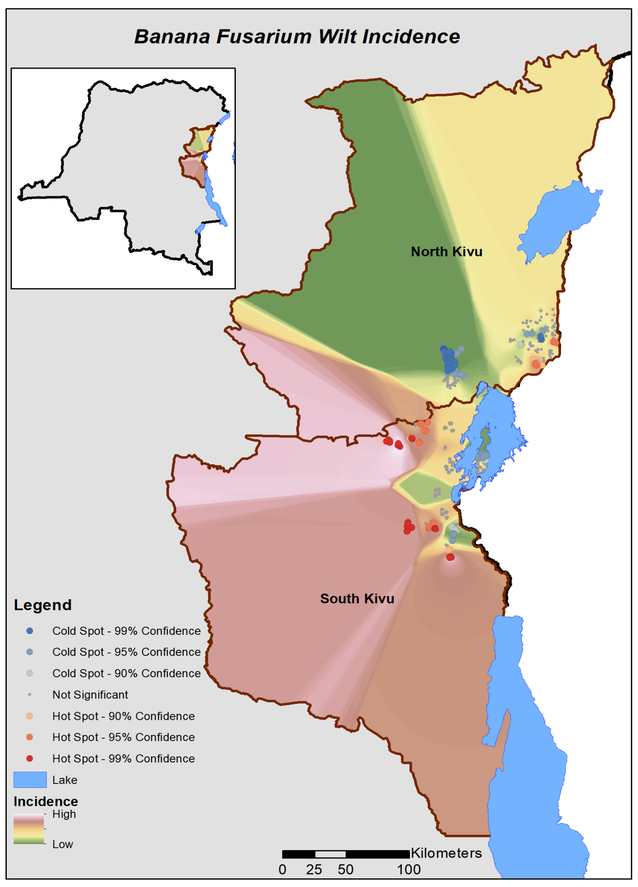

Fig.1 Incidence of Fusarium wilt banana in Kivu region

Methods

Soil samples from the rhizosphere of banana were collected in South and North Kivu highlands regions. They were diluted in water and spread onto PDA plates with antibiotic or LB Agar, CFC Agar and King B Agar plates in order to isolate fungi or bacteria, respectively. The antagonistic activity of isolated fungal and bacterial strains was assessed *in vitro* on PDA plates by direct confrontation against the FOC strain F142 originated from Walungu (Kivu). The top 10 fungal and 10 bacterial BCAs were identified based on sequencing of the ITS region for fungi and 16S rDNA for bacteria. Five fungal and five bacterial strains with the highest potential biocontrol activities against FOC strain F142 were confronted *in vitro* to five strains of FOC in order to evaluate their spectrum of antagonistic activity.

Results and discussion

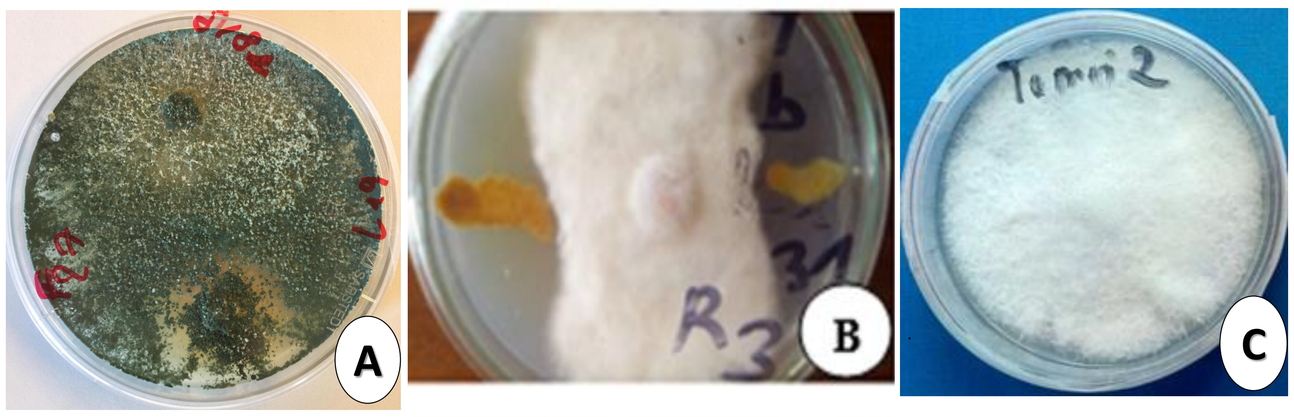

Fig. 2. Assessment of antagonistic activity of potential biocontrol agents (BCAs) against FOC by direct confrontation on PDA. A: BCA = *Trichoderma hazianum* with antagonistic activity; B: *Pseudomonas protegens* with antagonistic activity; C: Control growth of FOC

A total of 273 bacterial and 265 fungal strains were isolated from 80 soil samples. The screening of their antagonistic activity against FOC strain F142 revealed that 37 bacterial strains and 27 fungal strains showed high inhibition against the mycelial growth (>70%) (Fig. 2, 3 and 4). Three of the five selected bacterial strains and all of the five selected fungal strains exhibited high antagonistic activities against the five tested FOC strains (Fig. 5 and 6). The molecular characterization revealed that the efficient bacterial strains belong to three species of the genus *Pseudomonas: P. fluorescens*, *P. japonica*, *P. protegens*. The efficient fungal strains belong to *Trichoderma asperellum, T. atroviride* and *T. harzianum.* Several strains of these species are reported in other countries as efficient biological control agents (Chaves *et al*., 2016; Bubici *et al.*, 2019) .

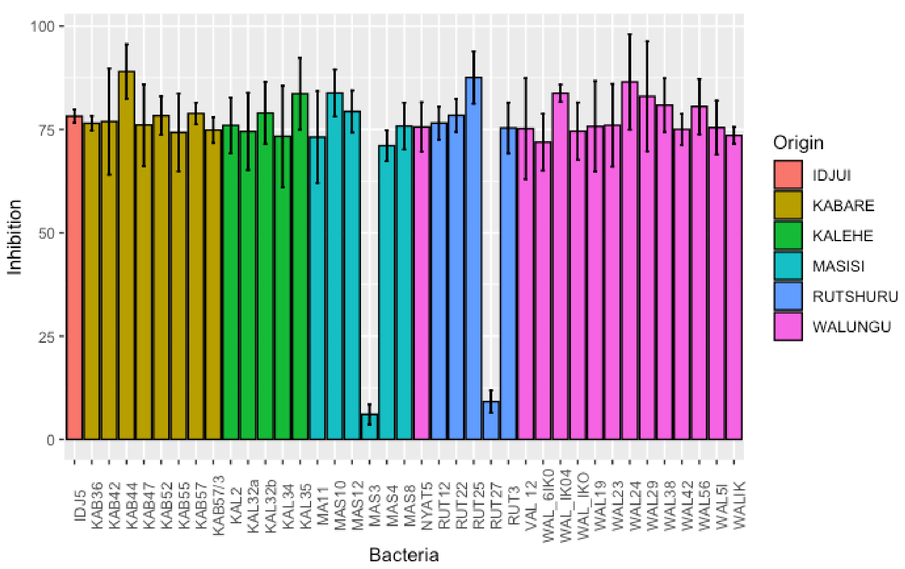

Fig. 3. Inhibition percentage of FOC strain 142 by -39 bacteria strains

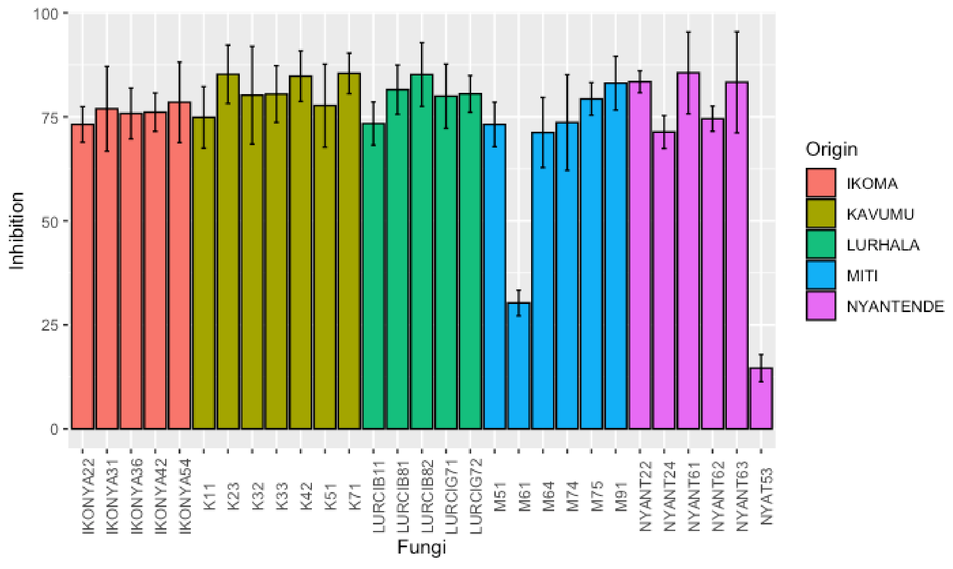

Fig. 4. Inhibition percentage of FOC strain 142 by 29 fungal strains

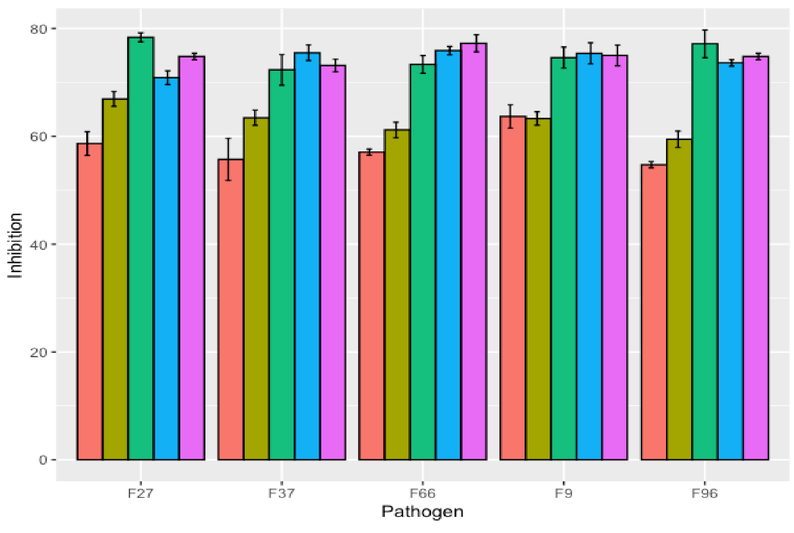

Fig 5. Inhibition percentage of five FOC strains (F27 to F99) by five bacterial strains (*P. fluorescens* (strain KAL35), *P. japonica* (strain KAB52), *P. protegens* (strains WAL29, RUT3 and MAS8)

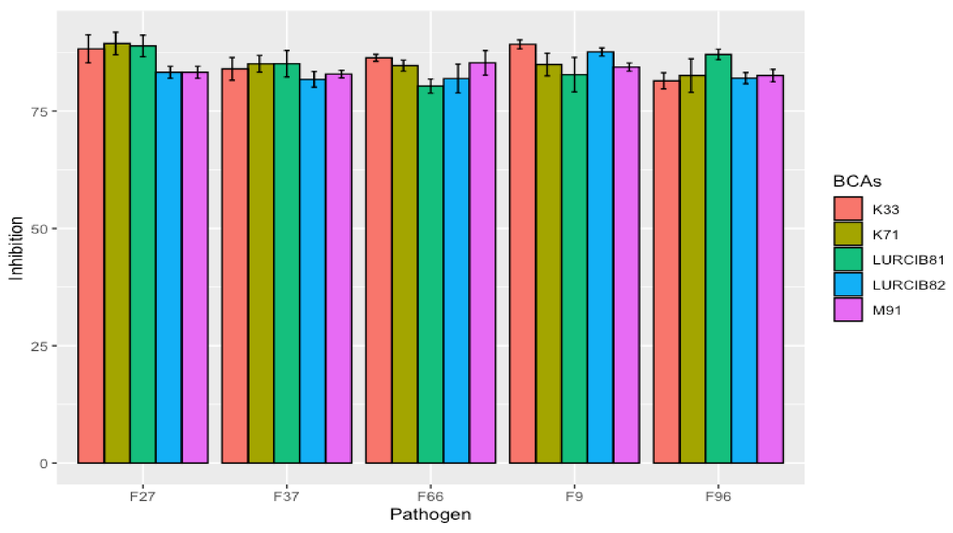

Fig 6. Inhibition percentage of five FOC strains (F27 to F99) by five fungal strains (*Trichoderma asperellum* (strain K33, LURCIB81 and M91), *T. atroviride* (strain K71), and *T. harzianum* (strain LURCIB82)

Conclusion and perspectives

Several strains of *Trichoderma* spp., namely *T. asperellum, T. atroviride, T. harzianum,* and of *Pseudomonas* spp. , *P. japonica, P. fluorescens,* and *P. protegens, originating from RDC* showed antagonistic activities against FOC isolates. Further characterization of their spectrum of activities and modes of action will be studied under controlled conditions and in fields in order to evaluate their potential in management of banana Fusarium wilt.

References
Karangwa P., Blomme G., Beed F., Niyongere C. and Viljoen A., 2016. The distribution and incidence of banana *Fusarium* wilt in subsistence farming systems in east and central Africa. *Crop Protection*, 84 (2016) 132-140.
Chaves, N. P., Staver, C., and Dita, M. A. 2016. Potential of Trichoderma asperellum for biocontrol of Fusarium wilt in banana. *Acta Horticulturae*, *No.1114*, 261–265. https://doi.org/https://www.actahort.org/books/1114/1114_35.htm,
Bubici G., Kaushal M., Prigigallo M.I., Gómez-Lama Cabanás C. and Mercado-Blanco J. 2019. Biological Control Agents Against Fusarium Wilt of Banana. *Front. Microbiol*. 10:616. doi: 10.3389/fmicb.2019.00616

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