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Short communication

Microbial Inoculant Success in Soil: Native Superiority and the Importance of Tracking Inoculative Species

A. Manfredini[1], E. Malusà[2,3] and L. Canfora[1]

Abstract

The use of microbial inoculants in agricultural soils holds great promise for improving crop productivity and sustainability, but their successful application requires reliable monitoring tools to ensure persistence and effectiveness. Recent advances have introduced innovative molecular approaches for tracking inoculated species in complex soil environments. Real-time PCR (qPCR) remains the most widely used technique due to its high sensitivity and specificity, enabling accurate detection and quantification of target microorganism. However, its effectiveness depends on the design of species-specific primers and probes based on genomic markers of the target organism. The selected microbial strains might not necessarily find the conditions necessary to establish and proliferate under all pedoclimatic conditions. The present study presents results from the EXCALIBUR project that highlight the importance of considering the exploitation of the native soil microbial community, which cannot be disregarded.

Keywords: microbial inoculant, detection, Real time, marker, soil

Introduction

Recent European projects such as EXCALIBUR demonstrated the potential of microbial inoculants, but also highlighted strong variability linked to soil–crop context. The 32 field trials of the EXCALIBUR project highlighted both the complexity of soil–plant–microbe interactions and the need for careful biostimulant selection to improve soil quality without negatively affecting yields, the key factor for farmers. Results also showed that crop performance depends strongly on geographic area, soil characteristics (physical, chemical, biological), and nutrient status. These findings clearly indicate that a “one-size-fits-all” solution is ineffective and underscore the need to develop tailor-made microbial products that exploit native microorganisms adapted to specific crops, soils, and production systems.

The EXCALIBUR experience also provides solid evidence that, to fully understand the potential of microbial inoculants, it is crucial to monitor and track the persistence of inoculated species in soil. Indeed, native microorganisms generally have greater opportunities to colonise, persist, and express growth-promoting functions in host roots compared with non-native strains. This aspect can only be properly assessed through dedicated diagnostic analyses, which not only allow a better understanding of the efficacy and efficiency of microbial inoculants but also help guide their application and support formulation optimisation. Monitoring the fate and persistence of microbial inoculants in soil is essential for their registration, safe use, and effective performance. Such monitoring has both ecological and technical implications, as it helps minimize environmental risks and optimize application strategies. Effective tracking also prevents unnecessary applications, improving cost efficiency and enabling evaluation of the inoculants’ effectiveness under specific pedo-climatic conditions.

[1] CREA Centro di Ricerca Agricolutra e Ambiente, IT-00184 Roma; andrea.manfredini@crea.gov.it; loredana.canfora@crea.gov.it
[2] CREA Centro di Ricerca Viticoltura e Enologia, IT-31015 Conegliano
[3] National Institute of Horticultural Research, PL-96-100 Skierniewice

Detection and quantification of microbial inoculants in complex matrices like soil require highly specific and sensitive analytical methods. Among these, real-time PCR (qPCR) is currently the main approach used for specific detection and quantification of introduced microorganisms, due to its high sensitivity and discrimination capacity. However, its effectiveness depends on the design of species-specific primers and probes based on genomic markers of the target organism. Molecular techniques are progressively replacing conventional monitoring methods. The selected microbial strains might not necessarily find the conditions necessary to establish and proliferate under all pedoclimatic conditions. The present study presents results from the EXCALIBUR project that highlight the importance of considering the exploitation of the native soil microbial community, which cannot be disregarded.

Material and Methods

T. asperellum FC80 was isolated from re-used perlite and perlite-peat substrates in soilless tomato cultivation (Clematis *et al.* 2009; Liu *et al.* 2009). Field trials were carried out at a tomato farm in Moretta (CN, Italy; 44.7823 N- 7.5155E) and a strawberry farm in Boves (CN, Italy; 44.3440 N- 7.5732E). In both locations, plants were transplanted into mulched soil and irrigated using a drip irrigation system. The same treatments were carried out in Cesena in a strawberry farm. Standard cultivation practices in the region were followed. Specifically, tomato plants of the “Cuore di bue” type (cv. Meneghino) was planted in April 2021, 2022, and 2023. Samples collected from the field were treated with a nucleic acids preservation solution, following the manufacturer's protocol (LifeGuard®, Qiagen, Italy). Subsequently, all treated samples were stored at -80°C until nucleic acids extraction. For RNA extraction, the ZymoBIOMICS™ DNA/RNA Miniprep Kit (Zymo Research, Irvine, CA, USA) was used, following the manufacturer's protocol. The extracted RNA was reverse-transcribed into cDNA using SuperScript™ IV VILO™ Master Mix (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's instructions. A Qubit® 2.0 Fluorometer with DNA HS Assay Kit (Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA) was employed to quantify the obtained cDNA, following the manufacturer's instructions. The quantified cDNA was diluted to a concentration of 10ng μL^{-1} and stored at -20°C for subsequent downstream analyses.The Real-time PCR experiments were conducted on the QuantStudio™5 Real-Time PCR System (QuantStudio™ 5, Thermo Fisher Scientific, Inc., Waltham, MA, USA) using the QuantStudio™ Design and Analysis Software ver. 1.5.1. The qPCR reactions were carried out using the primers designed in Manfredini et al., (2025).

Results and Discussion

The successful adoption of biocontrol agents by farmers depends on assurance of their reliability and consistent efficacy. The effectiveness of these agents is influenced by environmental conditions, farming practices, application methods, and the biological properties of the microorganisms (Bardin and Pugliese, 2020). While microbial antagonists may show good efficacy in controlled experimental settings, their effectiveness under field conditions can be less pronounced (Nicot *et al.,* 2011). Monitoring the population dynamics of these agents is crucial not only for regulatory purposes but also for understanding the success of their application over time. The efficacy of microbial antagonists in controlling plant pathogens strongly depends on the dosage applied, and continuous monitoring facilitates improvements in their effectiveness under field conditions.

Trichoderma is a genus of fungi commonly found in soil. Various strains of Trichoderma are typically present in soil samples (Li et al. 2015). All soil samples collected in 2021, 2022, and 2023 were used for detection. Positive results for our target were obtained in all the

samples inoculated with *T. asperellum* FC80, and the quantitative PCR analysis of the active population using RNA samples synthesized into cDNA revealed high values, with a few exceptions. Remarkably, by the end of 2022, the active population of *T. asperellum* FC80, measured in terms of b-tub2 copies per gram of dry soil, significantly increased in the tomato field soil (Figure 1 A). Conversely, a different trend was observed in strawberry experiments (Figure 1 B), highlighting the accuracy of this TaqMan assay.

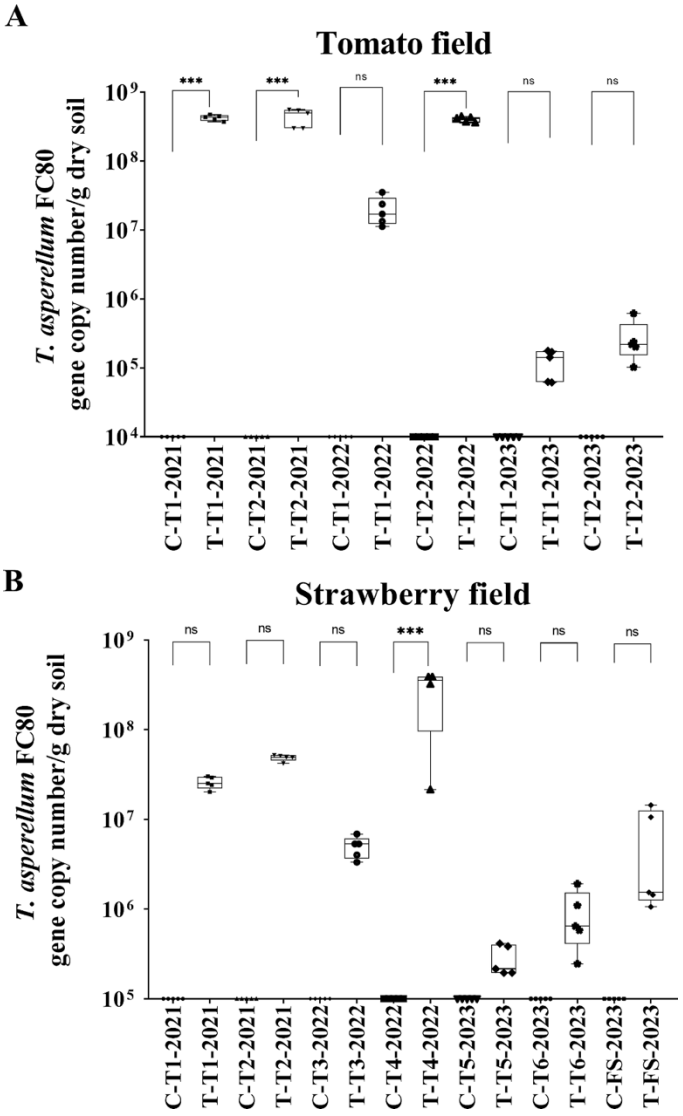

Figure 1: Trend of the gene copy number of T. asperellum FC80 in the two soils under analysis.

While a decrease of *T. asperellum* FC80 was expected in both tomato and strawberry trials, the experimental tomato fields showed an increase in the quantity of active *T. asperellum* FC80 in the soil three months after the treatment with the biological control agent (BCA). In contrast, the persistence and the activity of *T. asperellum* FC80 in strawberry trials remained stable after the first treatment in 2021, except for the T1_2022 time point, corresponding to the flowering stage (BBCH 60). Despite a single application to strawberries in 2021, *T. asperellum* FC80 was consistently detected in 2022, with a high number of gene transcript copies. These results demonstrate the persistence of the biological control agent in the soil of the inoculated plants. Its activity is likely triggered by the presence of pathogens and influenced by environmental conditions. A pronounced decline in gene copies of approximately three orders of magnitude was observed in the strawberry trial. A growth trend was observed at T5, where the fungus was reinoculated, and at the final sampling, the fungus's gene copy number was increased. The soil samples collected in Cesena showed no persistence of the inoculant (data not shown). Several tests have been carried out to exclude any bias or problems during the co-extraction of RNA and DNA. Considering that we did not observe the presence of the inoculants after two weeks from the treatments, we argued that the establishment of the inoculants was probably unsuccessful once applied in these soils.

The findings presented in this paper display distinct behaviours of *T. asperellum* FC80 on the two crops studied, highlighting the importance of determining the colonization of *T. asperellum* FC80 inoculant in soil. Specifically, in tomatoes, an annual crop, the population of *T. asperellum* FC80 increased by 3 months after the initial application, surpassing the starting point. In contrast, on strawberries, which involved a 2-year cropping period, the population of *T. asperellum* FC80 remained stable for approximately 450 days. Similarly, in a field experiment with *the T. atroviride* strain SCI, Stummer et al. (2020) reported good persistence of *Trichoderma* up to 18 weeks after inoculation. This prolonged persistence of

the biocontrol agent is particularly significant, as it aligns with good agricultural practices, which typically recommend re-inoculation after 1 year. However, in this case, the extended effectiveness of *T. asperellum* FC80 obviates the need for a new inoculation within the given timeframe. These observations underscore the practical implications of monitoring the population dynamics of biocontrol agents, as they can inform farmers' decisions and optimise the efficacy of biological control strategies in sustainable agriculture. We are currently using these data to define agroecological factors that affect the establishment and persistence of the Trichoderma inoculant's efficacy in tomato and strawberry cropping systems.

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