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## Qualitative-quantitative protocol for studying the interaction between fungi and between fungi and bacteria to produce bio-fertilising co-inoculants

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The production, formulation and use on agricultural soils of biofertiliser or biostimulant mixtures containing several species of microorganisms is becoming increasingly common. The study of the interactions between the biological components of such products is less frequent. Often the marketed mixtures combine a high microbial load, in the form of both spores and propagules of fungi and bacteria whose positive or negative synergies are tested only to a limited extent, mainly because of the costs involved in field trials. The *in vitro* study of interactions between fungi or between fungi and bacteria is very important for understanding what can be expected *in vivo*, i.e. once microorganisms are released in large quantities into soils that already have local microbial communities. The use of innovative techniques such as digital qPCR and phenotype microarrays now makes it possible to rapidly test thousands of interactions between two or more microorganisms. In this work, a method is proposed to evaluate the positive or negative effects of co-inoculums of different microorganisms on some main biological functions, and in particular the use of defined nutritive sources. The proposed protocol combines the application of commercial Biolog<sup>R</sup> microplates with the quantification of the biomass of the individual co-inoculated strains by means of molecular tracing techniques and digital qPCR analysis. *In vitro* analysis of the effects of interactions is crucial because competition or commensalism can give rise to new compounds (i.e. enzymes, antibiotics, allopathic molecules) or behaviours (production of resistant structures, cellular apoptosis, changes in life cycle as perfect state inhibition or stimulation, etc.), which in turn can have definite effects on both the efficacy of the product and the variability of its properties.