

THE ASSOCIATED UNIT MBG-CSIC / CiQUS-USC BIOCONTROL OF CROPS

Antonio M. DE RON^{1,3*}; Fernando J. LÓPEZ^{1,2,3}

¹ Misión Biológica de Galicia (MBG), Spanish National Research Council (CSIC). Pontevedra, Spain.

² Center for Research in Biological Chemistry and Molecular Materials (CiQUS), University of Santiago de Compostela (USC). Santiago de Compostela, Spain.

³ Unidad Asociada (Associated Unit) Biocontrol de Cultivos. MBG-CSIC / CiQUS-USC. Pontevedra, Santiago de Compostela, Spain.

* amderon@mbg.csic.es

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Abstract

Sustainable agriculture faces a relevant challenge: providing food for growing human demands and, at the same time, minimizing the impacts, fundamentally of agrochemicals, on agrosystems whose biodiversity has often been impoverished by intensive and aggressive agricultural practices. The Associated Unit (AU) Biocontrol of Crops intend to identify the most beneficial metabolites for crops and their function. Simpler analogues that can exert the same behaviour on crops (MBG) will also be designed and synthesized in the laboratory (CiQUS). The achievement of the objectives set out in this AU requires the participation of researchers from distant scientific fields and only close collaboration between them will allow the proposed objectives to be successfully achieved, which can have a significant impact on both scientific areas and on society, through the improvement of crops.

Resumen

La agricultura sostenible se enfrenta a un desafío relevante: proporcionar alimentos para las crecientes demandas humanas y, al mismo tiempo, minimizar los impactos, fundamentalmente de los agroquímicos, sobre los agrosistemas cuya biodiversidad a menudo se ha visto empobrecida por prácticas agrícolas intensivas y agresivas. La Unidad Asociada (UA) Biocontrol de Cultivos pretende identificar los metabolitos más beneficiosos para los cultivos y su función. También se diseñarán y sintetizarán en el laboratorio (CiQUS) análogos más simples que puedan ejercer el mismo comportamiento en los cultivos (MBG). La consecución de los objetivos marcados en esta UA requiere de la participación de investigadores de campos científicos diversos y una estrecha colaboración entre ellos permitirá alcanzar con éxito los objetivos propuestos, que pueden tener un impacto significativo tanto en los ámbitos científicos como en la sociedad, a través de la mejora de los cultivos.

Background

The current structure of agrosystems facilitates the appearance, evolution and dispersal of plant parasites and pathogens and, therefore, crops are continually subject to new threats. Sustainable agriculture faces a relevant challenge: providing food for growing human demands and, at the same time, minimizing the impacts, fundamentally of agrochemicals, on agrosystems whose biodiversity

has often been impoverished by intensive and aggressive agricultural practices. Agricultural ecosystems may be exacerbated by climate change, which increases the frequency of disturbances, changes the suitability of habitats, and changes the way species interact.

In this context, the biological control of biotic factors of plant-soil microbiota systems contributes to the stability and diversity of agricultural communities. The control mechanisms exerted by biocontrol agents are very varied and can include mycoparasitism, antibiosis and competition for nutrients with the pathogenic element. In addition, many biocontrol agents secrete a series of secondary metabolites that play an indispensable role in the protection and growth of the plant. In the plant rhizosphere, where soil microorganisms interact with each other and with plant roots, secreted secondary metabolites can significantly affect the plant. For example, root-colonizing fungi can directly synthesize plant phytohormones such as abscisic acid and gibberellins to affect the balance between plant growth and stress responses. Additionally, microbes that benefit plants can biosynthesize antimicrobials to limit the growth of pathogens and thus contribute to the health of the plant.

The deep understanding of the secondary metabolites of the rhizosphere, including their biological activities, will contribute to the discovery of new biological agrochemical agents and principles, not harmful to the ecosystem. Likewise, access to synthetic analogues of said secondary metabolites of interest could lead to improving their protective and/or plant growth-promoting activities and could even improve the nutritional quality of the crop in question.

The study of the activities of these metabolites on crops and access to them, as well as to more effective analogues, requires the contribution of specialists in different disciplines. Specifically, the participation of agrobiologists specializing in the genetics and improvement of crops and their microbiota is necessary, as well as synthetic chemicals that allow access to the metabolites and analogues of interest. The R&D&i actions of the Associated Unit (AU) Biocontrol of Crops will be framed in the keys of Cutting-edge Science and Multidisciplinary, within the Life Area, with intersection with the Subject Area of the CSIC, aligning with the Sustainable Development Goals (SDG: 2, 3, 9, 12, 13, 15 and 17).

Use of Trichoderma and its secondary metabolites in the protection and growth of crops

Soil fungi such as endogenous mycorrhizae and species of the genus *Trichoderma* contribute to defence against diseases and promote plant growth. *Trichoderma* is a genus of fungi in the Hypocreaceae family, which are found in the soil and can grow on the walls, roots, and stems of different plants. The genus *Trichoderma* is valued for its important beneficial effects on crops important for agricultural production, as well as for its ability to protect crops against diseases and promote plant growth of crops under field and greenhouse conditions (De Ron et al. 2019; Harman et al. 2004).

The most efficient strains of *Trichoderma* spp. exert biocontrol through several complementary strategies, which can include mycoparasitism, antibiosis and competition for nutrients with the potential pathogenic fungus, but they show more than one biocontrol strategy (Howell et al. 2003). In fact, many *Trichoderma* strains produce bioactive metabolites that have growth-promoting and/or antimicrobial activities when applied directly to plants (Vizcaino et al. 2005). Thus, the purified secondary metabolites of *Trichoderma* spp. They can be potentially effective in controlling bacterial infections, exhibiting faster antibiotic activity than that observed with the application of the live microorganism under field conditions (Fravel et al. 1988).

On the other hand, some recent studies demonstrated that the application of *Trichoderma* bioactive metabolites to certain types of growing plants provided effects equivalent to those observed with the direct application of *Trichoderma* strains producing said metabolites, which reduces the drawbacks

of use the microorganism (e.g. limited useful life of the microorganism, specific maintenance conditions and/or variability of its effectiveness depending on the type of soil, geolocation and meteorological conditions (Sivasithamparam et al. 1998; Vinale et al. 2014; Keswani et al. 2014). For example, it was recently shown that the application of different bioactive metabolites to soybeans not only stimulated plant growth, but also increased the nutritional properties of the harvested grain (Marra et al. 2019). Similarly, a positive correlation was also demonstrated between the application of selected bioactive metabolites of *Trichoderma* to the vine and the increase in polyphenol content and antioxidant activity in the corresponding fruits (Pascale et al. 2017), or between the application of these metabolites to strawberry plants and the quality and quantity of the cultivated strawberries (Lombardi et al. 2020).

Additionally, *Trichoderma* is also considered an efficient producer of extracellular enzymes, some of which are involved in the biological control of plant diseases (Monte 2001; Harman et al. 2004), and also produce plant growth regulators and solubilize minerals in the soil, promoting plant growth.

Use of Rhizobium and similar microbiota in crop growth and climate change mitigation

Rhizobia and similar microbiota are bacteria known for their ability to establish symbiotic associations fixing atmospheric N₂ with legumes. As a consequence of the plant-bacteria interaction in the roots, a new organ is formed, the nodule, where the bacteria reduce N₂ to NH₄⁺ through the enzyme nitrogenase. The NH₄⁺ that is produced is available to the plant for its growth and development. Hence, these plants survive in arid, infertile soils, with little or no nitrogen content. When it dies, much of the nitrogen assimilated by the plant returns to the soil where the next crop can take advantage of it. Despite the wide range of rhizobia with which the bean establishes N₂-fixing symbioses, it is considered a poor plant with respect to N₂ fixation capacity compared to other grain legumes, a problem attributed to the inefficiency of the rhizobia that nodulate it. In previous experiments at the MBG, the presence of bacterial nodules was observed in roots of plants not inoculated with rhizobia, and the Nitrogen Metabolism Group of the Zaidín Experimental Station (EEZ-CSIC) of Granada verified that the bacteria isolated from the nodules was *Burkholderia alba*, a species that was isolated for the first time not from nodules of a legume but from soils in South Korea (Lee et al. 2018). The MBG and EEZ Research Groups have been the first to observe the ability of *B. alba* to establish N-fixing symbiosis with the common bean. The symbiotic effectiveness of atmospheric N fixation of this species is unknown, opening new opportunities for crop improvement.

Objectives of the AU

The AU is framed in the aforementioned contexts and is aimed at studying the beneficial and sustainable effects of biocontrol agents and their secondary metabolites in different types of crops. Specifically, and based on the experience of the BAS group (MBG), efforts will initially focus on the use of nitrogen-fixing bacteria such as rhizobia (and similar) and the fungus *Trichoderma* as biocontrol agents in bean cultivation (*Phaseolus vulgaris* L.) as a model species. In addition, the possibility of improving their benefits through the additional incorporation or substitution of secondary metabolites of these biocontrol agents will be analysed.

The final objective is to increase the protection of the cultivation of this and other species against diseases, promote their growth and improve the properties of their fruits and seeds, as well as mitigate the effects of the impact of agricultural production on the environment, through limitation of the use of agrochemicals.

On the other hand, the AU also aims to identify effectors of pathogens and understand their activity in blocking the plant defence signalling pathway. This knowledge will allow the development and synthesis of selective chemical treatments for the treatment of diseases of interest by blocking the

activity of the effector. This strategy would allow to avoid the environmental effects associated with most of the agrochemical treatments available on the market.

Expected outcomes of the AU

In short, despite this background, the study of the influence of the secondary metabolites of these biocontrol agents on plant cultivation is still little explored, both from the point of view of the different types of metabolites and the type of plant that is grown in our ecosystems. Consequently, the treatment of plants, such as the common bean, with the secondary metabolites secreted by *Trichoderma* and/or rhizobia and *B. alba*, will allow the evaluation of changes at the molecular, metabolic and macroscopic level, in the plants, their fruits and seeds, opening new opportunities for crop improvement.

This Associated Unit intend to address that objective by identifying the most beneficial metabolites and their function. Simpler analogues that can exert the same behaviour on crops (MBG) will also be designed and synthesized in the laboratory (CiQUS). The achievement of the objectives set out in this proposal requires the participation of researchers from distant scientific fields and only close collaboration between them will allow the proposed objectives to be successfully achieved, which can have a significant impact on both scientific areas and on society, through the improvement of the studied crops.

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