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Follow-up and monitoring microbial inoculants applied in combination with *Lumbricus terrestris* as a tool for the bioremediation of plastics

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Introduction

Annually, the agri-food industry generates millions of tons of plastic waste, especially associated with primary agriculture production. In this sector, synthetic plastics derived from petroleum are mainly used due to the durability of this material. However, this characteristic is its greatest environmental disadvantage, since it is associated with a low biodegradability. The limited availability and effectiveness of technologies for management and treatment of plastics waste has led to their accumulation in the natural environment, causing serious imbalances in ecosystems. In this work, sustainable alternatives for the biodecontamination of plastic polluted soil through the use of earthworm *Lumbricus terrestris* in combination with microorganisms were analyzed. The aim was to develop an effective method for the detection of two microbial consortia used, including microorganisms destined to fortify the *L. terrestris* gut microbiota and for direct application to the soil. Both inocula together with the earthworms, make up a specialized biological cocktail, useful in bioremediation tasks in environments contaminated with plastics.

Experimental

Two types of plastic-degrading microbial consortia (PMC) were used to be incorporated together with *L. terrestris* as a biological cocktail for the bioremediation of soils contaminated with plastics. ENDO-PMC, consisted of microorganisms isolated and identified from the intestine of earthworms exposed to agri-food waste plastic (AWP): *Pseudomonas putida* PL7 and *Pseudomonas alkylphenolica* PL6. EXO-PMC, consisted of microorganisms isolated and identified from different environments in based on their abilities to degrade plastics: *Bacillus subtilis* RBM2 and *Pseudomonas putida* REBP7. ENDO-PMC was inoculated through *Morus alba* leaves to act as probiotics to strengthen the gut microbiota of *L. terrestris*. EXO-PMC was inoculated through a carrier consisting of a mix of vermicompost, biochar, and urea. The

earthworms fed with ENDO-PMC and the pre-inoculated carrier with EXO-PMC were incorporated into a contaminated soil with a mixture of plastics (LLDPE, LDPE and recycled mulching). After 60 days, the soil microbiota and the *L. terrestris* gut microbiota of each sample were analyzed with culture and molecular methods to trace and detect the microorganisms used. Selective and differential culture media were used for the detection and quantification of each bacterial species studied. Chromo Select Agar+Polymyxin B (CSA+P) for *B. subtilis*, and Pseudomonas Agar Base (PSA) + Cetrimide/Fucidin/cephalothin (selective CFC supplement) to differentiate *P. putida* and *P. alkylphenolica.* Quantitative PCR (qPCR) was used for monitoring by culture-independent methods using Girase B and 16S rRNA as target gene for *Bacillus* (Xie et al., 2020) and *Pseudomonas* (Fontanazza et al., 2021), respectively.

Results and Discussion

In this work, among the available tools, a combination of classical microbiological analysis (culture techniques) and molecular (q-PCR) methods was selected for the development of a protocol that guarantees the necessary level of discrimination required to track and monitor microbial inoculants in complex samples. Plate counts and q-PCR quantification presented complementary results. According to the results, both techniques are necessary to obtain an accurate information on the presence and persistence of the inoculated bacteria over time.

Conclusions

The use of a combined approach for the tracking of bioinoculants allowed obtaining more complete and accurate information on the persistence of the consortia in the different treatments studied in comparison to the use of a single method, cultivar or molecular, as a sole approach.

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Biography

Macarena M. Jurado has a PhD in Agronomy Engineering since 2015 and is a member of the BIO-175 Research Group of the University of Almeria (UAL) since 2010. She has participated in numerous research projects, national and European. Her work has focused on the use of microorganisms as biotechnological tools of agronomic and environmental interest. For the control of plant diseases, the improvement of plant growth, and the bioremediation of contaminated environments and biodegradation and transformation of waste, respectively. Currently, with the contractual figure of Assistant Professor, teaches classes in different subjects of the Unit of Microbiology at the UAL. She has participated as author and co-author in more than 30 scientific articles, published in international impact journals, as well as in more than 50 communications to national and international congresses. She also has participated in several scientific divulgation activities such as Pint of Science or Researcher´s Night.