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In vitro demonstration of the role of earthworms on the growth of PGPR and PGPF microorganisms. Phylogenetic determination of bacteria isolated from the coelomic fluid of the earthworm *Aporrectodea molleri* and characterization of their promoter properties on soil fertility and on the growth and the metabolism of maize (*Zea mays*) under abiotic stress conditions.

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Abstract

Since earthworms represent the first animal biomass in the rhizosphere, we were interested in examining the chemical and biological impacts of these annelids and the microorganisms associated with their coelomic fluid (CF) on the soil and its biological components.

The results shown that the efficacy of crude extracts and cutaneous excreta of earthworms on microbial growth is supported not only by the high growth rates, but also by the concentrations employed, which are many times lower than those of conventional media.

The molecular investigation, using 16S rDNA gene sequencing technique, revealed that 16 bacteria isolated from the CF of *Aporrectodea molleri* were phylogenetically distinct. 87 percent of these bacteria show favorable PGP (Plant growth-promoting) characteristics, including the production of siderophores and indole-acetic acid (IAA), the solubilization of phosphate (P) and potassium (K), nitrogen fixation (N), and the prevention of *Fusarium oxysporum* development.

The greenhouse culture of maize plants treated with PGPR isolates and grown in alkaline soil deficient in N, P, and K demonstrated that CF bacteria (CFB) significantly improve soil quality by increasing microbial biomass, the availability of essential elements (N, P, and K), and the concentration of enzymes (phosphatase and urease). We were able to observe a substantial increase in the growth of maize, as evaluated by plant biomass and chlorophyll content. Lipidomic study by GC-MS (gas chromatography-mass spectrometry) of CFB-treated plants revealed the resilience of abiotic stress defense systems.

Keywords: Earthworm, coelomic fluid, PGPR, fertility, plants, abiotic stress, lipidomic.

Introduction

It is now widely acknowledged that sustainable farming methods are crucial to addressing future global agricultural needs (Prasad et al., 2019). Consequently, it is of the utmost significance to investigate the function of soil and natural components to boost agricultural productivity without causing harm. The terrestrial environment relies heavily on the activities of creatures to function. Indeed, soil organisms play an important role in the breakdown and recycling of materials (Hayat et al., 2010; Raaijmakers et al., 2009).

Soil is considered as a living system due to the enormous number of microorganisms and animal interactions that govern plant nutrition availability. According to Prasad, agricultural productivity cannot be sustainable without a microbial community in the soil. The most promising of these possible soil microorganisms are bacteria known as PGPR (Zainab et al. 2021). Numerous analyses have proved that PGPR is an excellent alternative to hazardous chemical fertilizers for ecologically friendly and sustainable agriculture. Rhizosphere soils supplemented with diverse groupings of PGPR bacteria enhance plant development either directly or indirectly (Zainab et al., 2021). PGPRs promote plant development by mobilizing soil nutrients, generating many plant growth regulators, protecting plants from plant pathogens, and decomposing xenobiotics (Wang et al., 2021). In addition, several studies have demonstrated that a variety of PGPR species, such as *Bacillus, Pseudomonas, Rhizobium, Azotobacter, Enterobacter, and Azospirillum,* perform admirably against biotic and abiotic influences (Aliyat et al. ., 2020; Jamal et al., 2018; Munir et al., 2019). In extreme circumstances, a crop's production might be lowered by 50 to 82 percent due to the influence of abiotic factors (Anami et al., 2020; Prasad et al., 2017). Recent studies have demonstrated that agricultural plants grown in alkaline soil are shorter and produce leaves at a slower rate (Gentili et al., 2018). Alternatively, a deficiency in one of the necessary nutrients (NPK) might reduce plant development and yield (Carvalhais et al., 2011).

As bacteria have limited mobility, they rely on vectors for migration across the various rhizosphere levels (Edwards, 2004). Indeed, earthworms, often known as "soil engineers," are burrowing creatures that influence the dynamics of bacterial populations and assure their dispersion in the soil via intestinal transit (Aira et al., 2018; Picón et al., 2015). They also increase the quantity of bacteria in the surrounding soil by a factor of five (McLean et al., 2006). On the other hand, they play a significant role in temperate terrestrial ecosystems; they nourish soils by changing their physicochemical and biological characteristics (Edwards, 2004). These annelids enhance plant biomass (aerial and root biomass) in 75% of instances, with an average increase of 57% for aerial parts and 36% for seed output (Brown et al., 2000). However, the connection between earthworms and microorganisms is still difficult to comprehend and complicated.

The soil's vast population of earthworms offers an excellent setting for the enhanced activity or growth of particular microorganisms. Therefore, the most studied earthworm-microorganism associations were limited to microbial communities ingested with soil and passing through the earthworm's intestine, with the intestine being the only internal organ in permanent contact with soil microorganisms (Kim et al., 2011; Kim et al., 2004; Picón et al., 2015).

The coelom, which develops in the shape of a huge cavity in various species of invertebrates, including earthworms, would be a perfect site for colonization by microorganisms if it were not protected by an efficient immune system. Typically, bacterial clearance following experimental injection of bacteria into coelomic fluid (CF) is complete within 48 hours (Bilej et al., 2018). This liquid might therefore be deemed aseptic. However, the CF of earthworms is in continual contact with the external environment via their excretory systems, nephridia, and pores (Bilej et al., 2018).

Analyses have demonstrated that CF implies the presence of antifungal and plant hormonal substances (Nadana, Selvaraj, et al., 2020). Thus, CF can trigger genes involved in defense against fungi. (Chaoui et al., 2003; Plavin et al., 2017) There is evidence that CF can inhibit the growth of *Fusarium oxysporum*, which is harmful for the most important crops, including wheat, maize, and rice. In addition, CF has recently demonstrated its ability to enhance seed germination and plant development characteristics (Nadana et al., 2020a, 2020b). However, the method by which coelomic fluid affects plant development remains unknown.

According to the scientific literature, these saprophagous organisms make up an immense mass of between 0.5 and 5 tonnes/hectare (fresh weight), which accounts for 60 to 80 percent of the soil's animal biomass. In this regard, they are the first animal population of the newly-emerged areas. This mobile mass stirs organic and mineral substances into the soil (Edwards & Fletcher, 1988; Hameed et al., 1994; Lavelle, 1988). Annually, earthworms excavate thousands of kilometers of tunnels every acre. These galleries are actual aeration and drainage valves for the soil. Earthworms consume many tons per hectare each year of organic detritus mixed with soil microorganisms when excavating galleries (Zhao et al. 2010). The excavation of the network of galleries in the soil is impossible without the creation of lubricating and viscous cutaneous excretions, which protect earthworms from dryness and facilitate their mobility by crawling and sliding (Zhao et al., 2010). It has never been observed that earthworm biomass and excreta stimulate the development of beneficial soil microbes after death.

Our laboratory was interested in the *in vitro* research of the effect of earthworm’s crude extracts and cutaneous excreta on microbial growth, specifically on soil PGPR and PGPF (plant growth promoting fungi). The second purpose was to identify the microorganisms associated with the earthworm *Aporrecrodea molleri* at the coelomic fluid level. In the third section, we sought to determine the effect of bacteria isolated from earthworm’s CF on various PGP traits by conducting qualitative and quantitative tests revealing the potency of siderophore and AIA production, phosphate and potassium solubilization, nitrogen fixation, and inhibition of pathogenic fungi (*Fusarium oxysporum*). Under abiotic stress conditions, we sought to assess the impact of bacteria isolated from CF with high PGP potentials (CFB) on the development and metabolism of maize (*Zea mays L*.) and the biochemical parameters of the soil (alkalinity and NPK deficiency).

GENERAL RESULTS AND DISCUSSION

The fertility of a soil is determined by the influence of several biological activities, particularly those of the mesofauna and the microflora. We were interested in researching the chemical and biological consequences of earthworms and the microorganisms associated with their coelomic fluid, as they are the first animal mass in the rhizosphere.

1. Effects of Earthworm's Crude Extracts and Cutaneous Excreta on Microbial Development

We were interested in the *in vitro* research of the post-mortem influence (earthworm's crude extract) and cutaneous excreta on the proliferation of soil microorganisms in the first section of this thesis. The development of bacteria and fungi was analyzed after crude extracts and cutaneous excreta were separately prepared at various concentrations. The bacterial strain (*P. fluorescens*) and fungal strains (*Trichoderma sp, Melanocarpus sp, Acaulospora sp*) utilized are known as PGPR (Plant Growth Promoting Rhizobacteria) and PGPF (Plant Growth Promoting Fungi), respectively, because they perform a vital function in enriching the soil and stimulating plant development directly or indirectly (Clark, 1997; Naseby et al., 2000; Siddiqui & Shaukat, 2004; Zin & Badaluddin, 2020; Elekhtyar, 2016; Kong, Deng and al. 2017; Saranraj, 2014; Siddiqui & Shaukat, 2004; Pavlova and al. 2017).

According to the results, the crude extracts of freshly-harvested earthworms (FHE) and those deprived of soil and food for 10 days (FE) as well as cutaneous excreta (EX) promote microbial growth. The effectiveness of crude extracts and e cutaneous excreta on microbial growth is supported not only by strong growth rates, but also by concentrations that are many times lower than those of the conventional medium (BN, PDA, and/or SAB). Media preapared based on FHE, FE, and EX extracts would have a greater variety of nutrients and growth factors. These findings demonstrate that the decomposition of earthworm corpses, on the one hand, and the continued production of cutaneous excreta, on the other hand, enrich the soil with nutrients and growth factors with high added value for the activity and development of PGPR and PGPF groups, which are essential for soil fertility and plant growth.

In addition, it was discovered that the contents of the earthworms' digestive tract had no influence on the enhancement of microbial growth, since the efficacy of the extract from FE (empty digestive tract) earthworms was comparable to that of the extract from FHE earthworms (full digestive tract). The FE extract would be of higher quality than the FHE extract. The influence of the fasting period on the physiology of the earthworms might explain this surprising finding. In fact, throughout the period of fasting, the activity of earthworms deprived of soil and food slows, yet they stay active and continue to eliminate casts. Therefore, the mobilization of the reserves held as big insoluble molecules into more small soluble molecules, recoverable by maceration in water and easily digested by microbes, would explain the increased richness and variety of the crude FE extract. Various laboratories have investigated the chemical composition of earthworms using a variety of methodologies. Depending on the species, the protein content ranges from 32.6% to 67.2% (Damayanti et al., 2008; Julendra., 2003), the fat content from 7% to 10%, the carbohydrate level from 8% to 20%, and the mineral content from 2% to 3% (Ghatnekar et al., 1995).

The fact that earthworm extract-based media accelerate the development of fungal spores is an intriguing finding, as spores are the primary vector for the dispersal of fungi, enabling their dispersion in the environment and contributing to the colonization of new ecological niches (Dahlberg, 1982). The evolution of the quantity of spores released in earthworm extract-based culture medium (FHE and FE) differed significantly from the evolution of germinated spores and mycelial growth. It is known that sporulation is triggered when the growth rate of hyphae drops, and the process of spore germination and the growth of mycelia are both driven by the same culture conditions, thus the results were consistent with previous findings (Dahlberg, 1982).

The cutaneous excreta of earthworms, which consist of mucus, coelomic fluid, and urine, are likely a source of biogenic components that may be assimilated by both microbes and plants. These fascinating findings are consistent with what has been directly observed in the natural system. Mucus has been shown to stimulate microbial activity and the breakdown of plant residues in the soil (Bityutskii et al., 2012; Phillips et al., 2019). Oleynik and Byzov (2008) proved that earthworm surface excretions promote the development of soil microbial communities. According to Huang and Xia (Huang and Xia, 2018), during decomposition, "mucus" increases the proliferation of proteobacteria (Chen et al., 2016; Huang et al., 2020; Wei et al., 2020). The favorable findings achieved by our laboratory and those reported in the scientific literature attest to the richness and diversity of the excreta in nutrients assimilable by bacteria as well as chemicals and growth factors supporting their development.

The biomass of earthworms in the soil ranges from 0.5 to 5 tonnes per hectare, depending on soil type and temperature (Lavelle, 1988; Lavelle et al., 1997). At the conclusion of their life cycle, this substantial biomass decomposes, releasing chemicals of high nutritional value for plant development and microbial activity that is vital for soil fertility. Therefore, nutrient inputs resulting from the decomposition of earthworm corpses would serve as a replacement biological amendment, especially in soils that were initially deficient or depleted due to contemporary agricultural methods (chemical fertilizers, pesticides, etc.). It should be mentioned that the water content of earthworms was determined to be 75% in this study. If this content is represented relative to the earthworm biomass, we find a range of 0.37 to 3.75 tonnes of water per hectare. This substantial volume of water might assist sustain rhizosphere relative humidity.

2. Effects of microorganisms associated with the earthworm *Aporrectodea molleri* on plant growth, metabolism, and chemical and biological properties of soil

The COI (Cytochrome oxidase I) mitochondrial barcoding approach enabled us to establish that the abundant earthworm in the Bou Regreg-Akrach area is closely related to *Aporrectodea molleri*, an understudied species in comparison to other genera of *Aporrectodea*.

Based on the fact that coelomic fluid is a non-negligible component of the cutaneous excretions of the earthworms, we were interested in a second stage in the isolation and phenotypic and genotypic investigation of earthworm's bacteria associated at the level of this fluid. The isolated bacteria were then evaluated for their capacity to increase soil fertility and influence plant growth and metabolism. Lastly, their function in suppressing the growth of phytopathogenic fungi is discussed (*Fusarium oxysporum*).

16 out of the 28 bacteria identified from the LC of *Aporrectodea molleri* are distinct from one another phylogenetically. If the earthworm were not protected by an efficient immune system, it would be a suitable habitat for the development of external bacteria and saprophytic fungi. Several scientific studies have evaluated the antibacterial activity of earthworm's coelomic fluid (Brown, 1995; Kathireswari et al., 2014; Roubalová et al., 2015). It has been discovered that the CF of earthworms contains compounds with antibacterial action against Gram-positive and Gram-negative bacteria, but especially against pathogenic earthworm strains (Roch et al., 1991; Valembois et al., 1985). In the present investigation, only 38 percent of the total bacteria identified were Gram-positive bacteria. This leads us to hypothesize that selection may have developed based on either the compositions of the bacterial cell wall or the unique interactions between species. The extremely low number of fungi isolated from CF (*Penicillium griseofulvum* CBS 185.27 and *Penicillium polonicum* CBS 222.28) can be explained by the fact that, with the exception of a few species,

earthworms have a detrimental effect on fungi. Some fungal species appear to be favoured while others are rejected, according to Edwards (2004) (Brown, 1995).

Bacteria belonging to the families *Bacilliaceae, Enterobacteriaceae, Pseudomonas, and Aeromonacae* are the most prevalent in the intestines of several earthworm species, similar to what was found for the CF (Picón et al., 2015). The results of this microbiological analysis indicate that the CF that fills the coelomic cavity is not aseptic. In fact, excretory organs and dorsal apertures allow microorganisms to enter the CF in each segment of the earthworm hollow (Bilej et al., 2018). Consequently, the CF interacts with microbes in the soil. Microorganisms that breach the epithelial barrier in the coelomic fluid are eliminated by the efficient innate defensive mechanisms, which are provided by the coelomocytes and by numerous humoral antimicrobial substances involved in the direct elimination of invading germs. However, a mutual symbiotic relationship may have allowed certain bacteria to live under these conditions and be unaffected by the antibacterial compounds present in this system. For the earthworm species *Aporrectodea molleri*, from which the isolation was conducted, it is hypothesized that the 16 isolated bacterial species were selected by the earthworm and that they exist in a symbiotic relationship at the level of its CF.

The test findings demonstrated that 87 % of the isolated bacteria possess favourable PGP characteristics, including the synthesis of siderophores and AIA, the solubilization of phosphate and potassium, the fixation of nitrogen, and the inhibition of *Fusarium oxysporum* development (Biocontrol). Given the well-established role of iron-chelating siderophores in boosting plant development (Morris et al., 1992), the result suggests that CF bacteria release iron-binding ligands (siderophores) that can bind to ferric iron and make it accessible to plants. Bacteria's solubilization of phosphate and potassium molecules in the rhizosphere is another crucial aspect of plant growth promotion (Mehta and Nautiyal, 2001). Since bacteria isolated from CF were able to solubilize considerable amounts of phosphate and potassium under *in vitro* conditions, it is predicted that their P and K solubilizing abilities will boost plant development. In the present investigation, *Pseudomonas and Bacillus* isolates (LC71 and LC12) can dissolve a substantial quantity of tricalcium phosphate, respectively. Phosphate solubilization efficiency by *Pseudomonas sp and Bacillus sp* has also been reported in several research (Roychowdhury et al., 2015). PGPR are an essential source of phytohormone for root growth, and we discovered that isolate LC12 (*Bacillus sp*.) produced the highest amount of AIA *in vitro* compared to prior studies (Godinho A, Ramesh R, 2010; Vicene CS, Nascimento F, Espada M, Barbosa P, Mota M, Glick BR, 2012). Additionally, nitrogen is one of the scarcest vital plant nutrients. *Bacillus* is the genus of bacteria that showed a favourable outcome for nitrogen fixing with a much greater concentration of total nitrogen (LC9, LC47 and LC12). Numerous *Bacillus* strains have been identified as nitrogen fixers (Susilowati et al., 2015). In addition, earthworms directly contribute to the soil mineral nitrogen pool by the excretion of nitrogen molecules in their mostly coelomic fluid-based excretions (Salmon, 2001). Therefore, earthworms may readily impact N transformations such as mineralization, nitrification, and denitrification by means of bacteria linked with their coelomic fluid.

F. oxysporum is a very pathogenic fungus for several plant species (Normander and Prosser, 2000). In addition, substantial data suggests that CF can inhibit the development of *Fusarium oxysporum* (Chaoui et al., 2003; Plavin et al., 2017). However, there is no indication that bacteria associated with earthworms contribute to this inhibitory mechanism. Since two isolates were shown to limit mycelial growth, this lends our discovery a great significance.

Earthworm’s CF can inhibit the growth of *Fusarium oxysporum* (Chaoui et al., 2003; Plavin et al., 2017). There is a strong correlation between the potential of bacteria isolated from CF and the determined actions of earthworms in the soil, including the enrichment of soils with nutrients and the fact that earthworm’s CF can inhibit the growth of *Fusarium oxysporum*. Bacteria inhabiting the CF may have a significant influence in the earthworm's positive soil activities.

On the basis of their remarkable performance *in vitro* for PGP features, seven bacteria (CFB1, CFFB2, CFB3, CFB4, CFB5, CFB6, and CFB7) were chosen for greenhouse testing in an alkaline, NPK-deficient soil (abiotic Stress). CFB greatly increase soil quality (biofertilizer) by boosting microbial biomass, the availability of important biogenic elements such as N, P, and K, and the concentration of phosphatase and urease enzymes. In addition, we were able to see a considerable improvement in the growth of maize compared to the control, as measured by plant biomass and chlorophyll content (a and b). Thus, the current trials demonstrated the potential of these bacteria as microorganisms that promote plant development. In general, the majority of plant-associated PGPRs contain several PGP traits (i.e. production of IAA, siderophores and ACC deaminase, phosphate solubilization, etc.). Numerous scientists have demonstrated the function of PGPRs as growth-promoting agents under diverse stressors (Jamal et al., 2018; Moreira et al., 2016; Yang et al., 2021). According to studies, *Bacillus and Pseudomonas* genera can increase corn production (Egamberdiyeva, 2007). In addition to promoting plant development, CFB isolates also boost plant water content compared to the control.

Alkalinity and nutrient deficit circumstances are characterized by stunted plant development and chlorophyll deficiency, as evidenced by the uninoculated soil (Carvalhais et al. , 2011; Egamberdiyeva, 2007). This demonstrates that CFB-inoculated plants are more resistant to abiotic stress. In addition, it is previously known that abiotic influences can affect crop output by up to 82% in extreme circumstances (Anami et al., 2020; Prasad et al., 2017). It is an encouraging result that inoculating plants with CFBs can mitigate plant damage induced by a variety of stressors.

In the current investigation, the bacterial strains *Buttiauxella gaviniae* S1/1-984 and *Aeromonas hydrophila* JCM1027 stimulated the development of maize plants in nutrient-deficient alkaline soil significantly more effectively. Insofar as these isolates do not significantly affect the biochemical characteristics of the soil, it is reasonable to conclude that the stimulatory effectiveness is connected to other, more direct processes, such as the synthesis of phytohormones (AIA) (Lata et al., 2006). Munir et al., 2019; Pavlova et al., 2017). The potential indirect impact might also be explained by the induced systemic tolerance in the plant (IST). In recent years, it has been discovered that IST-inducing PGPRs can trigger physiological and morphological responses in plants to nutrient deprivation (Maheshwari et al., 2012; Romera et al., 2019). The strains CFB2: *Pantoea vagans*, CFB5: *Staphylococus epidermidis,* CFB4: *Pseudomonas aeruginosa*, CFB6: *Bacillus paramycoides,* and CFB7: *Bacillus thuringiensis* showed a considerable impact on soil biochemical characteristics, which may have boosted plant development directly.

In fact, PGPRs can directly interact with plants under stressful situations by improving the availability of vital nutrients and the creation and the control of chemicals needed in plant development. They can also influence plants indirectly by shielding them from abiotic stress by inducing systemic responses (Oleska et al., 2020). The ability of CF-associated PGPRs to promote plant development is of essential relevance, particularly under abiotic stress conditions when bacteria can boost plant fitness and stress tolerance.

Notably, earthworms can modify soil pH and boost alkaline phosphatase activity (Lim et al., 2015; Raouane and El Harti, 2016; Wu et al., 2012). We hypothesize that the capacity of CFBs to survive and flourish in alkaline soil is similar to that of the earthworm, which can not only withstand but also neutralize alkaline or acidic soil pH. (Raouane and El Harti, 2016).

Different molecular pathways may be implicated in the response of plants to various biostimulants, as shown by the varied metabolic profiles identified. Compared to the control, bacterial treatments induce a more significant metabolic reprogramming. In contrast to the control, abiotic stress had no effect on the composition and concentration of lipids in CFB-treated plants, indicating the robustness of the protective mechanisms.

The statistical analysis based on the Pearson correlation between the metabolites of the leaves of plants treated with CFBs and their growth parameters revealed a positive correlation between the length of the aerial part, the surface of the leaves, and the biomass (fresh and dry weight of the aerial part) and certain metabolites belonging to fatty acids and derivatives, carboxylic acids and derivatives, benzene derivatives, and alkanes. Possible explanation for this observation is that positively linked metabolites are positive signals that regulate plant development and stress resistance. The fact that the metabolites present in all treatments are abundant in the bacterial treatments relative to the control treatment suggests that the bacteria triggered the overexpression of these metabolites. CFB2 (*Pantoea vagans*), CFB4 (*Pseudomonas aeruginosa*), CFB6 (*Bacillus paramycoides*), and CFB7 (*Bacillus thuringiensis*) isolates resulted in a considerably high synthesis of diverse metabolites belonging to distinct chemical classes.

The quantity of metabolites of benzene origin, which are present in the composition of lignin, suberin, pigments (flavonoids, anthocyanins), and other phenolic chemicals (amec et al., 2021), may suggest a control of their production in bacterial treatments. By controlling cellular nutrient efflux, sterols and glycerols found solely in bacterial treatments (CFB1, CFB2, CFB4, and CFB6) play a crucial role in plant development and abiotic stress tolerance (Hull et al. , 2018). Moreover, heptadecane, octadecane, and azelaic acid, which are often observed in plants in response to stressful situations (Mzibra et al., 2021), were accumulated to significant amounts in plants treated with coelomic fluid isolates, which likely increased their tolerance.

GC-MS lipidomic analysis indicated a rise in the concentration of unsaturated fatty acids, particularly linolenic acid (C18:3), which is responsible for the flexibility of chloroplast membranes and plays a role in photosynthesis. Thus, changes in the lipid composition of plant leaves in response to abiotic stress were associated with modifications in photosynthetic activity (Phamthi, 1984); this explains why the chlorophyll content of plants treated with CFB was not changed by abiotic stress as it was in the control group. In bacterial treatments, palmitic acid and oleic acid levels increased, which are known to promote plant growth and development under stressful conditions (Zhukov, 2015).

Submission of metabolites to the KEGG database demonstrated that the isolates commenced fatty acid biosynthesis, which includes a sequence of condensation processes leading to the production of C16:0, which can be extended to C18:0 and desaturated. These fatty acids can be deposited in the cell wall to limit water movement and protect plants from abiotic stressors (Farid et al., 2019; Holapek et al., 2018; Karim et al., n.d.). According to previous research, overexpression of metabolites involved in the tricarboxylic acid (TCA) cycle and glyoxylate and dicarboxylate metabolic pathway in plants might modify key biological processes, such as hormone signal transmission, and boost the abiotic stress tolerance of plants (Xu et al., 2018). The activity of the pathways involved in the reinforcement of tolerance, which was greater in the CFB-inoculated plants than in the control plants, explains why the aerial sections of the treated plants were less severely damaged than those that were not treated.

Overall, and in agreement with the existing literature, the results gained in this study may be utilized in the natural environment and can be projected in the natural environment. The zone between a plant's root system and the soil is frequently referred to as the rhizosphere. This zone is where the interactions between plants and microbes occur. When earthworms become involved, the rhizosphere is referred to as the drilosphere. In fact, the drilosphere is a crucial biotope for the interaction between plants, microbes, and earthworms. During the movement of the earthworms in this zone, the coelomic fluid and associated bacteria (PGPR) are released via the coelomic pores by contractions of the metamers. The influence on soil microorganisms can boost the biomass and activity of helpful strains (biofertilizers) or suppress parasitic species (biocontrol). The direct effect on plants can have a favorable impact on growth, promotion of new root development, and activation of the production of numerous metabolites involved in growth and abiotic stress tolerance.

Scientific publications and communications

Published articles:

1. **Lamia Yakkou**, Sofia Houida, Jorge Domínguez, Mohammed Raouane, Souad Amghar, and Abdellatif El Harti. Identification and Characterization of Microbial Community in the Coelomic Fluid of Earthworm *Aporrectodea molleri* May 2021. Korean Journal of Microbiology and Biotechnology
2. **Lamia Yakkou**, Sofia Houida, Mohammed Raouane, Souad Amghar, and Abdellatif El Harti. Study of the Effect of the Earthworm (*Aporrectodea molleri*) Cutaneous Excreta on Bacterial Growth May 2021. International Journal of Ecology
3. Sofia Houida, **Lamia Yakkou**, Serdar Bilen, Mohammed Raouane, Souad Amghar, and Abdellatif El Harti. Taxonomic and functional characteristics of aerobic bacteria isolated from the chloragogenous tissue of the earthworm *Aporrectodea molleri* October 2021. Archives of Microbiology
4. **Lamia Yakkou**, Sofia Houida, Jorge Domínguez, Mohammed Raouane, Souad Amghar, and Abdellatif El Harti. The Effect of Endogenous Earthworm Extracts (*Aporrectodea molleri*) on the Growth of Beneficial Soil Bacteria October 2021. Acta microbiologica Bulgarica
5. Zakaria Hafidi, **Lamia Yakkou**, Fatima-Ezzahra Guouguaou, Souad Amghar and Mohammed El Achouri. Journal of Dispersion Science and Technology Aminoalcohol-based surfactants (N-(hydroxyalkyl)- N, N-dimethyl Nalkylammonium bromide): evaluation of antibacterial activity and molecular docking studies against dehydrosqualene synthase enzyme (CrtM) December 2019. Journal of Dispersion Science and Technology
6. Sofia Houida, **Lamia Yakkou**, Leyla Okyay Kaya, Serdar Bilen, Mouhcine Fadil, Mohammed Raouane, Abdellatif El Harti, Souad Amghar. Biopriming of Maize seeds with plant growth-promoting bacteria isolated from the earthworm *Aporrectodea molleri*: Effect on seed germination and seedling growth. March 2022. Letters of applied microbiology
7. **Lamia Yakkou**, Sofia Houida, Serdar Bilen, Leyla Okyay Kaya, Mohammed Raouane, Souad Amghar, Abdellatif El Harti. Assessment of earthworm (*Aporrectodea molleri*)'s coelomic fluid-associated bacteria on different plant

growth-promoting traits and maize germination and seedling growth. April 2022. Biocatalysis and Agricultural Biotechnology.

Submitted :

1. Earthworm *Aporrectodea molleri (oligochaeta*)'s coelomic fluid-associated bacteria modify soil biochemical properties and improve maize (*Zea mays L*) plant growth under abiotic stress conditions.

Communications:

1. Les Journées de l'ARCUS-E2D2 L'Environnement à travers les sciences, Université Mohammed V, Rabat- MAROC. 9-11 Avril 2018, Présentation poster
2. The Fourth International American Moroccan Agricultural Sciences Conference – AMAS Conference IV, Qualipôle-Agropolis, Meknes, Maroc. 9 au 11 mai 2018, Présentation poster
3. International Agricultural, Biological and Life Sciences Conference (AGBIOL Conference) Université Trakya, Edirne, Turquie. 2 au 6 septembre 2018. Présentation orale et présentation poster
4. 7 éme édition Ecole Internationale de Recherche, Faculté des Sciences d'Agadir, Université Ibn Zohr, Agadir, Maroc. 25 au 27 avril, 2019, Présentation orale
5. DOCTORIALE - CERNE2D Water, Energy & Environment Nexus 2019, ENSET RABAT, Maroc.12- 14 Juin 2019. Présentation orale
6. International Agricultural, Biological and Life Sciences Conference (AGBIOL Conference) Université Trakya, Edirne, Turquie. 1 au 3 septembre 2021. Présentation orale
7. 1st International Conference Sustainable Agriculture: Tools and Innovations "AgriNov 2021", Beni Mellal, Maroc. 27 au 30 octobre, 2021. Présentation poster
8. 8ème Ecole Internationale de Recherche, Faculté de Sciences Appliquées/Centre de Formation à la Faculté de Médecine et de Pharmacie/Faculté des Sciences, Agadir, Maroc. 20 au 22 décembre 2021. Présentation poster.

9. 2nd Edition of MAASI Scientific Week (Hybrid), Istanbul, Turquie. 23-30 Mai 2022. Présentation orale