

Research Article

Control of Cultivable IAA-Producing Bacteria by the Plant *Arabidopsis thaliana* and the Earthworm *Aporrectodea caliginosa*

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Some soil microorganisms are involved in the complex interactions with plants and earthworms, through the production of indole acetic acid (IAA) which modifies plant growth and development. In a factorial experiment testing the impact of the presence/absence of plants and earthworms on IAA production by cultivable bacteria, we observed that plants were decreasing IAA production of 43%, whereas earthworms were increasing it of 46%. In the presence of both plant and earthworms, IAA production was as low as in the presence of plant control, showing that plants influence on IAA production by microorganisms prevails on earthworm influence. We discuss functional reasons which could explain this result.

1. Introduction

Soil microorganisms can be closely associated with soil animals such as earthworms for the delivery of ecosystem functions such as organic matter mineralization [1–3]. The release of nutrients due to this dual interaction has consequences on plant growth [4–6]. In addition to nutrients, interdependence between microorganisms, plants, and earthworms has led to the selection of diversified signal molecules, from low-molecular weight compounds such as auxin (e.g., IAA), amino acids, and secondary metabolites, to high-molecular weight compounds such as proteins and polysaccharides (for review see [7]). Emission of signal molecules by microorganisms can be deleterious for plants in the case of pathogenic bacteria and invertebrate herbivores, or positive with mycorrhizas, nitrogen-fixing symbiotic bacteria, or free living bacteria such as plant growth promoting rhizobacteria (PGPR) [7–9]. Auxin-like compounds have also been detected in earthworm dejections [10, 11], and could be responsible for the positive effect of earthworms [12] and vermicompost [10] on plant growth. The same molecule can have positive or negative effects according to its concentration and plant sensitivity [13].

There are very few studies which consider simultaneously the impact of plants and earthworms on microbial activities. However, it is of prior importance to establish a hierarchy in biotic factors influencing soil microorganisms. The purpose of our study was to determine the hierarchy of biotic factors on IAA-producing bacteria: do microorganisms obey to plants or earthworms?

2. Materials and Methods

To answer this question, we set up an experiment with 4 treatments: soil without macroorganisms (S), with the plant *Arabidopsis thaliana* (P), and with the earthworm *Aporrectodea caliginosa* (E), with both plant and earthworms (PE). Nine cm diameter-10 cm high PVC experimental units ($n = 5$ per treatment) were filled with 1 kg of dry sandy soil from the CEREEP research station (Saint-Pierre-Lès-Nemours, France).

It was dried at 25°C for a week and sieved at 2 mm mesh size. This soil has the following properties: total organic carbon content, 14.7 g kg⁻¹; total nitrogen content, 1.19 g kg⁻¹; pH, 5.22; CEC, 4.08 cmol kg⁻¹; texture: 6.9% clay, 19.0% silt, 74.1% sand. During the experiment, it was then maintained

at 80% of the field capacity. Three earthworms ($1.60 \text{ g} \pm 0.06$) were introduced one week after soil moistening in treatments E and PE. One seed of *Arabidopsis thaliana* cv. Columbia was sown per experimental unit two weeks after soil watering in treatment P and PE. Plants were grown for 42 days under controlled conditions (Conviron Growth Chamber, Canada) at $20 \pm 1^\circ\text{C}$ and $18 \pm 1^\circ\text{C}$ day and night temperatures, $70\% \pm 5\%$ relative humidity, and $200 \mu\text{mol m}^{-2} \text{ s}^{-1}$ photon flux for 10 h per day.

At the end of the experiment, plant shoots and roots were retrieved, dried at 45°C , and weighted. As extraction and purification of IAA from natural soils is very difficult due to soil adsorbing complex (organic matter, humus, clays etc.), we extracted bacterial communities from the soil of the different treatments and quantified the production of IAA due to cultivable bacteria present in these extracts. Soil samples (100 g) were collected from experimental units and dried at 40°C . For all treatments (S, P, E, and PE), microbial populations were extracted from these dried soil samples in aqueous solution (5 g L^{-1} NaCl) at 1:10 soil:water ratio with 40 rpm agitation for 1 h. After centrifugation (3 min, 1000 rpm), 1 mL of bacterial suspension was inoculated on 6.5 mL of liquid Czapek media (with only 5 g L^{-1} sucrose). Two hundred $\mu\text{g L}^{-1}$ of L-Tryptophan (Sigma, USA) was added as precursor for IAA production. Bacteria culture was performed in the dark at 27°C with shaking at 180 rpm. At 24 h, 48 h, and 72 h, 500 μL of the bacterial culture supernatant were retrieved. Bacterial cells were removed by centrifugation at 5000 rpm during 1 min. Part of the supernatant (250 μL) free of bacterial cells was mixed with 750 μL of Salkowski's reagent (50 mL HClO_4 at 35% and 1 mL of 0.5 M FeCl_3). IAA concentration was determined by absorbance at 530 nm with a Novaspec III spectrophotometer (Amersham Biosciences, USA), with the help of a standard curve obtained from serial dilutions of IAA stock solution (Sigma, USA).

3. Results

IAA production by cultivable soil microorganisms extracted from soil without macro-organisms (S) was about $8.8 \times 10^{-5} \text{ mol L}^{-1}$, whereas the presence of earthworms in soil (E) was responsible for a significant 46% ($12.7 \times 10^{-5} \text{ mol L}^{-1}$) increase in IAA production by cultivable microorganisms (Figure 1). In the presence of plants (P) or in the presence of plants and earthworms (PE), IAA production was at the same level, respectively, 4.9×10^{-5} and $4.5 \times 10^{-5} \text{ mol L}^{-1}$ (Figure 1), suggesting that plants halved IAA production by soil microorganisms. A two-ways ANOVA confirmed the significance of plant negative effect (P value < 0.001 , $F = 58.33$), and earthworm positive effect (P value = 0.02, $F = 6.8$) on IAA production by microorganisms (Table 1). The reason for this inhibition of IAA production by plants could be linked to the fact that too high IAA exogenous concentration can inhibit root growth and prevent plant development [14] (see Section 4). This was supported by the significant negative correlation ($R^2 = 0.71$, P value = 0.005) between plant biomass and IAA production (Figure 2). As far as the interaction between plants and earthworms is concerned,

TABLE 1: Impact of the factors “plant,” “earthworm,” and their interaction on IAA production by cultivable microorganisms.

	IAA concentration		
	Df	F	P value
Plant	1	58.33	<0.001
Earthworm	1	6.80	0.02
Plant * earthworm	1	8.25	0.01
Residuals	13		

$n = 17$.

we observed that the positive effect of earthworms on IAA production observed in treatment E disappeared in treatment PE (Figure 1). A two-ways ANOVA showed that the statistical interaction between the factors of plants and earthworms was significant (P value = 0.01, $F = 8.25$), which means that earthworm effect on IAA production was dependant on the presence of plants.

Note that the discussed IAA concentration was due to the production of IAA by cultivable microorganisms, which represent only a small fraction of microorganisms living in a soil, despite the large spectrum of microorganisms growing on the Czapek medium. An alternative approach would be to extract all the IAA from the soil, but a given extraction protocol would not have the same extraction efficiency for soils deeply modified by plants or earthworms. Moreover, protocols developed to detect IAA in complex soils [10, 11, 15] do not provide reliable methods to quantify absolute amount of IAA in soil samples.

4. Discussion

Interactions between organisms in soil are mainly based on trophic relationships and exchange of signals. Among signal molecules, auxins have been shown to play a major role in plant-microorganism interactions; for example, rhizobacteria producing high amounts of IAA are generally responsible for a negative effect on plant growth and have been called “Deleterious Rhizobacteria” [16–18]. Rhizobacteria producing low amounts of IAA are generally responsible for a positive effect on plant growth and have been called “Plant Growth Promoting Rhizobacteria” [9, 19, 20]. This important effect of IAA can be related to its role in cell division and elongation: it is demonstrated that the auxin dose-response curve is bell shaped, with a maximal elongation rate reached for medium exogenous auxin concentration [14].

Many soil bacteria use signals to direct plant carbon for the build-up of additional root structures [21–23], with a subsequent positive effect on root exudates and bacteria populations. As earthworms are supposed to consume soil microorganisms, the increase in IAA production observed in the presence of earthworms could be explained as a way to increase root exudates, bacterial biomass, and thus earthworm resources. This is supported by previous observations of higher IAA content in earthworms-worked soils as compared with soil without earthworms [10, 11]

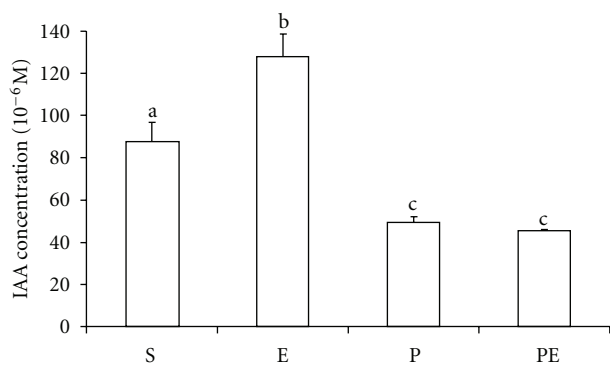


FIGURE 1: Average IAA concentration at 24, 48, and 72 h by cultivable soil bacteria from soil without macroorganisms (S), with the plant *Arabidopsis thaliana* (P), with the earthworm *Aporrectodea caliginosa* (E) and with both plant and earthworms (PE). Means \pm s.e., $n = 17$, different letters indicate a significant difference, Tukey HSD, $P < 0.05$.

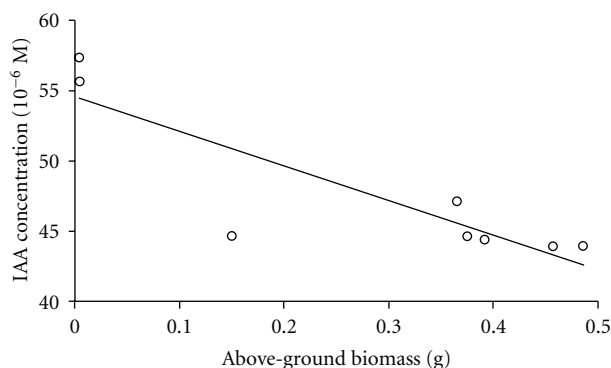


FIGURE 2: Relationship between IAA concentration and plant above-ground biomass ($y = -24.35x + 54.53$, $R^2 = 0.75$, $P = 0.005$).

as well as the hormone-like effects observed in compost made by earthworms [24–26].

However, initiation of additional root structures or increased exudation is probably not always beneficial to plants. Plants have indeed developed mechanisms to regulate IAA production by microorganisms. There are very few evidences of a direct control of IAA producing bacterial communities by plants. Nevertheless, it has been shown that transcription of *ipdC*, an *Erwinia* IAA biosynthetic gene, is induced in response to bean and tobacco compounds [27]. This kind of mechanism could be at the origin of the repression of IAA producing bacteria in the presence of plants observed in our study.

5. Conclusion

In this study, we confirmed that earthworms can be responsible for an increase in IAA production by microorganisms. We also observed a repression of IAA production in the presence of plants. In the presence of both earthworms and plants, we found that plants took the advantage on earthworms in the control of cultivable IAA-producing bacteria, by

maintaining a weak level of IAA production by cultivable microorganisms.

Plants are responsible for energy inputs in terrestrial ecosystems (about 90% of soil organic matter). They are major providers of substrates to microorganisms through root exudates and dead roots, which could represent 20 to 50% of the total assimilated carbon [28, 29], or to earthworms through dead leaves and roots [30]. This could explain the fact that they control many soil processes such as the production of IAA, but also free-living bacteria nitrogenase activity [31], amoebae community structure [32], and hydrostructural stability of soil [33].

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