Azospirillum affects Eh and potential denitrification in a soil

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Abstract

The effect of inoculation with Azospirillum brasilense (strain 7001) on soil Eh under anaerobic conditions (N₂ flux) was examined during 144 h at 26°C and 230 h at 18°C. The Eh values of the control (not inoculated) soil decreased to approximately 80 or 140 mV in both cases, whereas after 24 h of anaerobic incubation, the Eh of the Azospirillum-inoculated soil remained at higher values. After 144 and 230 h of anaerobic incubation, the denitrifying activity (measured in anaerobiosis with excess of e⁻ acceptor and donor) in the inoculated soil was seven and three times lower respectively, than in the non-inoculated soil. This indicates that Azospirillum may affect the soil Eh and consequently any highly Eh-dependent microbial activity, such as denitrification.

Introduction

Inoculation of plants with Azospirillum significantly affects a plant growth. It has been suggested that the changes are associated with the following factors: (i) nitrogen fixation, (ii) hormonal effects, (iii) general improvement in root growth resulting in improved mineral and water uptake, and (iv) activity of bacterial nitrate reductase inside the roots (Bashan and Levanony, 1990), which all involve the direct action of bacteria on root growth. However, none of these hypotheses deals with the possible influence of Azospirillum on the plant through the improvement of soil parameters, such as pH or redox potential, despite the demonstrated effect of inoculation with Azospirillum on both plant and rhizosphere bacterial parameters. For example, changes in N₂ fixation in naturally rhizobial-colonized legumes (Sarig et al., 1986) or in the nodulation ability of rhizobial strains (Schmidt et al., 1988) have been observed after inoculation with Azospirillum in plant-soil systems. A synergistic interaction between Azospirillum and vesicular-arbuscular (VA) mycorrhizal fungi can also occur, resulting in a significant increase in P content in plants (Pacovsky, 1988; Subba Rao et al., 1985). Straw decomposition was also increased by inoculation of Azospirillum (Halsall and Gibson, 1985) suggesting that this microorganism can affect the number and/or the physiology of the cellulolitic microflora.

In the case of Rhizobium or VA mycorrhizal fungi, the observed interaction may be the indirect result of morphogenic changes induced by Azospirillum. However, this hypothesis cannot be applied to cellulolitic activity because no plants are involved in the system. The influence of Azospirillum may therefore be general, affecting both plants and soil bacteria, with some of the observed interactions partly explained by a global modification of the soil environment.

In a previous work, Pidello et al. (1989)
reported that Eh values measured in inoculated soil plant system at saturation were higher than in control (non-inoculated) soil.

The objective of this work is to examine the influence of *Azospirillum* inoculation on the alteration of the redox potential (Eh) and Eh-dependent microbial activity, such as denitrification, in an anoxic soil.

**Materials and methods**

**Soil**

The loamy clay soil used in this study is a vertic Mollisol ("serie Peyrano") located in Casilda – Provincia de Santa Fe – Argentina, with the following characteristics: O.M., 37 g kg⁻¹; N, 2.3 g kg⁻¹; pH, 6.1; CEC, 21.7 cmolₑ · kg⁻¹ (INTA, 1979); N-NO₃⁻, 0.34 g kg⁻¹; N-NH₄⁺, 4.16 g kg⁻¹ (Pidello and Menéndez, unpublished data).

**Bacterial strain**

The bacterial strain used in this study was *Azospirillum brasilense* strain 7001 (streptomycin-resistant derivative of *A. brasilense* sp 7-ATCC 29145) obtained by C. Elmerich, Pasteur Institute, France). Cultures were performed in liquid NFb (Nitrogen Free broth) medium (Baldani et al., 1986).

**Procedure**

The experimental apparatus used to maintain the soil under N₂ flux and to monitor the soil Eh during the incubation is shown in figure 1.45 g of air-dried soil (sieved to 2 mm) were placed on a filter tissue in a plastic ring (60 mm diameter, 30 mm high) divided into 12 compartments. In order to perform the Eh measurements, 12 platinum microelectrodes (surface area 1.57 mm²) were radially inserted into the dry soil of the 12 compartments. Then, the soil was remoistened at 50% (w/w) with an *A. brasilense* sp 7001 suspension in distilled water for the inoculated treatment or with distilled water or with the same autoclaved bacterial suspension for the non-inoculated treatment (Exp. 1 and 2).

The bacterial suspension was obtained by centrifugation at 3000g for 10 min and washed twice with sterile distilled water. The initial bacterial concentration was determined by direct counts and the final concentration adjusted in order to obtain 10⁸ cells g⁻¹ dry soil.

The plastic ring with soil was then hermetically sealed between the two parts of a glass column (65 mm diameter, 170 mm high) as shown in Figure 1. The glass column allows the entrance of N₂ gas (50 mL min⁻¹) at the bottom and the insertion of a double junction reference electrode (calomel type-Orion 92-02) at the top. The reference electrode was put in contact with the superficial soil layer at the center of the plastic ring only when Eh measurements were performed to avoid undesirable diffusion of the electrolyte into the soil.

The electrodes were connected to an Orion potentiometer 407 and the saturated calomel cell as the reference electrode.