Plant Physiology and Biochemistry 83 (2014) 285–291

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Cytokinin producing bacteria stimulate amino acid deposition by wheat roots

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ARTICLE INFO

Article history:
Received 6 March 2014
Accepted 18 August 2014
Available online 27 August 2014

Keywords:
Bacillus subtilis
Cytokinin
Plant/microbe interaction
Rhizodeposition
Wheat

ABSTRACT

Phytohormone production is one mechanism by which rhizobacteria can stimulate plant growth, but it is not clear whether the bacteria gain from this mechanism. The hypothesis that microbial-derived cytokinin phytohormones stimulate root exudation of amino acids was tested. The rhizosphere of wheat plants was drenched with the synthetic cytokinin trans-zeatin or inoculated with *Bacillus subtilis* IB-22 (which produces zeatin type cytokinins) or *B. subtilis* IB-21 (which failed to accumulate cytokinins). Growing plants in a split root system allowed spatial separation of zeatin application or rhizobacterial inoculation to one compartment and analyses of amino acid release from roots (rhizodeposition) into the other compartment (without either microbial inoculation or treatment with exogenous hormone). Supplying *B. subtilis* IB-22 or zeatin to either the whole root system or half of the roots increased concentrations of amino acids in the soil solution although the magnitude of the increase was greater when whole roots were treated. There was some similarity in amino acid concentrations induced by either bacterial or zeatin treatment. Thus *B. subtilis* IB-22 increased amino acid rhizodeposition, likely due to its ability to produce cytokinins. Furthermore, *B. subtilis* strain IB-21, which failed to accumulate cytokinins in culture media, did not significantly affect amino acid concentrations in the wheat rhizosphere. The ability of rhizobacteria to produce cytokinins and thereby stimulate rhizodeposition may be important in enhancing rhizobacterial colonization of the rhizoplane.

1. Introduction

Introducing plant growth promoting rhizobacteria (PGPR) into the rhizosphere has increased plant yield in numerous studies (Verma et al., 2010; Dodd and Ruiz-Lozano, 2012). Species of soil bacteria known as PGPR can stimulate plant growth by a plethora of mechanisms including production of phytohormones (Weyens et al., 2009; Aroca and Ruiz-Lozano, 2009; Dodd et al., 2010). However application of PGPR to different crop production systems remains underemployed, likely due to poor understanding of their ecology (Vessey, 2003). Gaining more knowledge about interactions between PGPR and plants is crucial for improved management of agricultural systems.

Biological processes in the rhizosphere are strongly influenced by microbial degradation of organic carbon compounds including organic acids, sugars and amino acids excreted by plant roots. Root exudation of soluble compounds, specifically amino acids (Moe, 2013), attract and stimulate microbial growth thereby coupling plant and microbial productivity (Albareda et al., 2006; Bais et al., 2006; Houlden et al., 2008). However, much remains to be learnt about the regulation of rhizodeposition, and its ecological significance regulating plant/microbe interactions (Jones et al., 2004). Despite the dominant ecological view that microorganisms and roots passively compete for amino acids in the soil solution (Schobert et al., 1988; Owens and Jones, 2001; Sauheitl et al., 2009), it was suggested that root exudation is actively manipulated by microorganisms themselves by means of some yet unidentified enhancers (Phillips et al., 2004). Several microbial products like phenazine or zearalenone significantly enhanced the net efflux of amino acids from roots (Phillips et al., 2004). However their

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http://dx.doi.org/10.1016/j.plaphy.2014.08.015
0981-9428/

effective concentrations (200 μM) were rather high, making the possible carbon gain by microorganisms almost equal to the expenses of their production. Plant hormones are normally biologically active at much lower concentrations making them a more likely candidate for influencing root exudation. Involvement of microbially derived phytohormones in controlling rhizodeposition was previously suggested, but not studied (Pishchik et al., 2002).

The function of phytohormones in plant/microbe interactions has been thoroughly studied for symbiotic (Frugier et al., 2008) microbes, but less is known about free living microorganisms. There has been much interest in both microbial phytohormone production, and to a lesser extent phytohormone degradation, and its effects on plant growth (see references cited in the review of Dodd et al. (2010)). However there is relatively little information about what free living microorganisms themselves gain from their capacity to produce plant hormones. Although dependence of heterotrophic microbes on root exudates is obvious, the importance of microbe-synthesized phytohormones in stimulating root exudation was not frequently addressed. Although auxin stimulated sugar release by roots into the rhizosphere (Wittenmayer and Merbach, 2005), the significance of microbial auxin production on root exudation of amino acids seems not to have been studied, even though it was hypothesized that microbial-derived IAA stimulated the exudation of ACC (an amino acid and the immediate precursor of the phytohormone ethylene) into the rhizosphere (Glick et al., 1998). To our knowledge, the role of cytokinins (of either plant or microbial origin) on rhizodeposition has not been assessed.

This research aimed to determine if introducing PGPR into the rhizosphere influenced deposition of amino acids by wheat roots. *Bacillus subtilis* IB-22 was selected for its ability to synthesize zeatin-type cytokinins (Arkhipova et al., 2005) and since soil inoculation with this bacterium stimulated lettuce and wheat growth (Arkhipova et al., 2006, 2007). Maintaining high rhizobacterial colonization of wheat roots (above 10^5 CFU g^{-1} root fresh weight) throughout the vegetative period increased wheat productivity by *circa* 30% (Melent'ev et al., 2000; Kuz'mina and Melent'ev, 2003). Moreover, amino acids modulated the expression of genes in *B. subtilis* that promoted competence and sporulation (Mader et al., 2002). To test whether bacterial cytokinin production increased rhizodeposition of organic substances, the effect of bacterial inoculation on amino acid concentrations in the soil solution was compared to the impact of soil drenches with the cytokinin zeatin. Amino acids were measured since they are sometimes major components of root exudates (Jones et al., 2004), while cytokinins are known to influence nitrogen metabolism in plants (Sakakibara et al., 2006) by increasing activity of nitrate reductase (Sykorová et al., 2008). To avoid microbial utilization of root exudates interfering in the determination of amino acid concentrations in the soil solution, plants were grown with their roots divided into two compartments, allowing spatial separation of inoculation and sampling of soil solution from different roots of the same plant (Fig. 1).

2. Methods

2.1. Bacterial strains and culture media

The aerobic spore-forming bacterium *B. subtilis* strain IB-22, selected for its high level of cytokinin production (Arkhipova et al., 2005) and IB-21 which failed to accumulate cytokinins (Fig. 2B), were obtained from the Laboratory of Applied Microbiology of the Institute of Biology, Ufa Science Centre, Russia. The bacteria were cultivated on a rotary shaker (160 rpm) for 48 h at 37 °C in flasks with liquid medium containing 1% starch, 0.3%

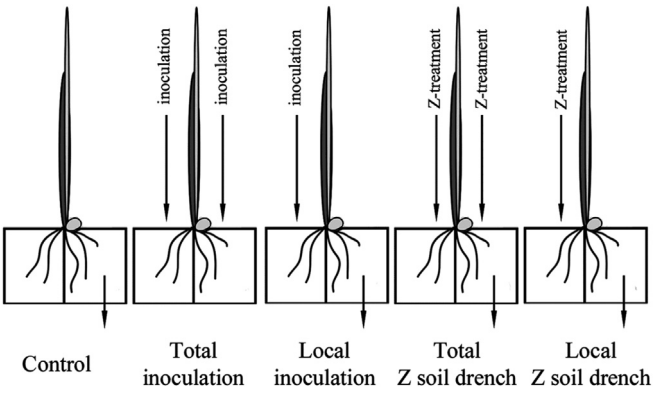

Fig. 1. Scheme of split root experiments. Seedlings were fixed inside plastic tubes on the divider between containers, while roots of each plant were evenly distributed between containers containing moist sand. Bacterial suspensions or zeatin solution (Z) (indicated with the arrows in the upper part of the figures) were introduced either into one (local treatments) or both (total treatments) root compartments. Soil solutions were sampled from the untreated (for local treatments) or a randomly selected (total treatments) root compartment (arrows in the lower part of the figures).

peptone, 0.3% yeast extract, 0.3% maize extract, 0.3% $(NH_4)_2SO_4$, 0.2% K_2HPO_4, and 0.2% $(NH_4)_2HPO_4$. Cytokinin concentrations in the culture media were measured (see below) during bacterial cultivation. Bacterial cultures were collected at the stationary growth

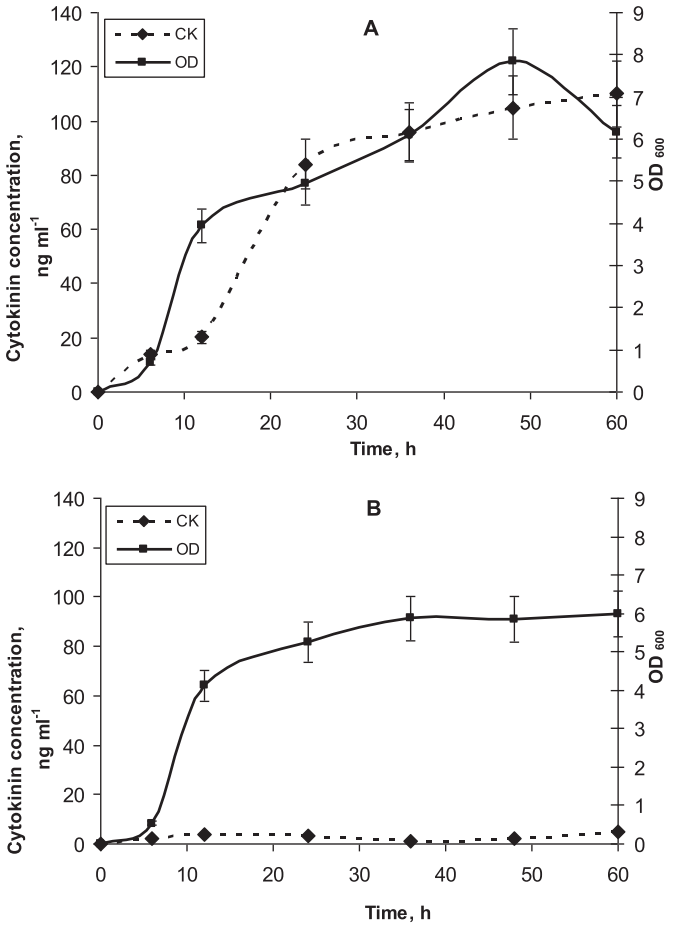

Fig. 2. Growth (expressed as optical density of culture media, OD) and accumulation of cytokinins (zeatin + its riboside and nucleotide) in culture media of *Bacillus subtilis* IB-22 (A) and IB-21 (B). Data are means ± SE of 9 replicates.