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Root cap influences root colonisation by *Pseudomonas fluorescens* SBW25 on maize

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Abstract

We investigated the influence of root border cells on the colonisation of seedling *Zea mays* roots by *Pseudomonas fluorescens* SBW25 in sandy loam soil packed at two dry bulk densities. Numbers of colony forming units (CFU) were counted on sequential sections of root for intact and decapped inoculated roots grown in loose (1.0 mg m^{-3}) and compacted (1.3 mg m^{-3}) soil. After two days of root growth, the numbers of *P. fluorescens* (CFU cm^{-1}) were highest on the section of root just below the seed with progressively fewer bacteria near the tip, irrespective of density. The decapped roots had significantly more colonies of *P. fluorescens* at the tip compared with the intact roots: approximately 100-fold more in the loose and 30-fold more in the compact soil. In addition, confocal images of the root tips grown in agar showed that *P. fluorescens* could only be detected on the tips of the decapped roots. These results indicated that border cells, and their associated mucilage, prevented complete colonization of the root tip by the biocontrol agent *P. fluorescens*, possibly by acting as a disposable surface or sheath around the cap.

Keywords: Zea mays; Border cell; *Pseudomonas fluorescens*; Biocontrol; Root cap; Confocal microscopy; Rhizosphere

1. Introduction

Root border cells, and their associated root cap mucilage, are thought to have roles in a number of processes including aiding root penetration into compacted soils [1,2], regulating root cap mitosis [3] and in establishing rhizosphere communities [4]. Recently, research has focused on the role of border cells in protecting plant health by controlling the growth of microorganisms in the rhizosphere [5]. Border cells synthesise and export a diverse range of low molecular weight proteins [6] and supply a readily available source of carbon into the extracellular environment [7], which could influence the activity of microbial communities in the rhizosphere. In this way, border cells potentially have the capacity to promote the growth of beneficial microorganisms and repel or inhibit the growth of pathogenic organisms [4,5,8]. The quantity and quality of exudates produced by border cells depends on the plant genotype. The microbial communities present in the rhizosphere will be influenced by the ability of these organisms to respond to and utilise particular compounds released from border cells [9].

Mechanisms by which border cells protect plant health have been suggested to include: acting as a decoy to attract fungal infection away from the vulnerable root

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tip, thereby leaving the tip free from infection when the border cells detach [10,11]; attracting and then inducing a transient quiescent state in root knot nematodes, allowing time for the root tip to grow past the area of danger [12]; and producing mucilage that repels pathogenic bacteria [11]. Border cell production has also been positively correlated with arbuscular mycorrhizal (AM) colonisation. AM fungi mainly benefit plant growth by improving immobile nutrient uptake from soil. Plants producing large numbers of border cells tend to show greater mycorrhizal root colonisation [13,14].

One group of beneficial rhizosphere microorganisms, that have received significant attention, is the plant growth promoting rhizobacteria (PGPR). Some PGPR can suppress the growth of pathogenic organisms directly by producing either antibiotic compounds [15], or siderophores that allow effective iron capture from the rhizosphere, depriving pathogenic organisms of this necessary element [16]. They can also indirectly promote plant growth by inducing systemic resistance in the plant [17,18]. Many PGPR belong to the fluorescent pseudomonads, including *Pseudomonas fluorescens*, which have been used successfully to control soil-borne plant pathogens [19]. Fluorescent pseudomonads are also capable of exploiting a variety of compounds exuded by roots, have a fast growth rate and are motile, [20,21]. Despite this, biological control of plant pathogens in the field is often inconsistent, partly due to poor root colonisation [19,20], leading to decreased biocontrol activity [22]. Successful biological control requires the density of the introduced biocontrol bacteria to be at the sufficient level, time, and location favourable for potential pathogenic attack irrespective of environmental conditions [23]. Soil physical conditions determine the size ranges of soil pores present and whether they are water-filled. This is relevant both for organism movement, and root growth rate (controlled by soil strength and matric potential). These factors will determine the impact of bulk soil density on the spread and location of biocontrol bacteria.

The aim of this paper was to determine the influence of border cells, and their associated mucilage, on the growth and distribution of *P. fluorescens* SBW25, when grown on *Zea mays* roots, in loose and compacted soil. Root colonisation by *P. fluorescens* was determined in intact and decapped roots at two soil bulk densities.

2. Materials and methods

2.1. Seed sterilisation

Caryopses of *Z. mays* KX0141 (KWS, Germany) were surfaced sterilised by soaking in a 10-ppm solution of oxytetracycline for 20 h at room temperature and then in 0.1% (w/v) silver nitrate solution for 10 min. Once drained, the seeds were rinsed in a 0.5 % sodium chloride solution to precipitate the remaining silver ions and finally, washed with vigorous shaking in three changes of sterile distilled water (SDW) [24]. The seeds were germinated between sheets of moist, sterile filter paper (Whatman No.1) in 14 cm diameter Petri dishes, sealed with laboratory film and incubated at 25 °C at an angle of 40° to the vertical.

2.2. Root treatment

After approximately 44 h incubation, when the roots were between 2.5–3 cm long, the root caps were removed from 20 seedlings under a stereomicroscope in a flow cabinet, using a new sterile scalpel blade to lever off the cap at the junction between the translucent cap and root. The seedlings were returned to the Petri dishes, sealed, replaced in the incubator at 25 °C at an angle of 40° to the vertical for 4 h, to allow time for the wound to heal. Seedlings with their cap removed are denoted as decapped and roots without their caps removed as intact.

2.3. Root border cell counts

A further 15 roots were decapped and returned to the Petri dishes along with 15 intact roots, the plates were sealed and replaced in the incubator at 25 °C at an angle of 40° to the vertical. Border cell counts were performed in triplicate on 5 intact and 5 decapped roots after 4, 24 and 48 h. Each seedling was placed over a micro-centrifuge tube containing 500 μl of SDW so that the root tip and approximately 10 mm of root penetrated the water. The tips were left to hydrate for 20 min, with agitation of the water using a micropipette after 10 min. Fifty microlitres of cell suspension was pipetted into a counting dish, stained with 3 μl of Toluidine blue (0.1% in 0.1 M phosphate buffer) and mixed with 500 μl of SDW. The cells were allowed to settle for 5 min before counting with a dissecting microscope, as previously described [25].

2.4. Plant growth medium

PVC tubing (220 mm $h \times 21$ mm d), which had been sawn in half longitudinally, was sterilised by soaking in 1% bleach overnight. The tubes were rinsed thoroughly in SDW and left to dry in a laminar airflow cabinet. Once dry, the original halves were sealed back together using insulating tape with plastic lids taped to one end to provide a base. Ten tubes were packed with sterile (autoclaved twice, 1 day apart at 121 °C for 20 min at 1 atm.) sandy loam soil (sand 56%, silt 36%, clay 8%) sieved to <2 mm at a dry bulk density of 1 mg m^{-3} with a gravimetric water content of 22%. A further 10 tubes were packed at a dry bulk density of