The role of bacterial motility in the survival and spread of *Pseudomonas fluorescens* in soil and in the attachment and colonisation of wheat roots

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Abstract

Motile and non-motile strains of *Pseudomonas fluorescens* SBW25 were constructed using different combinations of the *lacZY*, *xylE* and *aph* marker genes which allowed their detection and differentiation in soil, root and seed samples. The survival of motile and non-motile strains was investigated in both non-competitive and competitive assays in water and non-sterile soil. Although there was no difference between strains in water, the motile strain survived in significantly greater numbers than the non-motile strain after 21 days in soil. There was no significant difference between competitive assays, where motile and non-motile cells were co-inoculated into soil, and non-competitive assays where strains were inoculated separately. Bacterial survival decreased as matric potential increased from $-224$ to $-17$ kPa but matric potential had no significant effect on motile compared to non-motile strains. Vertical spread of both motile and non-motile strains was detected 6.4 mm from the inoculum zone after 14 days in the absence of percolating water. There was no significant difference, for either strain, in distance moved from the inoculum zone after 14, 26 or 40 days. The motile strain had a significant advantage in attachment to sterile wheat roots in both non-competitive and competitive studies. When the spatial colonisation of wheat root systems was assessed in non-sterile soil, there was no significant difference between the motile and non-motile strain from either seed or soil inoculum. However, when the whole root system was assessed as one sample unit, differences could be detected. Bacterial motility could contribute to survival in soil and the initial phase of colonisation, where attachment and movement onto the root surface are important.

Keywords: Bacterial motility; Survival; Spread; Attachment; Colonisation; *Pseudomonas*

1. Introduction

Commercial exploitation of *Pseudomonas* species as biocontrol agents of soil-borne plant pathogens, or as promoters of plant growth, has been restricted by inconsistent results and poor root colonisation [1]. One of the main problems appears to be the inability of the introduced organisms to move from the site of inoculation (usually the seed) and establish effective populations in the rhizosphere and on plant roots [2]. Root colonisation is a complex process and knowledge of general ecological characteristics, such as motility, in rhizosphere microorganisms is needed to optimise the development of effective biocontrol agents, and facilitate the risk assessment process prior to the release of genetically modified strains. In addition, pseudomonads are considered ubiquitous, play a key role in nutrient cycling and are a major component of the soil microbial community [3]. Further information is needed to understand the factors affecting the survival, spread and root-colonising ability of these species in the environment.

*Pseudomonas fluorescens* SBW25 used in our studies has been extensively studied under contained conditions and during a field release experiment [4–6]. A non-motile strain was constructed in this study and used to compare the survival and spread of motile and non-motile *P. fluorescens* co-inoculated into soil. Spread of bacteria through soil has implications for the persistence of genetically modified organisms [7,8] and contamination of groundwater supplies by microorganisms present in sewage from soil-based waste disposal systems [9]. Understanding bacterial movement in soil is also important for manipu-
lating colonisation of plant roots [10,11] and for optimising the dispersal of inoculants used in biocontrol and bioremediation strategies [12,13]. Previous studies have investigated the numerous factors affecting movement of bacteria in soil [7,10,14–16] but few have attempted to differentiate between passive spread of bacteria and active motility involving flagella [11,17,18]. Many workers have established that soil water flow is a major factor in the dispersal of bacteria in soil [8,10,19] and that plant roots can enhance water-facilitated transport [7,20]. Most of these studies have investigated the spread of bacteria in relatively large soil sections, whereas active motility using flagella may be more important for localised bacterial movement [12,15,17].

Attachment kinetics are important in the colonisation process, since successful attachment of rapidly colonising species would leave fewer attachment sites available to competitors [21]. Previous workers have studied the role species would leave fewer attachment sites available to competitors [21]. Previous workers have studied the role of chemotaxis and motility in plant colonisation but conflicting reports have been produced. De Weger et al. [22] demonstrated that flagella of P. fluorescens WCS374 were required for colonisation of potato roots and Catlow et al. [11] showed that active motility, not just possession of flagella, influenced the distribution of Rhizobium trifolii TA 1 on clover roots. Chemotaxis assays in non-sterile soil also demonstrated that Pseudomonas putida RW3 could migrate 1 cm towards soybean seeds in 12 h whereas a non-motile strain, P. putida RW3−, was not detected after 48 h [23]. However, other studies of motile and non-motile strains of P. fluorescens and P. putida found no effect of motility on the colonisation of roots, and have led to the conclusion that other factors are more important [12,18,23,24].

This paper presents results from experiments designed to investigate small-scale differences between motile and non-motile strains of P. fluorescens SBW25 in terms of survival and spread in soil and in attachment and colonisation of wheat roots.

2. Materials and methods

2.1. Bacterial strains

The motile strain, P. fluorescens SBW25 EeZY-6KX, was constructed by Bailey et al. [6], and contained the lacZY, xylE and aph gene cassettes on the chromosome. A strain made during the construction of P. fluorescens SBW25 EeZY-6KX, with only the lacZY gene cassette, was subjected to Tn5 transposon mutagenesis to create the non-motile strain used in this study. A tri-parental mating method was used to introduce Tn5 into P. fluorescens SBW25 EeZY using the plasmids W3110 [25] and pNJ5000 [26]. After 18 h, cells were streaked over the surface of succinate minimal medium [27] containing 50 μg ml⁻¹ kanamycin for selection of Tn5 mutants.

Attachment kinetics are important in the colonisation process, since successful attachment of rapidly colonising species would leave fewer attachment sites available to competitors [21]. Previous workers have studied the role of chemotaxis and motility in plant colonisation but conflicting reports have been produced. De Weger et al. [22] demonstrated that flagella of P. fluorescens WCS374 were required for colonisation of potato roots and Catlow et al. [11] showed that active motility, not just possession of flagella, influenced the distribution of Rhizobium trifolii TA 1 on clover roots. Chemotaxis assays in non-sterile soil also demonstrated that Pseudomonas putida RW3 could migrate 1 cm towards soybean seeds in 12 h whereas a non-motile strain, P. putida RW3−, was not detected after 48 h [23]. However, other studies of motile and non-motile strains of P. fluorescens and P. putida found no effect of motility on the colonisation of roots, and have led to the conclusion that other factors are more important [12,18,23,24].

This paper presents results from experiments designed to investigate small-scale differences between motile and non-motile strains of P. fluorescens SBW25 in terms of survival and spread in soil and in attachment and colonisation of wheat roots.

2.2. Detection and differentiation of motile and non-motile P. fluorescens SBW25

Motile and non-motile colonies were isolated on NA containing 50 μg ml⁻¹ kanamycin and 25 μg ml⁻¹ X-gal (NAKmX) at 30°C for 24–48 h. Both motile and non-motile strains produced blue colonies. Plates were sprayed with 1% (w/v) catechol and the motile strain (containing the xylE gene) produced a green–blue appearance to the colony surrounded by a very distinct yellow halo. Between 30 and 150 motile (yellow) and non-motile (blue) colonies were counted on each agar plate.

2.3. Inoculum preparation

Pseudomonas strains were inoculated onto the surface of NA containing 50 μg ml⁻¹ kanamycin (NAKm) and incubated at 30°C for 18 h. Cells were resuspended in sterile H₂O for seed and soil inoculum, or 0.01 M phosphate buffer containing 2.25 g l⁻¹ NaCl, pH 7.0 for attachment assays. The optical density of the suspension was adjusted to an OD₆₀₀ nm value of 0.23 (equivalent to 2×10⁸ cfu ml⁻¹) and this information was used to adjust samples to the final cell density required. For competition studies, motile and non-motile cell suspensions were mixed to give a 50:50 ratio (apart from initial survival experiments where 60:40 and 40:60 ratios were also set up) and diluted to give the same cell density as the individual inocula for the non-competitive experiment. Prior to inoculation, cell suspensions were observed by light microscopy at 400× magnification to assess motility of the strains.

2.4. Soil preparation and inoculation

The soil used in this project was a sandy loam (Wick series, wQ2) collected from an uncultivated grassland site (Grid Reference SP272576) at Horticulture Research International, Wellesbourne, Warwickshire, UK. The soil had a pH of 6.8, an organic carbon content of 0.9% (w/w) and available water content of 13.3% (w/w). The particle size distribution (w/w) was 67.1% sand (60 μm–2 mm), 16.6% silt (2–60 μm) and 16.3% clay (<2 μm). Samples were taken from the Ap horizon (0–25 cm depth), air-dried to 10% (w/w) gravimetric water content when necessary, sieved through a 2-mm mesh and stored at 10°C for a maximum of 48 h before use. Cell suspensions were diluted in the required volume of sterile H₂O needed to attain a matric potential of −33 kPa (or for the range of matric potentials stated in Section 3.1.3). The cell sus-
pension was added slowly to soil in a polyethylene bag and mixed thoroughly for 10 min until fully incorporated. The soil was inoculated with \(2 \times 10^7\) to \(2 \times 10^8\) cfu g\(^{-1}\) dry wt soil as stated in the relevant results section. Uninoculated soil was prepared using sterile H\(_2\)O instead of the cell suspension. Soil was packed to a bulk density of 1.25 g cm\(^{-3}\).

### 2.5. Survival experiments

Survival in water was assessed in 100 ml sterile H\(_2\)O in triplicate 250-ml Erlenmeyer flasks sealed with cotton-wool bungs and aluminium foil. The flasks were inoculated with \(7.9 \times 10^6\) cfu ml\(^{-1}\) in ratios of 60:40, 50:50 and 40:60 motile:non-motile strain and incubated at 18°C for 100 days. Flasks were swirled briefly before 1-ml samples were removed for dilution plate counts at appropriate intervals during the 100-day study. Survival of strains in soil was assessed by packing inoculated soil (ca. 8.0 \(\times\) 10\(^7\) cfu g\(^{-1}\)) into 100-ml Duran bottles to give a bulk density of 1.25 g cm\(^{-3}\). Controls consisted of uninoculated soil and all experiments were set up in triplicate. The bottles were covered loosely with aluminium foil and incubated at 18°C with 80% relative humidity (RH) for 100 days. Samples of soil (1.2 g (wet wt) soil (equivalent to ca. 1 g dry wt) were placed in 9 ml sterile 1/4 strength Ringer’s solution and vortexed for 20 s.

### 2.6. Spread experiments

The spread of cells was assessed using triplicate soil columns held in place using 25 polyethylene washers (28 mm internal diameter, 1.6 mm height, John Dewhurst Ltd, UK) secured together with black insulating tape. Soil at -33 kPa was packed in these columns to a bulk density of 1.25 g cm\(^{-3}\). A 5-cm pipe (polypropylene wastepipe, 32 mm internal diameter, Marley Extrusions, UK) was attached to the top of each column with tape and packed with uninoculated soil to a bulk density of 1.25 g cm\(^{-3}\). Control experiments used pipes packed with uninoculated soil. Model systems were covered loosely with aluminium foil and incubated at 18°C and 80% RH for up to 70 days. These soil systems were destructively sampled in triplicate by carefully removing the insulating tape and using a stainless steel knife to separate the pipe containing the inoculum from the column of washers. Each washer was sequentially removed using the knife and the soil section placed in 4.5 ml sterile 1/4 strength Ringer’s solution.

### 2.7. Root attachment assays

Wheat seeds (Triticum aestivum L. cv. Slepjner) were sterilised with chlortetracycline (10 \(\mu\)g ml\(^{-1}\)) and silver nitrate (0.1% w/v) using the method of Speakman and Krüger [29], grown in plant culture solution [30] and assays performed as described previously [31]. Briefly, 5-cm root pieces were placed in bacterial suspension (\(10^7\) cfu ml\(^{-1}\)), removed at 15-min intervals and washed five times in 0.01 M phosphate buffer containing 2.25 g l\(^{-1}\) NaCl, pH 7.0. Root pieces were macerated in a pestle and mortar and attached bacteria enumerated on NA\(K\)nX.

### 2.8. Colonisation experiments

#### 2.8.1. Seed inoculum

Wheat seeds (cv. Slepjner) were sterilised [29] and pre-germinated on tap water agar (15 g l\(^{-1}\) Technical Agar No. 1 in tap water) at 18°C for 48 h in the dark. To check sterility, 50 seeds were placed on two nutrient agar plates and incubated at 25 and 30°C for 48 h. In addition, five seeds were placed in 1 ml 1/4 strength Ringer’s solution, macerated in a pestle and mortar and five 100-µl samples spread onto nutrient agar. The plates were incubated at 25 and 30°C and assessed for contaminants after 48 h. Pre-germinated wheat seeds were submerged in sterile 1% (w/v) carboxymethylcellulose containing \(3.8 \times 10^{10}\) cfu ml\(^{-1}\) of motile and non-motile strains in equal proportions for 60 min. This resulted in a total inoculum of \(3 \times 10^8\) and \(3 \times 10^8\) cfu seed\(^{-1}\) for the spatial and total colonisation studies, respectively. Control seeds were prepared in the same way using sterile 1% (w/v) carboxymethylcellulose containing sterile H\(_2\)O instead of cell suspension.

#### 2.8.2. Soil inoculum

Soil was co-inoculated (see Section 2.4) with \(1.6 \times 10^8\) cfu g\(^{-1}\) dry wt soil of the motile strain and \(1.5 \times 10^8\) cfu g\(^{-1}\) dry wt soil of the non-motile strain to assess spatial colonisation, and with \(2.0 \times 10^7\) cfu g\(^{-1}\) dry wt soil of the motile strain and \(1.8 \times 10^7\) cfu g\(^{-1}\) dry wt soil of the non-motile strain to assess colonisation of the whole root system.

#### 2.8.3. Colonisation assay

Pre-germinated wheat seeds were planted in soil to a depth of 1 cm in plastic pots (7 cm i.d.) at a density of two seeds per pot. (Inoculated seeds were planted in uninoculated soil for seed colonisation experiments and uninoculated seeds were planted in inoculated soil for soil colonisation experiments.) Each experiment was set up in triplicate. Pots were placed on damp capillary matting, sealed in a polyethylene bag to reduce water loss, and incubated at 18°C with 80% RH and a 16-h photoperiod (32 W m\(^{-2}\)) for 5–9 days in a growth cabinet (Fisons Scientific Equipment, UK).

#### 2.8.4. Sampling procedure

Plants were sampled by gently removing the plastic pot and carefully breaking away the bulk soil. Samples of bulk soil (ca. 1.2 g wet wt) were placed in 9 ml sterile 1/4 strength Ringer’s solution and shaken using a wrist action shaker (speed setting 2, Stuart Scientific, UK) for 10 min. Plants were held just above the seed and shaken gently to
remove all but the tightly adhering soil (rhizosphere soil) from the roots. The seed was removed and macerated in 1 ml sterile 1/4 strength Ringer’s solution using a sterile pestle and mortar. For spatial colonisation studies, the roots were carefully placed on a sheet of polyethylene next to a ruler and cut into sections with a scalpel blade. Root sections, or whole root systems, were placed in 9 ml sterile 1/4 strength Ringer’s solution and shaken using a wrist action shaker (Stuart Scientific, speed setting 2) for 10 min. The roots were removed and the rhizosphere soil solution serially diluted separately. Roots were washed briefly in sterile H₂O to remove any rhizosphere soil solution and macerated in 1 ml sterile 1/4 strength Ringer’s solution.

2.9. Dry weight measurements

Soil samples (ca. 6 g wet wt) were placed in pre-weighed glass vials, weighed, and dried at 80°C for 48 h. In addition, soil or macerated root samples suspended in 1/4 strength Ringer’s solution were measured by pipetting 5 or 3 ml, respectively, of the primary dilution into a pre-weighed universal bottle. The sample was dried at 80°C for 18 h and the dry weight calculated.

2.10. Statistical analysis

Data was analysed using analysis of variance (ANOVA) [32], and a logit transformation used to assess the behaviour of the motile compared to the non-motile strain [33]. The logit (l) is defined as \( l = \log \frac{p}{1-p} \) where \( p \) is the proportion of a value in relation to another value in a mixture. Logit analysis provides a sensitive method for examining how the relative proportions of two components change. In experiments assessing spatial colonisation from seed inoculum, where substantial inter-replicate variation resulted in a large residual error, a sign test [34] was used instead of ANOVA.

3. Results

3.1. Survival experiments

3.1.1. Survival of motile and non-motile strains of \( P. \) fluorescens SBW25 in sterile water

In sterile water bacterial numbers remained very stable and overall only declined to \( 1.24 \times 10^6 \) cfu ml\(^{-1} \) for the motile strain and \( 1.15 \times 10^6 \) cfu ml\(^{-1} \) for the non-motile strain after 100 days (50:50 ratio). When inoculated in different proportions, no significant decline in numbers was detected after 100 days. Analysis of the proportion of motile to non-motile cells at each sample point compared with the proportion initially released showed that, overall, there was no significant difference in their survival in water. This indicated that the differential marking system had not resulted in survival differences between motile and non-motile strains.

3.1.2. Survival of motile and non-motile strains of \( P. \) fluorescens SBW25 in non-sterile soil

Survival of cells in soil can be seen in Fig. 1A. After 59 days in soil, motile and non-motile strains had declined to \( 4.8 \times 10^4 \) cfu g\(^{-1} \) dry wt soil and \( 2.0 \times 10^4 \) cfu g\(^{-1} \) dry wt soil, respectively, from a 50:50 inoculum, and had declined below the limits of detection (\( 1 \times 10^2 \) cfu g\(^{-1} \) dry wt soil) at subsequent sampling times. Similar rates of decline were observed when motile and non-motile cells were co-inoculated into soil in different proportions. The proportion of motile to non-motile cells progressively increased during the experiment and motile cells survived significantly better than non-motile cells after 21 days (Fig. 1B).

3.1.3. Effect of matric potential on survival of motile and non-motile strains of \( P. \) fluorescens SBW25 in non-sterile soil

When motile and non-motile strains were co-inoculated into soil at matric potentials of −224, −38, −25, −19 and −17 kPa, bacterial survival decreased as matric potential increased (Fig. 1C,D). After 42 days, the motile strain survived better than the non-motile strain at matric potentials of −38, −25, −19 and −17 kPa, although no significant difference of matric potential on the survival of motile or non-motile strains was observed when all time points were analysed.

3.2. Non-competitive and competitive vertical spread of motile and non-motile strains of \( P. \) fluorescens SBW25 through soil

The vertical spread of bacteria down the soil system over 40 days can be seen in Fig. 2. On day 70 vertical spread of these strains could only be detected in the sample 1.6 mm below the zone of inoculation, in numbers just above the detection limit (\( 1 \times 10^2 \) cfu g\(^{-1} \) dry wt soil). Therefore, data from day 70 have been excluded from statistical analysis and graphical representation. Overall, there was no significant difference between motile and non-motile strains in the distance moved from the inoculation zone after 14, 26 or 40 days.

In non-competitive assays, vertical spread of the motile strain was detected at 6.4, 3.2 and 9.6 mm on days 14, 26 and 40. The non-motile strain was detected at 4.8, 3.2 and 6.4 mm on days 14, 26 and 40 in similar studies. When the strains were co-inoculated into soil in a competitive assay, both motile and non-motile strains were detected at 6.4 mm on day 14 and day 26, and at 9.6 mm on day 40. Overall, there was no significant difference in the distance moved from the inoculation zone, of either motile or non-motile strains, when non-competitive and competitive assays were compared.

The number of motile and non-motile cfu detected at
Fig. 1. Survival of co-inoculated motile and non-motile *P. fluorescens* SBW25 at different ratios (A,B) and at different matric potentials (C,D,E) in non-sterile soil. Error bars represent the least significant difference (l.s.d. at *P* < 0.05). (A) CFU motile (•) and CFU non-motile (□) inoculated in equal numbers; and (B) logits of motile strain when introduced as 60% (white columns), 50% (striped columns) and 40% (hatched columns) of the initial inoculum (l.s.d. = 0.28). (C) Motile CFU; (D) non-motile CFU and (E) logits of motile strain when co-inoculated into soil at different matric potentials (l.s.d. = 9.78).

Fig. 2. Vertical spread of motile and non-motile *P. fluorescens* SBW25 through non-sterile soil after 14 days (A), 26 days (B) and 40 days (C) in non-competitive and competitive assays. Error bar represents the l.s.d. = 0.873 (*P* < 0.05).
each distance was compared with the number of cfu detected in the inoculum zone at each sample point to overcome any differences in the survival ability of the motile and non-motile strains. In general, there was no significant difference in numbers of cfu of motile and non-motile strains, at any distance from the inoculum zone, in non-competitive or competitive assays. However, the non-motile strain was detected in significantly greater numbers at 1.6 mm on days 14 and 26 in the non-competitive assay compared with the competitive assay. These results were not due to a large variation in matric potential, or a difference in the amount of soil sampled, as dry weight measurements were similar in all samples. Also, similar numbers of the non-motile strain were detected in each replicate in the 1.6 mm samples on days 14 and 26. Therefore, it is unlikely that these results were due to inter-replicate variation.

Fig. 3. Non-competitive (A) and competitive (B) attachment of motile (○) and non-motile (■) *P. fluorescens* SBW25 to sterile wheat roots. Error bar represents the l.s.d. = 0.373 (P < 0.05).

Fig. 4. Spatial colonisation of wheat root sections by *P. fluorescens* SBW25 after 9 days from a seed inoculum. Motile and non-motile strains were co-inoculated onto seeds and monitored in rhizosphere soil (A) and on roots (B). Values in each key represent the range from 1.0 to 9.9 × 10^6 cfu g^-1 dry wt soil, or cfu cm^-1 root; nd, not detected. * indicates a significant difference between motile and non-motile strain.

Fig. 5. Colonisation of complete wheat root system by *P. fluorescens* SBW25 from a seed inoculum. (A) Motile cfu (dotted columns) and non-motile cfu (striped columns) and (B) logit of motile strain. Error bar represents the l.s.d. = 1.32 (P < 0.05). Values for inoculum (inoc), upper rhizosphere soil (u.r.s.) and lower rhizosphere soil (l.r.s) are in cfu log_{10} g dry wt soil^-1, upper roots (u.root) and lower roots (l.root) are in cfu log_{10} cm root^-1 and seed is in cfu log_{10} seed^-1.
3.3. Non-competitive and competitive attachment of motile and non-motile P. fluorescens SBW25 to sterile wheat roots

The effect of motility on the attachment of P. fluorescens SBW25 to sterile wheat roots was compared in non-competitive (Fig. 3A) and competitive (Fig. 3B) attachment assays. The motile strain attached in significantly greater numbers than the non-motile strain in both assays. Over the first 60 min the motile strain showed a significant increase in attachment, which was also true for competitive assays. Thereafter, the number of motile cells attached to the roots remained relatively constant. In contrast, as well as not attaining such a high population on the roots, the numbers of non-motile cells remained constant with time in both non-competitive and competitive assays. Since time had an effect on the attachment of motile cells a comparison of the average number of cells attached to roots in non-competitive and competitive assays was not possible. However, the number of motile cells attached to the roots at the end of the experiment was not significantly different in both assays. In contrast, the average number of non-motile cells attached to roots was significantly greater in the non-competitive assay (1.3 × 10^4 cfu per root) compared with the competitive assay (5.3 × 10^3 cfu per root).

3.4. Colonisation experiments

3.4.1. Spatial colonisation of wheat roots by motile and non-motile strains of P. fluorescens SBW25 co-inoculated onto seeds

After inoculation and a 9-day growth period at 18°C, colonisation of an entire seminal root and associated rhizosphere soil was assessed in 10 sections of variable length. On the roots, the furthest distance from the seed where the inoculated strain was detected was 12–15 mm for the motile strain, and 9–12 mm for the non-motile strain (Fig. 4). The numbers of cfu on most root sections were close to the limits of detection (1 × 10^2 cfu). Both motile and non-motile cfu were detected in all rhizosphere soil samples and 1.2 × 10^4 cfu of each strain were present in the 140-mm root tip section. ANOVA could not be performed due to considerable inter-replicate variation and data were subjected to a sign test instead. This test indicated that there was no significant difference between motile and non-motile strains in rhizosphere soil or on roots from a seed inoculation.

3.4.2. Colonisation of whole root system by motile and non-motile strains of P. fluorescens SBW25 co-inoculated onto seeds

After inoculation and a 6-day growth period at 18°C,
Colonisation of the entire root system and associated rhizosphere soil was assessed (Fig. 5A). The sample was divided into two sections, representing the first 5 cm from the base of the seed (upper), and 5 cm below the seed to the root tip (lower). Significantly greater numbers of both strains were detected in the upper rhizosphere soil compared with the lower rhizosphere soil. There was no significant difference between numbers of motile and non-motile cells on the seed, in the bulk soil, rhizosphere soil, or on the roots (Fig. 5A). However, compared to the initial inoculum, a significantly greater proportion of the motile strain compared with the non-motile strain was detected on the seed, upper roots and in the lower rhizosphere soil using logit analysis (Fig. 5B).

3.4.3. Spatial colonisation of wheat roots by motile and non-motile strains of *P. fluorescens* co-inoculated into soil

Non-sterile field soil was co-inoculated with motile and non-motile strains and planted with wheat seeds. After 9 days both strains were present in significantly greater numbers in the rhizosphere soil compared to the bulk soil (Fig. 6A,B). There was no significant effect of position in the rhizosphere soil, but significantly fewer motile and non-motile cfu were detected at the root tip compared with the other root sections. There was no significant difference in survival between motile and non-motile strains in the bulk soil. Logit analysis of the proportion of each strain detected in each section showed that the motile strain was only present in significantly greater numbers than the non-motile strain on root sections at 4–5 cm (Fig. 6C).

3.4.4. Colonisation of whole root system by motile and non-motile strains of *P. fluorescens* SBW25 co-inoculated into soil

Non-sterile field soil was co-inoculated with the motile and the non-motile strain and planted with wheat seeds. The motile strain was detected in significantly greater numbers in all the environments (Fig. 6D). However, analysis of the proportion of each strain showed that there was no significant difference between the ratio of motile and non-motile strains present in the bulk soil, rhizosphere soil, or on the roots and only showed a significant increase in numbers on the seed (Fig. 6E).

4. Discussion

Factors affecting bacterial survival in soils are known to be both abiotic, such as soil type and matric potential which influence pore space and water availability, and biotic, such as predation by protozoa and competition with indigenous microorganisms [35–37]. When inoculated into non-sterile soil, populations of *P. fluorescens* SBW25 declined steadily, and differences in survival were seen between motile and non-motile strains. Survival data reflected results from similar studies by de Leij et al. [4] and rates of decline were comparable with other strains of *P. fluorescens* [38–40]. Co-inoculation studies demonstrated that the motile strain survived significantly better than non-motile strain after 21 days. Significant effects observed in simple non-sterile systems indicate that motility can be important for the survival of bacteria inoculated into soil and suggests that motility may facilitate bacterial response to the numerous fluctuations experienced in the soil environment over much longer time intervals. If bacterial inoculants were introduced under conditions that permitted active cell motility, then movement into protective microniches may enhance the survival prospects of the introduced population.

Matric potential is thought to affect bacterial activity mainly by restricting bacterial movement, therefore preventing relocation to new nutrient sources, or by limiting metabolism of established colonies within microsites due to nutrient deficiencies caused by retarding substrate diffusion. Wong and Griffin [15] reported that bacterial movement becomes negligible between −20 and −100 kPa and that bacterial activity decreases sharply as matric potential falls between −50 and −300 kPa. However, no significant difference in bacterial survival between matric potentials of −17 and −224 kPa in the soil type used in these studies was observed when motile and non-motile strains of *P. fluorescens* SBW25 were compared, although survival of both strains decreased as matric potential increased. Meikle et al. [41] also found that increased matric stress appeared to reduce the survival of *P. fluorescens*, although differences were not statistically significant, which may have been due to variability between replicates.

There was no significant difference in the vertical spread of motile and non-motile cells when inoculated as single strains into the soil systems, which suggested that active motility had not influenced movement of bacteria in these assays. Results from competitive assays also demonstrated that there was no significant difference between strains, and that non-motile cells were not co-transported by motile cells further than they would move when applied alone. One of the main problems encountered in the experiments assessing the spread of bacteria through soil was the large amount of inter-replicate variation. This has been reported by other workers [7,8,17,19,42] and seems to be an inherent factor when studying heterogeneous particulate systems such as soil.

Previous work [31] showed that motile strains of *P. putida* PaW8 had an advantage in non-competitive and competitive attachment assays. Fewer cells of the equivalent non-motile strain attached in competitive compared to non-competitive assays indicating that both motile and non-motile cells were competing for the same attachment sites [31]. This suggested that motility could be very important under specific conditions, such as initial attachment to roots during the first stage of colonisation.
The motile strain of *P. fluorescens* SBW25 also showed a significant advantage in both competitive and non-competitive attachment assays to sterile wheat roots. Active motility could enhance colonisation ability by enabling cells to reach attachment sites more quickly, or increase the probability of finding potential binding sites, rather than relying on chance.

In order to investigate the effect of motility over relatively small distances, spatial colonisation of roots using 3-mm sections were sampled following seed inoculation. Although greater numbers of the motile and non-motile strains were detected in upper rather than lower sections, roots had not outgrown the seed inoculum as both strains were detected in rhizosphere soil down the entire root length. The absence of percolating water in these studies implies that motile and non-motile cells were transported by elongating roots, and detection of strains in rhizosphere soil, but not on corresponding root sections, suggests movement from the root surface into the rhizosphere soil. Use of root sections should increase the precision of detecting differences in colonisation ability between motile and non-motile strains, but this approach is labour-intensive and subject to greater variability among replications than use of whole roots as a sample unit [43].

When root systems were assessed in larger sample units (upper and lower roots) greater numbers of the motile strain were detected on roots and in rhizosphere soil. Logit-transformed data indicated that the motile strain was present in significantly greater numbers on the seed, upper roots and in the lower rhizosphere soil. This suggested that motility could provide an advantage in moving from a seed inoculum to colonise the root system and enable movement from the roots into the rhizosphere soil.

Colonisation of wheat roots was also assessed using soil inoculum and greater numbers of bacteria were detected on roots compared to colonisation assays using seed inoculum. There was no significant difference between the bulk soil, rhizosphere soil and roots on the sample day which suggested that the motile cells had a survival advantage, rather than a greater ability to move into rhizosphere soil and onto roots. Only the seed sample showed a significant increase in the motile strain which indicated that motility may enable *P. fluorescens* SBW25 to move towards nutrients, e.g. from germinating seeds or wounded roots, rather than conferring a significant advantage in root colonisation.

Whether bacteria have the ability to swim actively in soil has been questioned by soil microbiologists for almost a century [44,45]. Soil microorganisms that could detect, and move towards, a nutrient source would have an evolutionary advantage since nutrient supply is often limited in soil, and motile plant pathogens, nodulating bacteria and rhizosphere-associated microorganisms could locate their host organisms by directed behaviour rather than chance [28]. Most researchers have discounted the possibility that active motility contributes to the movement of bacteria in soil [10,12,18] whereas Soby and Bergman [17] provided one of the few reports that a bacterial motility-chemotaxis system could function in soil. Bacterial movement over relatively small distances by active motility could result in long distance dispersal of cells in soil. For example, movement out of a soil pore could result in the transport of a bacterium over considerable distances by percolating water. In addition, movement from a soil aggregate onto a root surface, and subsequent attachment to a suitable binding site, could be responsible for relocation of bacteria during root extension through soil. Overall, soil water flow or the movement of members of the soil fauna are still likely to be the main determinants of bacterial distribution in the soil matrix. However, active motility may contribute to the localised spread of bacteria within microenvironments, such as soil pores, or onto the root surface provided that there is a suitable water film present.

To our knowledge, there are only two reports to suggest that active motility plays a role in root colonisation [11,22]. De Weger et al. [22] investigated colonisation of potato roots by *P. fluorescens* JM 3741, and four non-motile mutants of this strain, inoculated as single strains on potato stem cuttings. After 8 days, the motile strain was found in significantly greater numbers than any of the non-motile strains 8 cm away from the inoculum which indicated that active motility or flagella had played a major role in the colonisation process. However, many workers have disputed that active motility is important in soil, usually on the basis that water films become too thin for flagella-mediated movement when soil is drier than −50 kPa [46]. There are two reports which indicate that bacteria are carried passively on elongating roots [18,47] whereas the majority of workers have concluded that downward percolation of water is more important for bacterial dispersal through the rhizosphere [48–50].

This paper demonstrated that motility provided a clear advantage to *P. fluorescens* cells in survival in soil and in attachment to wheat roots. Our experimental approach enabled the effect of bacterial motility to be assessed on the same root or soil sample and wheat plants were grown without percolating water in order to assess the role of bacterial motility, instead of passive movement, in the colonisation process. The results from colonisation experiments were less clear, although in some assays small differences between motile and non-motile strains were detected. However, even a small advantage observed in the motile strain may become more pronounced on a larger scale or when a longer timescale is considered.

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References


introduced into two soils of different texture in field microplots. FEMS Microbiol. Ecol. 38, 151–160.


