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The Response of Rhizobacteria Pseudomonas Fluorescens to Applied Electrical Fields

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Summary: *Rhizobacteria show electrotactic swimming in the rhizosphere as stimulated by plant roots. The electrotactic response of four strains of rhizobacteria ,Pseudomonas fluorescens (P.fluorescens)-P.fluorescens-290E,P.fluorescens-M,P.fluorescens- 292 and P.fluorescens-WCS 374 was observed in vitro conditions to different electrical fields in Phosphate Buffer Solution (PBS),root Exudate (RE) and Water (W).Electrical fields occurs in vitro condition is comparable in magnitude to those generated by plant root in the rhizosphere. The electrotactic response was greatest in phosphate buffer solution followed by root exudate and water. In the electrical fields tactic response of P.fluroscens-290E and P.fluroscens-WCS-374 was towards anode. However P.fluroscens-292 and P.fluroscens-M was towards cathode .Electrical field of physiological magnitude had little effect on the velocity of swimming but increased the turning frequency of P. fluroscens over two fold. The swimming behaviour of these rhizobacteria was greater at low electrical field strength as compared to high electrical field and control conditions. The results suggest that swimming behaviour of these rhizobacteria is related to endogenous electrical gradients generated by growing roots or other plant tissues .*

Key Words: *Electrotaxis, Electrical field, Rhizobacteria, Pseudomonas*

I. Introduction

It is well established that pseudomonas and other rhizobacteria may increase plant growth (Brown et. al, 1964) and have been demonstrated as Plant Growth Promoting Rhizobacteria (PGPR) (Kloepper et . al.,1980;Kumari and Srivastava, 1999 ; Gulati et. al. 2012), and Yield Increasing Bacteria (YIB) (Glick, 1995; Chaudhary et. al. 2008). Establishment of plant growth promoting rhizobacteria and disease suppressing bacteria on seeds and root systems is well recognised as critical step towards their effectiveness ((Hegan and Kolter, 2002).

Motility gives the organism opportunity for migration and it is therefore likely that chemotactic attraction plays an important role in the search for an appropriate ecological niche in nature(Arora and Gupta 1993;Gorvel,2000, Richard 2003). Rhizobacteria are attracted to many kinds of chemicals, such as carbon as well as N,P and S compounds (Cramer and Richard,1999,Tromans,2002). Roots are known to produce exudates containing a wide variety of substances resulting in positive chemotaxis (Currier and Strobel ,1976). Chemotaxis and motility play an important role in the symbiotic interaction of rhizobia with their hosts as contact and adherence to the host roots (Cactano-Anolles et. al.1988),formation of highly localized bacterial clouds on the infectible surface of the root, efficient nodule initiation, rapid infection development (Cactano – Anolles et.al, 1988) and competition for nodule occupancy (Gulash et. al.,1984).

Plant root generate electrical fields in the rhizosphere as a consequence of spatial heterogeneities in elctrogenic transport system in the root (Miller et. al, 1991) due to the current flow mainly by protons . The shape of the electrochemical profile of a root varies in different plant species (Gow et. al,1992;Miller and Gow,1989) and is influenced markedly by endogenous and exogenous factors ,including plant growth regulators ,soil acidity, salinity matric potential,source of nitrogen(Gow et al,1992;Miller et. al ,1991) and carbon (Cramer and Richards 1999). In most cases ,however, positive electrical current enters the meristematic tissue and zone of cell elongation and exists basipetally in the mature tissue (Miller and Gow, 1989).The magnitude of the electrical field resulting from these currents depends on the resistivity and salt content of the bathing medium.For a resistivity of 50Ωm typical of many soil,loams and clays,the resulting fields can be calculated between 0.05 to 5/vm with a nodal field of o.5 /vm and the elongation zone 2-5/ vm in the vicinity of wound sites (Miller and Gow ,1989 a,b; Gow et al, 1992). These sites are highly sensitive for colonization of this plant growth promoting rhizobacteria (Bowen and Rovira, 1976,Spaink,2002).It has been postulated that bacteria may utilize electrical gradients in addition to chemicals to determine the location of target sites for colonization or attachment (Scher et. al, 1988). Electrotaxis has been reported (Khew and Zentmyer 1974, Morris et. al, 1992 and Morris and Gow 1993) on fungal zoospores. However, there is no work on mechanism of electrotaxis in rhizobacteria P. fluorescens. In the present study a chamber has been used with agarose bridges separating the electrodes and bacteria to protect the cells from the products of electrolysis, and mechanism of electrotaxis has been observed in case of rhizobacteria,P.fluorescens for the first time.The importace of the topic in this field of research is to observe the response of rhizobacteria in in-vitro condition in electrical field.

II. Materials and Methods

Organism

Four strains of rhizobacteria,P.fluroscens WCS-374, P.fluroscens-2 92, P. fluroscens 290E and P. fluroscens M has been used for the experiment. The strains has been collected from the laboratory of Prof.D.K.Arora department of Botany B.H.U Varanasi. These strains have been maintained on King' s medium B at 25 ±5 ˚C temperature.

Culture for electrotaxis

The rhizobacteria were grown in 20 ml of liquid medium for 16 h(stationary phase;A 550 1.0- 1.2) at 25+-5*c in a temperature controlled rotary shaker (125 rpm).To obtain exponential growth phase of these bacteria one ml aliquot of 16- hrs old culture was transferred to 10 ml fresh liquid medium and incubated for approximately 1-3 hrs. Cells were harvested by centrifugation (1200 g for 10 min, 4*c) and the pellet was washed twice with cold (4*c) 50 m sodium phosphate buffer solution (pH 7.0). Cells were resuspended in 10 mm sodium phosphate buffer solution for overnight and cell density was adjusted to approximately 1×10^8/ ml (A550-0.06-0.12) by using a colorimeter. Before each experiment, motility of the bacterial cells were checked under Nikon optiphot' phase contrast microscope.

Root exudates

Root exudates were acquired from chick pea plant, grown in 1000 ml beaker containing 100 ml of nitrogen free medium and an aluminium screen covered with cheese close, was arranged so that the seeds were suspended above the liquid medium, and the chamber was covered with aluminium foil. After 2-3 days in the controlled environment with 1-1 hr light –dark cycle and 27˚C day ,16˚C night temperature cycle the liquid medium containing root exudates were filtered through Whatman no. 1 filter paper ,frozen and lyophilized .The dry root exudate powder was resuspended in distilled water and after microbial contamination test ,it was used for experiment.

Construction of chamber for electrotaxis experiment

Electrotaxis responses were measured with the modified method and apparatus of Morris et al 1992. A chamber was prepared from a glass microscope slide with a central channel formed between two sections of glass platinum wire electrodes were glued to both ends of the channel , molten agarose gel (1%w/v) was poured over the electrodes and both ends of the channel to form a central well,measuring 1 cm^2 x 0.1 cm deep. Both electrodes were connected with a system including battery (1.5 v), fixed resistance (1k) , a potentiostate (1k), with supplementation of a fixed electrical current which was fixed through a multimeter (fig 1). Rhizobacterial suspension($1x10^{-7}$cells/ml) was exposed to an electrical field for 60 min. At the end of an experiment the chamber was physically partitioned in to three section and the concentration of bacteria was measured by plating technique. The extent of eletrotaxis was determined by a Tactic Response Quotient(TRQ).

TRQ=(A-C)/(A+C+2M)

Where A=density of bacteria at the anode

C=density of bacteria at the cathode

M=density of bacteria at the centre

Measurement of swimming behaviour

Soil bacteria swam in counter clockwise rotation and tumbled in clockwise rotation. Turning frequency of bacteria in presence and absence of electrical fields was determined by recording their swimming pattern with time-laps video microscopy. Path of individual bacterium was traced by video monitor during frame by frame play back and the digitized with an image analysis system. A turn is defined as an abrupt change in direction greater than 15˚from a direct swimming path way. The number of turn per second was determined. Rhizobacterial suspension was prepared in 2mM sodium phosphate buffer solution (pH -7.0).

III. Results

Electrotaxis in rhizobacteria

The tactic response of the strains of pseudomonas fluorescens (Table1&2)tested were greater at 500 mv/cm than at 50 mv/cm(P - 0.01) .At a field strength of 100 to 500 mv/cm,greater than the electrical field normally generated by plants roots(50-100).P.fluorescens-290E & P.fluorescens WCS- 374 showed significant taxis(P-0.01) towards the anode. However P. fluorescens – 292 and P.fluorescens - M showed significant taxis towards the cathode. Significant was calculated by comparing density at the anode and cathode according to three way analysis of variance.The tactic response of all the strains of P.fluorescens showed that significant increase with increase in physiological field strength in buffer solution , root exudate and water respectively.

Rhizobacteria showed significant decrease in tactic response in water and root exudate in comparison to sodium buffer solution.

Swimming behaviour of Rhizobacteria in applied electric field

P.fluorescens –WCS-374 swam slightly faster in electrical fields than those of control (Table-3).The average swimming speed also varied slightly between experiments, however,possibly reflected a small differences in ambient temperature . The rate of tumbling was increased in electrical fields of 100 to 500 mv/cm. by a factor of two fold compared to control. No significant difference was found in the rate of tumbling or of swimming velocity for rhizobacteria moving towards the anode compared to those moving towards the cathode (p-0.05) according to a students t-test.

IV. Discussion

In the present study P.fluroscens 290 E & P. fluroscens WCS-374 exhibit electrotaxis towards the anode of an applied electrical field, however,P. fluorescens- 292 & P. fluorescens- M towards the cathode. This response may be dependent on population density,pH of the medium and the magnitude of the applied electrical field. Field strengths comparable to these, found in the rhizosphere are sufficient to elicit electrotaxis. The maximum electrical fields measured with vibrating microelectrodes around plant roots is 50-100 mv/cm, assuming a soil water resistivity 5000Ω/cm (Gow et. al. 1992;Miller&Gow 1989 a,b) in case of Phytophthora zoospores (Morris et. al.1992). My observations suggest that rhizobacteria are influenced by the natural electrical fields around root tips ,sites of wounds ,emerging lateral roots, or stomatal guard cells (Bowling et. al.1986).

The response of rhizobacteria P.fluorescens- 290 E & P.fluorescens-WCS-374 was anodic in an electrical field of 50 mv-500mv/cm where as that of in P.fluorescens - M &P.fluorescens- 292 was cathodic. The anodic and cathodic regions of plant roots vary according to the plant species (Miller &Gow,1989 b) source of combined nitrogen (Miller et. al.1991) and plant growth regulators (Miller &Gow,1989 b). Some evidence suggests that a positive correlation between the endogenous electrical polarity of a plant root and the zone of bacterial colonization (Ames,et.al.1988).

The result proposed that electrotaxis is the result of two processes: orientation of bacteria in the field according to their electrical dipole (electro-topotaxis) and voltage stimulation of the turning frequency (electro-klinokinesis). Field-dependent stimulation of the turning frequency of rhizobacteria also may be significant in the electrotactic response of rhizobacteria to the endogenous electrical fields of plants.

The magnitude of these fields decreases with increasing distance from the root surface. Therefore,bacteria approaching a root surface wild experience an increasingly large electrical field that can be estimated to be at least 250 mv/cm at 10 um from the root surface ,depending on the plant species. Frequent zoospore turning in the vicinity of a root has been reported (Jones et. al.1991) and could enhance the accumulation of zoospore at the root surface. Rhizobacteria are motile and swimming involves frequent changes in direction, there would not be sufficient time for electrical fields to redistribute proteins in the cell membrane thereby influencing ion transport and flagellar motion.

The results suggests that attraction of swimming bacteria to root is related in part to the sensing of swimming bacteria to root is related to the sensing of endogenous electrical gradients generated by growing roots or other plant tissues(Svitel et.al. 1998,Robinson,1985). Electrotaxis is nonspecific in so far as plant roots should stimulate it equally. The former may involve concerted and synergistic chemotactic and electrotactic mechanism. The present work and observations may be helpful in application of rhizobacteria in control of diseases in field as a biocontrol agent.

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Table-1 Sum (S),Sum of Squares (SS), Mean(X) and standard deviation (Sigma) ,N- number of reading of Pseudomonas fluorescens WCS -374,P.fluorescens -292,P.fluorescens –M of TRQ value.

Solution PBSRE Water

F = Field Strength , PBS = Phosphate Buffer Solution , RE = Root Exudate

Organism(Rhizobacteria) Statistical measures

F1 F2 F1 F2 F1 F2 Total

Pfl -WCS 374 N 3 3 3 3 3 3 18 S 0.18 0.43 0.14 0.24 0.10 0.18 1.27 SS 0.01 0.06 0.01 0.02 0.004 0.01 0.114 x 0.06 0.14 0.05 0.08 0.03 0.06 0.07 Sigma 0.02 0.02 0.03 0.01 0.02 0.03 0.036 Pfl. -292 N 3 3 3 3 3 3 18 S 0.84 1.49 0.52 0.62 0.36 0.47 4.30 SS 0.24 0.75 0.09 0.13 0.05 0.08 1.34 X 0.28 0.50 0.17 0.21 0.12 0.16 0.24 Sigma 0.02 0.04 0.02 0.05 0.02 0.02 0.061 Pfl -290E ≠µ N 3 3 3 3 3 3 18 S 0.32 0.75 0.25 0.51 0.22 0.32 2.370 SS 0.03 0.19 0.02 0.09 0.02 0.03 0.380 X 0.11 0.25 0.08 0.17 0.07 0.11 0.132 Sigma 0.02 0.04 0.02 0.05 0.02 0.02 0.61 Pfl -M N 3 3 3 3 3 3 18 S 0.22 0.45 0.19 0.35 0.13 0.25 1.590 SS 0.02 0.07 0.01 0.04 0.01 0.02 0.170 X 0.07 0.15 0.06 0.12 0.04 0.08 0.088 Sigma 0.02 0.03 0.02 0.03 0.03 0.02 0.041 Total treatment N 12 12 12 12 12 12 72 S 1.56 3.12 1.10 1.72 0.81 1.22 9.530 SS 0.30 1.07 0.13 0.28 0.08 0.14 2.004 X 0.13 0.26 0.09 0.14 0.07 0.10 0.132 Sigma 0.09 0.15 0.05 0.05 0.05 0.04 0.102

Table – 2: Summary of three way analysis of variance for Electrotaxis

Source of Variation df Sum of Squares Mean sum of squares

Frequency

Main Effect Organism(S) 3 0.308 0.103 126.691 ˣˣ Solution(S) 2 0.155 0.078 95.94 ˣ ˣ Field(f) 1 0.094 0.94 115.621 ˣˣ

Interaction Effect

O X S 6 0.095 0.016 19.680ˣ ˣ O X F 3 0.007 0.0023 2.829 ˣ O x S x F 6 0.015 0.0025 0.075 ˣ S x F 2 0.030 0.015 18.45 ˣˣ

Error(Within) 48 0.039 0.000813 Total 71 0.743

ˣ ˣP – 0.01 , ˣ P – 0.05

Table 3: Swimming behaviour of bacteria in applied electrical field :

N = 3 in each cell

50 mv 500 mv 0 mv t Mean S. D. Mean S.D. Mean S.D. Velocity /min 62.10 2.40 90.93 2.29 53.97 3.01 50mVvs500mv=12.29ˣ ˣ 50mV vs 0mv=2.99ˣ ˣ 500mv vs 0mv=13.82ˣ ˣ Turns/min 8.50 0.40 26.37 0.81 3.43 0.40 50mVvs500mv=27.97 50mv vs 0mv=12.68 500mv vs omv=35.91 Velocity to Anode 63.40 1.002 91.73 3.04 - - 12.49ˣ ˣ Turns/min to Anode 5.37 0.32 15.37 0.32 - - 31.25ˣˣ Turns/min to Cathode 5.73 0.21 6.27 0.25 - - 2.34 ≠

ˣP=0.05(Significant at 0.05 level)

ˣ ˣP=0.01(Significant at 0.01 level)

≠ P=0.05(Not Significant at 0.05 levelZ)