Evaluation of the role of fluorescent siderophore in the biological control of bacterial wilt in tomato using Tn^5 mutants of fluorescent *Pseudomonas* sp.

Fluorescent pseudomonads have been recognized as biocontrol agents against certain soil-borne plant pathogens. They are characterized by the production of yellow-green pigments termed pyoverdines, which fluoresce under UV light and function as 'siderophores'[1]. Pyoverdines chelate iron in the rhizosphere and deprive pathogens of iron which is required for their growth and pathogenesis[2]. Involvement of iron competition in biological control has been inferred from experiments in which mutants deficient in siderophore production were compared with the wild type with respect to biocontrol activity[3].

A number of fluorescent pseudomonads with biocontrol activity against *Ralstonia solanaceraum* causing bacterial wilt disease in tomato, have been reported[4]. In order to assess the mechanism of biocontrol in these strains, the forward genetic approach of developing random mutants was attempted using a tranposable element, Tn^5, borne on the suicidal plamid PGS 9 maintained in *E. coli* WA 803. The mutagenesis of fluorescent siderophore producing *Pseudomonas* strains, RBL 101 and RSI 125, was performed following the method of Mahesh Kumar *et al.*[5]. The putative mutants were checked for fluorescence under UV as well as for siderophore production following CAS plate assay[6]. The colonies that did not fluoresce were designated as Flu$^-$ mutants and those which fluoresced brighter than the wild type as hyperactive (Flu^{++}) mutants. The colonies which did not produce yellow zone of colouration on CAS plates were designated as Sid$^-$ mutants. Those which produced small zones were designated as Sid^d mutants and those which produce large-sized zones as Sid^{++} mutants. The putative mutants were tested for their *in vitro* inhibition of *R. solanacearum* following the dual culture assay[7] and for *in vitro* suppression of the bacterial wilt disease in tomato following the plant growth assay[8].

The Tn^5 mutagenesis of RBL 101 strain yielded seven mutants, which fluoresced under UV to a greater extent than the wild type (Flu^{++} mutants; Table 1). On CAS plates, these mutants produced high amounts of siderphores as evidenced by a large zone of colouration (Sid^{++}; Figure 1). And, in the dual culture assay, the mutants produced higher zone of inhibition of *R. solanaceraum* (7 and 8 mm dia), compared to the wild type (5 mm dia).

The Tn^5 mutagenesis of RSI 125 yielded two mutants (RSI 1256 and 1258) which did not fluoresce at all (Flu$^-$ mutants) and three mutants (RSI 1253, 1254 and 1257), which fluoresced to a lesser extent than the wild type (Flu^d mutants). On CAS plates, the Flu$^-$ mutants did not produce siderophores (Sid$^-$), while the Flu^d mutants produced

Table 1. Characteristics of Tn^5 mutants developed

Strain	Fluorescence	Siderophore	*In vitro* antagonism (ZOI in mm)
Wt (RBL 101)	Flu^{++}	Sid$^+$	5
Mutants			
RBL 1011	Flu^{++}	Sid^{++}	8
RBL 1012	Flu^{++}	Sid^{++}	7
RBL 1013	Flu^{++}	Sid^{++}	8
RBL 1014	Flu^{++}	Sid^{++}	7
RBL 1015	Flu^{++}	Sid^{++}	8
RBL 1016	Flu^{++}	Sid^{++}	7
RBL 1017	Flu^{++}	Sid^{++}	7
Wt (RSI 125)	Flu$^+$	Sid$^+$	6
Mutants			
RSI 1251	Flu$^+$	Sid$^+$	6
RSI 1252	Flu$^+$	Sid$^+$	6
RSI 1253	Flu^d	Sid^d	4
RSI 1254	Flu^d	Sid^d	4
RSI 1255	Flu$^+$	Sid$^+$	6
RSI 1256	Flu$^-$	Sid$^-$	0
RSI 1257	Flu^d	Sid^d	3
R SI 1258	Flu$^-$	Sid$^-$	0

Wt, wild type; ZOI, zone of inhibition.

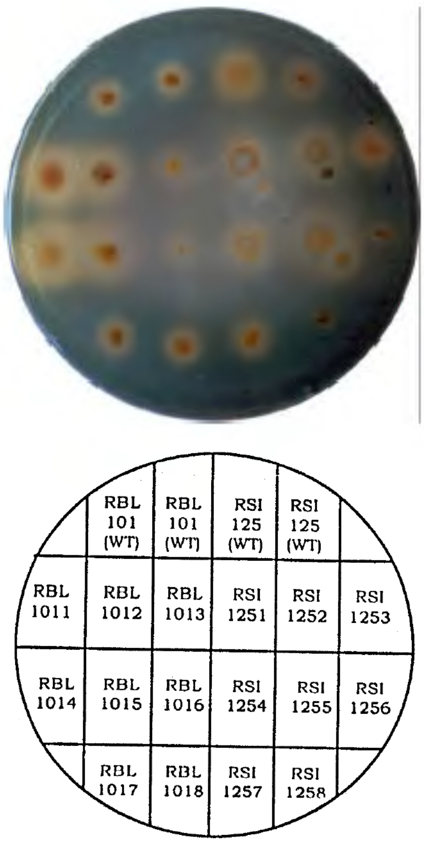

Figure 1. Siderophore production by Sid$^-$, Sid^d and Sid^{++} mutants.

Table 2. Suppression of bacterial wilt by the siderophore-minus, siderophore-delayed and siderophore-hyperactive mutants

Strain code	Per cent disease control
Check	
Wild type	
RSI 125	75.00
Sid^- mutants	
RSI 1256	25.00
RSI 1258	12.50
Sid^d mutants	
RSI 1253	25.00
RSI 1254	50.00
RSI 1257	37.50
Wild type	
RBL 101	12.50
Sid^{++} mutants	
RBL 1011	37.50
RBL 1013	25.00
RBL 1015	75.00

a smaller zone of colouration (Sid^d). And, Flu^- and Sid^- mutants did not inhibit the pathogen *in vitro*.

In the biocontrol experiment, Flu^- Sid^- (RSI 1256 and 1258) and Flu^d Sid^d (RSI 1253) mutants failed to control the disease as much as their wild type (Table 2), confirming the role of siderophores in the biocontrol mechanism. The involvement of the fluorescent siderophore (pyoverdin) in the suppression of *Pythium*-induced damping-off of tomato by *Pseudomonas aeruginosa* TNS K2, has been demonstrated using pyoverdine-deficient mutants[9]. The hyperactive mutants (Flu^{++} Sid^{++}) (RBL 1015 and 1011) with higher siderphore production suppressed the wilt disease to a greater extent than the wild type. Similar increase in the biocontrol potential of the fluorescent siderophore over producing mutant MPS 16 M-1 of *Pseudomonas* sp. against *Rhizoctonia solani* in chickpea has been reported[10]. Thus, the study has proved fluorescent siderophore production as a mechanism of biocontrol of the bacterial wilt disease in the fluorescent pseudomonad isolates, RBL 101 and RSI 125.

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A rare earthquake in Antarctica

The Antarctic continent is the largest apparently earthquake-free area on the earth, only a few earthquakes have been located by the Worldwide Standardized Seismograph Network (WWSSN). More than 10 seismological stations have been operating in Anatarctica since the International Geophysical Year (IGY), and the small local tremors recorded by these stations are commonly attributed to calving of the ice shelves or fracturing of the ice sheet. Small earthquakes, probably of volcanic origin, are associated with the active volcanoes at Mount Erebus on the Ross Island and at the Deception Island near the Antarctic Peninsula. The WWSSN detects almost all global earthquakes with magnitude > 4.0. Three such earthquakes have been recorded from Antarctica, one in 1952, another of magnitude 4.9 in 1974, both originating in the northern Victoria Land, close to a major glacier and ice tongue. The third earthquake, in 1985 of magnitude 4.7, was reported to have occurred in Dronning Maud Land. Seismologists suggest that, although the 1974 event had characteristics resembling those earthquakes generated by normal tectonic processes, it is likely to have been caused by movements within the ice. The 1985 event, in contrast, was attributed to normal tectonic processes, and remains the only unquestionable Antarctic earth-tremor[1].

The first seismograph station in Antarctica was operated as early as 1902–1903 at Scott-Base (SBA). Later on, some more stations were operated intermittently by various countries between 1940 and 1952. During IGY, 4 stations were operated, one each at MIR, SBA, WILKES and ADELIE. Two WWSSN stations are in operation from 1963 to 1964 at SBA and SPA. Subsequently, many countries have installed seismographs at their base stations in Antarctica. Due to difficult environmental and operating conditions, many stations have a very high downtime. For this reason, the first hypocentre in the region could be instrumentally located as late as 12 January 1995, when an earthquake of magnitude 4.7 occurred at 82.064°S, 43.993°N and depth of 10 km (ref. 2).

In order to monitor the seismic activity in Antarctica and the Indian Ocean, a reconnaissance survey for site selection and the feasibility of operation of