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PSEUDOMONAS FLUORESCENS IS AN ANTAGONIST TO *XANTHOMONAS CITRI* (HASSE) DYE, THE INCITANT OF CITRUS CANKER.

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THE terms 'antibiosis' and 'antagonism' refer to the reduction in growth and activities of organisms living in association[1]. Many bacteria and fungi are known antagonistic agents. Several studies have suggested that *Pseudomonas* spp. may play a role in reduction of plant diseases[2–7]. *Pseudomonas fluorescens* produces a siderophore which complexes the environmental iron making it less available to other pathogens and thus reduces disease severity[4].

From cankered citrus leaves collected from three locations of Madras, cultures of *Xanthomonas campestris* pv. *citri* (*X. citri*) were isolated by using nutrient agar plates. The surface-sterilized leaf bits and twigs yielded a fast growing yellowish green fluorescent bacterium which prevented the development of *X. citri*. Subsequently *X. citri* was isolated on NSCA (selective) medium[8].

Biochemical tests were made to characterize the yellowish green fluorescent bacterium abundantly associated with *X. citri* in mature cankers. The results from these biochemical tests (table 1) suggest that the organism is *P. fluorescens*. Further these two strains which were positive for levan production, denitrification, and used sorbitol and ethanol as sole carbon sources were identified as biotype I of *P. fluorescens* as outlined in Bergey's manual[9].

P. fluorescens strains were further characterized and they produced the siderophore (fluorescent pigment) only in low iron containing King's medium B (KB)[10]. When KB was amended with 1 μM of $FeCl_3$, the siderophore production was suppressed. Therefore this bacterium which showed properties of siderophore-producing rhizobacteria[11] was tentatively considered a plant growth-promoting rhizobacterium (PGPR).

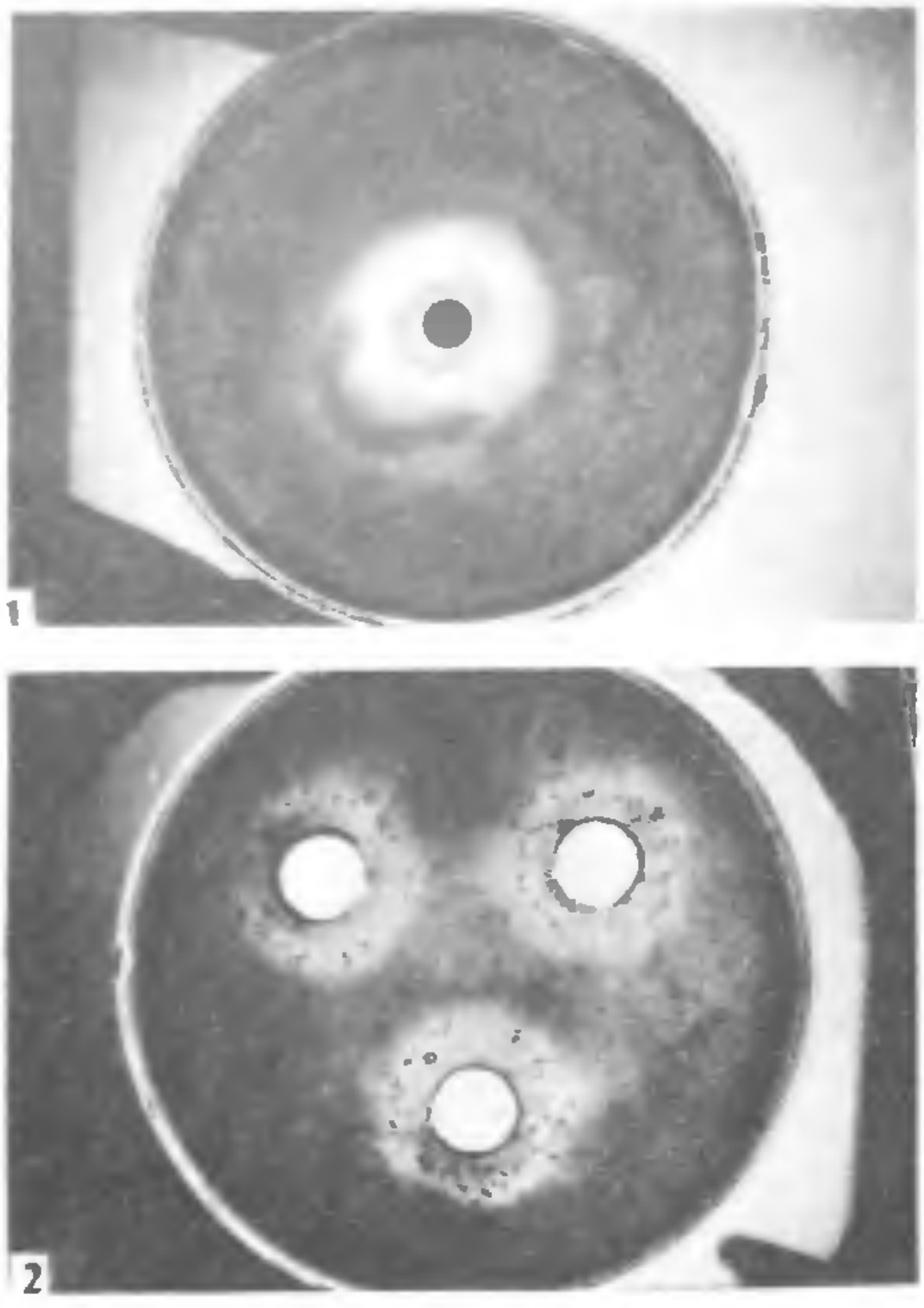
In two methods of *in vitro* bioassays (filter paper disc assay and agar well method), *P. fluorescens* cell suspension or a preparation of siderophore inhibited the growth of *X. citri* (figures 1 and 2). The siderophore was prepared following the method of Scher and Baker[10]. In another set of *in vitro* test, *X. oryzae*, the incitant of bacterial leaf blight of rice was also inhibited. Tests were also performed to check if this PGPR like *P. fluorescens* has growth-promoting activity following the method of bacterization of seeds described recently by Scher *et al*[12]. In rice seedlings (cv. IR-20) which were bacterized with *P. fluorescens* (2 strains) and grown in test tubes, shoot measurements were higher (average 5.9 cm) for one of the strains. In non-treated (control) plants, the average shoot length was 5.2 cm. The second strain of *P. fluorescens* did not have the growth-promoting activity and therefore the results are considered inconclusive.

The association of PGPR-like strains of *P. fluorescens* with citrus canker pathogen *X. citri* is reported for the first time from India. While this study was nearing

Table 1 *Biochemical tests for characterising fluorescent pseudomonas strains (of the plant growth promoting rhizobacteria (PGPR) type).*

	Pseudomonas fluorescens	
Test	AHS-PGPR strain*	UC-PGPR strain**
Gram reaction	– ve (small rods)	– ve (small rods)
Levan formation	+	+
Oxidase test	+ (1 sec)	+ (3 sec)
Gelatin liquefaction	+ (2 days)	+ (3–4 days)
Arginine dihydrolase production	+	+
Denitrification	+	+
Utilisation of carbon sources:		
a) Trehalose	+	+
b) Sorbitol	+	+
c) Ethanol	+	+
Antagonism to		
X. citri	+	+
X. oryzae	+	+

+ denotes a positive reaction to the test and – denotes a negative reaction; * AHS refers to strain collected from Agri-Horticultural Society Gardens and ** UC refers to strain collected from University Campus (Madras University).



Figures 1 & 2. Toxicity of *P. fluorescens* associated with citrus canker (*X. citri.*) **1.** Cell suspension (20 µl) of *P. fluorescens* was added to filter paper. **2.** Preparation of siderophore (pigment produced by *P. fluorescens*), 100 µl, was added to three agar wells.

completion in 1983 similar report of a *Pseudomonas* sp associated with *X. citri* in Japan has been made[13]. Although the growth-promoting activity of these strains contained in the present report needs further testing, the fact that they are inhibitory to the growth of *X. citri* (and also to *X. oryzae*) suggests their possible significance as biological control agents.

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THE METHOD OF STAINING POLLEN NUCLEI

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THERE is no suitable method known for staining and observing the nucleus inside the pollen. Microscopic examination of pollen nucleus is, in general, hindered by the presence of the enveloping exine. In the case of plants like the many members of Oleaceae where the pollen although stainable by dyes like carmine do not germinate[1] and their nuclei cannot be observed even during pollen tube formation. In such cases it is expedient to separate the exine from the inner protoplast. It is extremely inconvenient and difficult to achieve their separation by mechanical means. During our attempts to stain the pollen mother cells of jasmines for studying their chromosomes in meiosis after staining with lactopropionic orcein[2] (LPO) we have come across a noteworthy phenomenon wherein the inner contents (protoplast) of the pollen come out through the pollen pore. We have taken advantage of this phenomenon for an effective and convenient