out the study. Figure 2 also shows that ambient BC level responds immediately to the resumption (even though it was only partial and confined to within the state from 21 April onwards). The strike was called off nationwide on 23 April; BC had responded immediately to the increase in the influx of trucks and reached values as high as 4500 ng m^{-3} on 24 April and continued to do so on subsequent days.

Our study has shown that the exhaust from diesel trucks contributes significantly to the ambient BC concentration at the urban centre of Hyderabad. Even though there is a sudden decrease in the BC concentration associated with the cut-off of a potential source component, the full impact is felt much later due to the finite atmospheric residence time of BC. The recovery due to resumption of truck traffic is almost instantaneous.

1. Allen, G. A., Lawrence, J. and Koutrakis, P., Field validation of a semicontinuous method for aerosol black carbon (aethalometer) and temporal patterns of summertime hourly black carbon measurements in south-western PA. *Atmos. Environ.*, 1999, **33**, 817–823.
2. Babu, S. S. and Moorthy, K. K., Aerosol radiative forcing due to enhanced black carbon at an urban site in India. *Geophys. Res. Lett.*, 2002, **29**, 271–273.
3. Novakov, T., The role of soot and primary oxidants in atmospheric chemistry. *Sci. Total Environ.*, 1984, **36**, 1–10.
4. Ogren, J. A., Groblicki, P. J. and Charlson, R. J., Measurement of the removal rate of elemental carbon from the atmosphere. *Sci. Total Environ.*, 1984, **36**, 329–338.
5. Andreae, O. M., The dark side of aerosols. *Nature*, 2001, **409**, 671–672.
6. Reddy, M. S. and Venkataraman, C., Atmospheric optical and radiative effects of anthropogenic aerosol constituents from India. *Atmos. Environ.*, 2000, **34**, 4511–4523.
7. Hansen, A. P. A., Rosen, H. and Novakov, T., The aethalometer – an instrument for the real-time measurement of optical absorption by aerosol particles. *Sci. Total Environ.*, 1984, **36**, 191–196.
8. Liousse, C., Cachier, H. and Jennings, S. G., Optical and thermal measurements of black carbon aerosol content in different environments: variation of the specific attenuation cross section, sigma. *Atmos. Environ. A*, 1993, **27**, 1203–1211.
9. Chen, L. W. A., Doddridge, B. G., Dickerson, R. R., Chow, J. C., Mueller, P. K., Quinn, J. and Butler, W. A., Seasonal variations in elemental carbon aerosols, carbon monoxide and sulfur dioxide: Implications for sources. *Geophys. Res. Lett.*, 2001, **28**, 1711–1714.
10. Babu, S. S. and Moorthy, K. K., Anthropogenic impact on aerosol black carbon mass concentration at a tropical coastal station: A case study. *Curr. Sci.*, 2001, **81**, 1208–1214.

Received 17 July 2003; revised accepted 5 January 2004

Diacetylphloroglucinol-producing pseudomonads do not influence AM fungi in wheat rhizosphere

Rachna Gaur[†], Noam Shani[#], Kawaljeet[†], B. N. Johri[†,*], Pierre Rossi[#] and Michel Aragno[#]

[†]Department of Microbiology, CBSH, G.B. Pant University of Agriculture and Technology, Pantnagar 263 145, India
[#]Microbiology Laboratory (LAMUN), University of Neuchatel Case Postale 2 CH-2007 Neuchatel, Switzerland

Natural agroecosystems are directly dependent on beneficial microorganisms present in bulk soil and rhizosphere for soil health and plant productivity. With the current thrust on use of microbial inoculants, especially the biological control agents, it is necessary to assess their negative influence on such beneficial forms as AM fungi since they help the plant withstand various stresses. Among other molecules, pseudomonads present in the rhizosphere secrete the antifungal, 2,4-diacetylphloroglucinol (DAPG) and are currently in great demand as bioinoculants. Based on the cultural, biochemical, and molecular tools we show that DAPG producing pseudomonads recovered from wheat rhizosphere do not adversely affect AM colonization. Evaluation of such effects on non-target organisms will help early acceptance of microbial inoculants in future.

CONSIDERABLE research efforts are underway globally to exploit the potential of fluorescent pseudomonads (FLPs) in maintenance of soil health and as crop protectants, since they represent not only a dominant bacterial group in the rhizosphere ecosystem but are also metabolically and functionally most versatile[1]. Earlier studies with FLPs in our group have provided an effective background to support exhaustive exploitation of their gene pool for the benefit of the farming community[2–4]. It is well established that FLPs effectively colonize wheat rhizosphere and exert positive influence on plant growth[5]. However, current emphasis relies on the selection of promising isolates from the rhizosphere by screening of a large gene pool for multiple functional traits[6]. Among these traits, antagonism towards phytopathogenic fungi has been studied extensively[7]. However, it is only recently that concern has been shown in field release of isolates that secrete antifungals, including. 2,4-diacetylphloroglucinol (DAPG), since such isolates could also inhibit beneficial, naturally-occurring forms such as arbuscular mycorrhizal (AM) fungi[8]. Considering the likely negative influence of some FLPs on beneficial forms, here we describe characterization of DAPG producers from marginal wheat fields and their influence on indigenous fungi as a prelude to field use of effective isolates for improved soil health and plant productivity.

*For correspondence. (e-mail: bhavdishnjohri@rediffmail.com)

DAPG producers were isolated from three farmer's fields in Ujhani, Dist Badaun (Table 1). The fields were selected based on fertilizer input and grain yield and termed: (i) low input low yield (SLL), (ii) low input high yield (SLH), and (iii) high input high yield (SHH). These fields have a history of at least 20 years of continuous wheat–rice rotation and wheat variety UP2338 has been cultivated for several years. Dilution plating was performed on King's B (KB) agar to recover rhizosphere and endorhizosphere/rhizoplane bacteria. A gene pool of 840 FLPs was recovered from the three fields. For preliminary screening, all 840 isolates were subjected to dual culture plate assay on KB : PDA (1 : 1 v/v) against *Fusarium oxysporum*, *Helminthosporium sativum* and *Rhizoctonia solani*. Fungal cultures were grown on PDA for 3 days, 1 cm disk was removed and placed centrally on prepoured (KB : PDA) plates. Bacterial cultures were spot inoculated on the edge of the plate, and incubated at 28°C. Inhibition of fungal growth, if any, was scored after 4 days. A total of 224 gram-negative antagonistic bacterial isolates were subjected to colony hybridization to detect the presence of *phlD* gene.

For colony hybridization, bacterial colonies were transferred to nylon membrane lot no. IB7310 (Amersham) by standard protocol. The membranes were baked for 1 h at 42°C in 2 × SSPE (0.1% SDS, 100 µg/ml pronase; 2 × SSPE is 20 mM $NaH_2PO_4 \cdot 2H_2O$, 36 M NaCl, 2 mM EDTA). To remove bacterial cell debris from colony blot, the membranes were placed in SSPE solution without pronase at 65°C with agitation. The hybridization buffer contained 5 × SSC (1 × SSC + 0.5 M NaCl, 15 mM sodium citrate), 0.1% sodium lauryl sarcosine, 0.02% SDS, and 2% blocking reagent (Boehringer Mannheim). Pre-hybridization was performed for 1 h at 52°C and hybridization was performed overnight at 52°C. The membranes were washed twice in 5 × SSC–0.1% SDS for 15 min at 52°C (hybridization temperature) and twice in 2 × SSC–0.1% SDS for 5 min at room temperature. The probe used was 17 mer PhlD1 (5′ 496GTGATGAACTCCATCAAGA515 3′) and PhlD2 (5′ 496GTGATGAACTCCATCAAGA515 3′)[9]. The probe was labelled by non-radioactive digoxigenin system using the protocol recommended by the manufacturer (Boehringer Mannheim). The hybridized probe was detected with antidigoxigenin alkaline phosphatase Fab fragment and was visualized colorimetrically using the substrate nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolylphosphate, as per suppliers instruction. DAPG-producing isolates PILH1, CHAO, PGNR1, PGNR5 and TMA3 were used as positive reference strains, and *Escherichia coli* and *Pseudomonas aeruginosa* as negative reference to check specificity of the probe.

Table 1. Soil characteristics and distribution of antagonistic isolates in three farmers' fields cultivating wheat variety UP2338

Soil system	Soil characteristics			Percentage antagonistic isolates*	
	Available P (kg ha^{-1})	Available N (kg ha^{-1})	Percentage organic carbon	Rhizosphere	Rhizoplane
SLL	25.21	115.7	0.60	60.76	51.35
SLH	9.73	118.8	0.64	58.25	59.93
SHH	73.84	118.8	0.87	56.28	57.37

*Based on dual agar plate assay of 840 isolates against *Fusarium oxysporum*, *Rhizoctonia solani* and *Helminthosporium sativum*.

Hybridization-positive isolates were subjected to PCR amplification of *phlD* gene. DNA was extracted[10] and the first PCR was performed using the primers Phl 2a (5′GAGGACGTCGAAGACCACCA3′ 704–723) and Phl 2b[11]. This was followed by second nested PCR on the 1st amplification product using primers Phl 296 (chf)/chao group 5′CTGTGCTACCAGCCGGA3′ (286–302) Phl 296 (f) 5′CTCTGCTATCAACCMCA3′ (286–302) in 1 : 1 mixture as forward primer and Phl 486r 5′TTRATGGAGTTCATSAC3′ (496–512) as reverse primer[9]. Characterization of the isolates was done by amplifying the ITS region through *Pseudomonas*-specific ITS primers[9].

In vitro production of DAPG was tested by solvent extraction of the molecule from the culture broth and analytical HPLC. Isolates were grown in KB broth for 72 h and antibiotic extraction was carried out as described by Rodriguez and Pfender[12]. The ethyl acetate extracts were dried in vacuum and dissolved in methanol. Aliquots were filtered (0.2 µM sartorius filter) and analysed on an HPLC system (Waters Corp Milford, MA) fitted with a PDA detector (Water's model 501) and nucleosil C_{18} ET-250/8/4 (Macherey Nagel) column. Samples were run in an isocratic mobile phase (45% H_2O, 30% acetonitrile and 25% methanol) at a flow rate of 1.0 ml min^{-1}. The chromatogram was read at 272 nm for DAPG and spectra were compared with those of the antibiotic standard. DAPG positive isolates were functionally characterized for *in vitro* production of IAA and siderophore. Active culture (12-h-old) was inoculated in 10 ml minimal salt medium in 50 ml Erlenmeyer flask amended with 100 µg ml^{-1} tryptophan. Flasks were incubated at 28°C under shaking (100 rpm) for 48 h. Culture broth was centrifuged at 7500 rpm for 10 min. To 1 ml culture supernatant, 2 ml Salkovsky reagent was added and contents incubated at 30°C for 25 min. Absorbance was read at 530 nm and levels quantitated from standard curve of IAA. For quantitation of siderophore, actively grown culture was inoculated in standard succinate medium; inoculum size was maintained at 0.1%. Culture broth was centrifuged after 48 h growth at 28°C at 8000 rpm and absorbance of the fluorescent pigment was read at 400 nm[4]. The amount of siderophore was calculated according to Meyer and Abdallah[13]. Production of hydrolytic enzyme was qualitatively assayed in minimal medium containing skim milk, starch, pectin and carboxymethyl cellulose for protease, amylase, pectinase and cellulase respectively. Plates were incubated for 48 h at 28°C and formation of clear zone around bacterial colonies was

read as positive. Interaction of DAPG-positive isolates with native AM fungi was observed in a soil microcosm. Soil microcosm was prepared in plastic cups (volume, 250 g soil cup^{-1}). The experiment was laid in unsterile soil of (a) SHH, and (b) a mixed soil (SLL + SLH, 1 : 1, w/w). Seeds of wheat variety UP2338 were bacterized at a population size of 10^2 cells per seed. AM inoculum (1 g $seed^{-1}$) was placed in a hole and covered with a layer of soil over which bacterized seeds were sown. Each treatment was replicated thrice and arranged in completely randomized design in a net-house. Pots were watered when required. The experiment was terminated after 30 days. The bacterial population structure was assessed by dilution plating and mycorrhizal colonization by staining the roots in trypan blue[14]. The extent of colonization was determined based on frequency distribution of fungal hyphal length in randomly selected root segments (1 cm in length). The data were analysed by one-way ANOVA.

Though soil samples were different in terms of available P and N and in organic C content, no significant variation was observed in percentage distribution of antagonistic isolates (Table 1).

Colony hybridization tests followed by two-step PCR confirmed the presence of *PhlD* gene in six isolates (Figure 1). A 210 bp product was obtained which was also amplified in *PhlD*-positive reference isolate, CHAO. The positive six isolates were LHRE117, LHRE30, LHRE62, LLRE63, LHRS14 and HHRE81. Quantitative analysis of DAPG in liquid medium revealed that antibiotic levels ranged from 32 to 60 μg ml^{-1}. Maximum DAPG was produced in isolate LLRE63 and minimum in isolate LHRE-117. Siderophore levels ranged between 20 and 30 μg ml^{-1}; isolate LHRE30 was negative for siderophore. IAA levels varied between 12 and 34 μg ml^{-1}. No specific relationship was apparent in the production between the three metabolites (Table 2). *In vitro* production of such secondary metabolites is not normally reflected in their efficiency to secrete these molecules in the naturally competitive environment of the rhizosphere. Thus, levels of DAPG reaching 0.33 ng have been reported in soil microcosm[15]. *In situ* performance of a bacterial isolate is not only dependent on the expression of the desirable function/(s), but also on its genetic background and the prevailing environmental conditions.

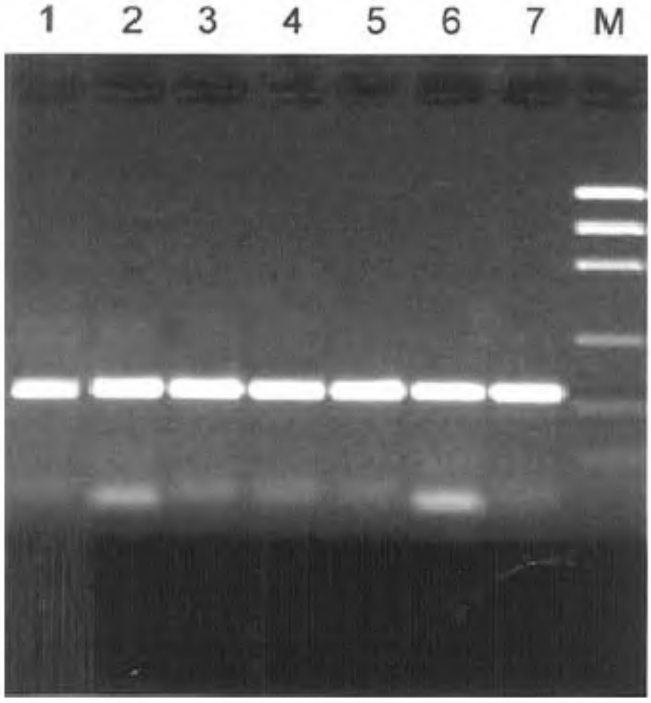

Figure 1. 210 bp gene product amplified after two-step PCR. Lane 1, CHAO; lane 2, LHRE30; lane 3, LHRE62; lane 4, LLRS63; lane 5, HHRE81; lane 6, LHRE117; lane 7 LHRS14; lane 8, Marker.

All six $PhlD^+$ isolates were able to secrete amylase and cellulase based on the relative zone size; LHRE30 produced greater amount of cellulase. Three isolates produced pectinase, viz. LLRE63, LHRS14 and HHRE81, whereas five were positive for protease (Table 3).

Amplification with *Pseudomonas*-specific ITS primer gave a band of 1.3 kb in all isolates except LHRE30 (Figure 2), which confirmed the identity of these isolates as *Pseudomonas*.

The interaction of AM fungi with bacterial population in soil microcosm resulted in a positive response. At the start of the experiment, FLPs population was 10^6 CFU g^{-1} in both the experimental soils. After 30 days of wheat growth, FLPs population on KB reached a level of 10^8–10^9 CFU g^{-1} soil. The total number of culturable bacteria was unaffected by inoculation with AM fungi both in rhizosphere and rhizoplane/endorhizosphere fraction. Since bacterial community analysis was not carried out, one cannot predict the change at this level. However, using fatty acid

Table 2. Production of DAPG, siderophore and IAA by antifungal antibiotic-producing FLPs isolates in standard succinate medium

Isolate	DAPG (μg ml^{-1})	Siderophore (mg ml^{-1})	IAA (μg ml^{-1})
LHRE117	32.0 ± 2.0	20.17 ± 2.0	32.0 ± 3.00
LHRE30	44.0 ± 3.0	n.d.	31.0 ± 2.0
LHRE62	56 ± 2.0	26.28 ± 2.0	32.0 ± 3.0
LLRE63	60.0 ± 2.0	21.26 ± 2.0	34.5 ± 2.0
LHRS14	35.0 ± 2.0	22.58 ± 2.0	25.0 ± 2.0
HHRE81	58.0 ± 2.0	38.88 ± 2.0	12.0 ± 1.0

Table 3. Secretion of hydrolytic enzymes by Phl D^+ isolates in agar plate assay

Isolate	Amylase	Protease	Pectinase	Cellulase
LHRE117	+	+	–	+
LHRE30	+	–	–	+++
LHRE62	+	+	–	+
LLRE63	+	++	+	+
LHRS14	+	–	+	+
HHRE81	+	+++	+	+

+, Relative size of the clearing zone in plate-based assay.

methyl ester analysis (FAME), Gesse-Mansfeld *et al.*[16] showed considerable shift in the community with the inoculation of AM fungi in *Cucumis sativus*. Andrade *et al.*[8] have also reported qualitative change in bacterial communities as affected by different AM fungi in both the rhizosphere and hyphosphere of *Medicago sativus*. Mycorrhizal colonization differed with each isolate (Figure 3). An increase in the level of mycorrhization was observed from 30 to 55%. Isolates with all three functional properties and hydrolytic enzymes produced prominent vesicles in roots in the mixed soil (SLL + SLH) system. A slightly higher level of root colonization was observed in this soil. However, in an earlier study by Singh *et al.*[17] it was found that levels of P had much lower effect on mycorrhization compared to root exudates which exerted the maximum influence.

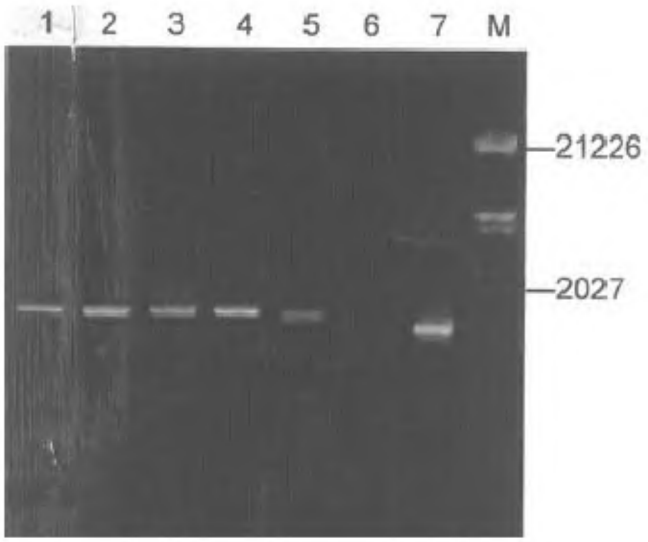

Figure 2. 1300 bp ITS amplification with *Pseudomonas* specific primers. Lane 1, LHRE117; lane 2, LHRE62; lane 3, LLRS63; lane 4, HHRE81; lane 5, LHRS14; lane 6, LHRE30; lane 7, CHAO; lane 8, Marker.

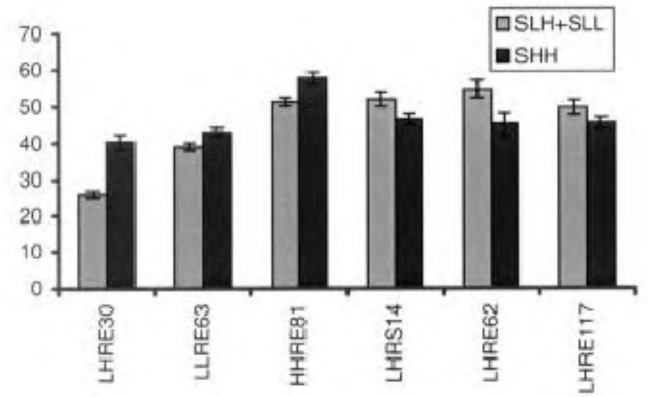

Figure 3. Per cent colonization of AM fungi in the presence of Phl D^+ isolates in two different soils.

Considering the known toxicity of DAPG[18] (Table 4), the results of this study show that the negative influence of DAPG-producing FLPs on mycorrhization could be dependent on antibiotic production levels. However, available data on *in vitro* synthesis do not provide a clear idea of expression of antifungal metabolite in the rhizosphere. It is to be noted that mycorrhizal colonization in the presence of FLPs was not related with the level of DAPG produced by these isolates under *in vitro* conditions. Therefore, colonization by AM fungi may or may not be directly influenced by such bacteria. Isolate HHRE81 with maximum enzyme activity and resultant higher AM colonization could be acting as mycorrhization helper bacteria. Studies are underway to understand the influence of these isolates on AM fungi in the rhizospheric community at farmers' field. It is, however, clear that none of the DAPG producers in this study exerted negative influence on mycorrhizal colonization, which is in contrast to our earlier observation on antifungal-producing strain GRP3A which inhibits mycorrhization[18].

Marschner[19] had reported negative influence of pseudomonads on AM fungi, whereas Barea *et al.*[20] reported neutral influence of globally used strain *P. fluorescens* CHAO on AM fungi, along with those of other commonly used bioinoculants, based on *Trichoderma viride* and *Gliocladium virens*. These workers had however cautioned that introduction of a new inoculant in the soil should be preceded by detailed analysis of the biological equilibrium. Marschner *et al.*[21] have recently shown that the converse is also true; AM infection altered community composition in maize rhizosphere, although synergistic interaction between AM and FLPs has also been reported[22]. In view of the above it is essential to assess the impact of a new bioinoculant on non-target organisms and on specific microbial species, including indigenous beneficial forms so that natural biological balance can be maintained in the rhizosphere environment. In this context, several studies have demonstrated that both wild type and genetically modified Phl overproducing pseudomonads do not interfere with symbiosis, but a reliance on culture-dependent approach for impact assessment has also been questioned. There are fundamental challenges which include use of consortia for increased PGPR properties and

Table 4. Toxicity level of synthetic DAPG on various organisms[18]

Test organism/plant	MIC (μM)	
	I_{50}	I_{100}
Wheat	100–200	> 3200
Pythium ultimum	200	400
G. graminis var *tritici*	50–100	200
Thielaviopsis basicola	100–200	400
Rhizoctonia solani	100–200	400
Fusarium oxysporum	50	400
Nematode	200	> 1000

development of universal formulations to increase inoculant survival during seed coating and storage. In addition, marketing of bioinoculant product as environmentally friendly alternatives to chemical fungicides will depend on the generation of essential biosafety data required for the registration of biocontrol agents in general[23].

1. Lugtenberg, B. J. J. and Dekkers, L. C., What makes *Pseudomonas* bacteria rhizosphere competent? *Environ. Microbiol.*, 1995, **1**, 9–13.
2. Rao, Ch V. S. and Johri, B. N., Seed and root colonization of soybean (*Glycine max*) by fluorescent pseudomonads. *Indian J. Microbiol.*, 1999, **39**, 31.
3. Rao, Ch V. S., Sachan, I. P. and Johri, B. N., Influence of fluorescent pseudomonads on growth and nodulation of lentil (*Lens esculantus*). *Indian J. Microbiol.*, 1999, **39**, 23.
4. Sharma, A. and Johri, B. N., Combat of iron-deprivation through a plant growth promoting fluorescent *Pseudomonas* strain GRP3 in mung bean (*Vigna radiata* L. Wilzeck). *Microbiol. Res.*, 2003, **158**, 77–81.
5. Srivastava, R, Johri, B. N. and Sharma, A., Colonization of wheat (*Triticum aestivum* L.) root by fluorescent pseudomonads. *Indian J. Microbiol.*, 1999, **39**, 205.
6. Antoun, H., Beauchamp, C. J., Govassard, N., Chabot, R. and Lalande, R., Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non legumes: Effect on radishes (*Raphanus sativus* L.). *Plant Soil*, 1998, **204**, 57–67.
7. Dowling, D. N. and O'Gara, F., Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. *Trends Biocontrol.*, 1994, **12**, 133–144.
8. Andrade, G., Azcon, R. and Bethlenfalvey, G. J., A rhizobacterium modifies plant and soil responses to the mycorrhizal fungus *Glomus mosseae*. *Appl. Soil Ecol.*, 1995, **2**, 195–202.
9. Shani Noam, 2,4-diacetylphloroglucinol (DAPG) producers in the rhizosphere of wheat: development of probes and primers to assess their presence and abundance in Indian rice–wheat crop rotations. MSc thesis, University of Neuchatel, Switzerland, 2002.
10. Bazzicalupo, M. and Fani, R., The use of RAPD for generating specific DNA probes for microorganisms. In *Methods in Molecular Biology* (ed. Clap, J.), Humana Press Inc., Totowa, NJ, 1994, vol. 50, pp. 155–175.
11. Raaijmaker, J., Weller, D. M. and Thimashow, L. S., Frequency of antibiotic producing *Pseudomonas* spp in natural environment. *Appl. Environ. Microbiol.*, 1997, **63**, 881–887.
12. Rodriguez, F. and Pfender, W. F., Antibiosis and antagonism of *Sclerotinia homoeocarpa* and *Drechslera poae* by *Pseudomonas fluorescens* PF-5 *in vitro* and in plant. *Phytopathology*, 1997, **87**, 614–621.
13. Meyer, J. M. and Abdallah, M. A., The fluorescent pigment of fluorescent pseudomonad in mycorrhizal and nonmycorrhizal pepper (*Capsicum annuum* L.). *J. Gen. Microbiol.*, 1978, **107**, 319–328.
14. Biermann, B. and Linderman, R. G., Quantifying vesicular arbuscular mycorrhizae: A proposed method towards standardization. *New Phytol.*, 1983, **87**, 63–67.
15. Maurhofer, M., Keel, C., Haas, D. and Defago, G., Influence of plant species on disease suppression by *Pseudomonas fluorescens* strain CHAO with enhanced antibiotic production. *Plant Pathol.*, 1995, **44**, 40–50.
16. Gesse-Mansfeld, K., Larsen, J. and Bødker, L., Bacterial populations associated with mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices*. *FEMS Microbiol. Ecol.*, 2002, **41**, 133–140.
17. Singh, C., Johri, B. N. and Sharma, A. K., Host genotype determines the impact of soil phosphorus on arbuscular mycorrhizal symbiosis in maize. *Symbiosis*, 2002, **33**,145–164.
18. Keel, C. *et al.*, Suppression of root disease by *Pseudomonas fluorescens* (CHAO): Importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol. *Mol. Plant–Microb. Interact.*, 1992, **5**, 4–13.
19. Marschner, P., Crowley, D. E. and Higashi, R. M., Root exudation and physiological status of a root-colonizing fluorescent pseudomonad in mycorrhizal and non mycorrhizal pepper (*Capsicum annuum* L.), *Plant Soil*, 1997, **189**, 11–20.
20. Barea, J. M. *et al.*, Impact on arbuscular mycorrhiza formation of *Pseudomonas* strains used as inoculants for biocontrol of soilborne fungal plant pathogens. *Appl. Environ. Microbiol.*, 1998, **64**, 2304–2307.
21. Marschner, P., Crowley, D. E. and Lieberei, R., Arbuscular mycorrhizal infection changes the bacterial 16S rDNA community composition in the rhizosphere of maize. *Mycorrhiza*, 2001, **11**, 297–302.
22. Garbaye, J., Helper bacteria: a new dimension to the mycorrhizal symbiosis. *Acta Bot.Gallica*, 1994, **141**, 517–521.
23. Walsh, F., Morrissey, P. J. and Gara, O'F., *Pseudomonas* for biocontrol of phytopathogens: from functional genomics to commercial exploitation. *Curr. Opin. Biotechnol.*, 2001, **12**, 289–295.
24. Bisht Rekha, Interactive study between AM fungi and PGPR using *Dalbergia sissoo* L. and *Vigna radiata* L. as hosts, PhD thesis. G.B. Pant University of Agriculture and Technology, 2001.

ACKNOWLEDGEMENTS. This work has been carried out under the Indo-Swiss Cooperation in Biotechnology project SA7 supported by the Department of Biotechnology and Swiss-Development Cooperation. We thank Dr Alok Adholeya and Ms Pragati Tewari, TERI, New Delhi for supply of indigenous AM consortium and laboratory support for mycorrhizal component.

Received 7 September 2002; revised accepted 20 October 2003

Ecobiology of the tropical pierid butterfly *Catopsilia pyranthe*

J. B. Atluri[†], S. P. Venkata Ramana[†] and C. Subba Reddi[#,*]

[†]Department of Botany and [#]Department of Environmental Sciences, Andhra University, Visakhapatnam 530 003, India

Successful and effective conservation management of butterflies depends on sound knowledge of their life history and host plant requirements in the wild. As part of an effort in this context of South Indian butterflies, the life history and the length of each life stage (egg, larva and pupa) of the pierid butterfly *Catopsilia pyranthe* (Linn.), which is a strong migrant, were studied. The development period from egg to adult emergence spanned over 22–29 days, thus opening up a possibility of at least 11–12 breeding genera-

*For correspondence. (e-mail: aulibra@md2.vsnl.net.in)