The broad band observed at 3436.9 cm\(^{-1}\) is characteristic of OH hydrogen bonded to the oxygen ions of the framework. In addition, an intense band at 1647.1 cm\(^{-1}\), which is characteristic of the bending mode in the water molecule, is also observed. The sharp and deep band of FASBC-1 corresponding to the water of hydration, indicates higher percentage of water of hydration. IR frequencies observed for various samples are given in Table 5.

From Table 5 it can be seen that the other FAZ-A samples also show the characteristic IR bands, in the range discussed for FASBC-1. IR spectra of zeolite-A standard procured from Degussa (Figure 2f) shows the characteristic IR bands at frequencies similar to those of FAZ-A samples. It may thus be concluded that IR spectra of the FAZ-A samples and standard zeolite-A match quite closely, indicating presence of similar structural units and formation of identical chemical moieties in the FAZ-A samples. This is also in agreement with the results obtained from XRD studies.

Comparative evaluation of IR and XRD methodologies used for estimation of per cent crystallinity values shows that they match closely with each other for almost all the samples. Hence it may be concluded that the IR method can be used for monitoring crystallinity in zeolite samples being synthesized from a complex matrix like flyash.

Table 6 depicts the chemical composition of the samples. The desired purity level of zeolite phase is dependent on the end-application. Flyash-based zeolite material is presently being used as an adsorbent and ion exchanger, wherein the presence of trace-level impurities of metals like Fe and phase impurities like sodalite, etc. do not show significant detrimental effects.

IR method is successfully used for identifying zeolite structure as a complementary technique to XRD. The crystallinity estimated using IR and XRD methods is quite comparable and hence can be used as a method for monitoring the synthesis of zeolite-A from a complex matrix like flyash. Further studies are in progress for determining the sensitivity and accuracy of the method. The phase purity of the sample is important from the point of view of its usage as a catalyst. In case of environmental remediation, wherein it is to be used as an adsorbent/exchanger, purity of the sample is not a significant issue and can be compromised as a trade-off between cost and efficiency. Ultimately, the adsorbent medium is being used as a phase transfer for the pollutant and may either be disposed-off safely or converted into some value-added ceramic precursor, which may differ substantially in phase.


ACKNOWLEDGEMENTS. We thank Dr N. Labhsetwar and Mr R. B. Bintwale, for their useful suggestions during the course of this work. We also thank Dr Garway for analysing zeolite samples for XRD analysis, and CDRI, Lucknow for recording IR spectra.

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Cold-tolerant fluorescent Pseudomonas isolates from Garhwal Himalayas as potential plant growth promoting and biocontrol agents in pea

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Four rhizospheric strains of Pseudomonas fluorescens (PF), viz. PF-102, PF-103, PF-110 and PF-173 were antagonistic against Fusarium solani f. sp. pisii (causal agent of root rot in pea). In liquid culture assay, PFs could inhibit the growth of F. solani f. sp. pisii by 60–100%, suggesting that their secondary metabolites were sufficient to antagonize the target pathogen. Mode of inhibition of F. solani f. sp. pisii by PF-102 and PF-103 was fungistic, while PF-110 and PF-173 were lytic in their action. Plant growth promotion and in vivo antagonism assays against the target pathogen revealed that a consortium of four test strains was the best. Also individually, strains PF-110 and PF-173, capable of solubilizing inorganic phosphate and producing siderophore, were consistently better than the others. These activities are relevant to ecological fitness of the producer strains under the framework of organic farming underway in Uttarakhand.

Keywords: Biocontrol agents, Garhwal Himalayas, Pseudomonas fluorescens, pea, plant growth promotion.

SEVERAL microbes in recent times have been a focus of attention as plant growth promotion or biocontrol agents against

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various phytopathogens. Among these *Azotobacter* spp., *Bacillus subtilis*, *Pseudomonas fluorescens*, *Rhizobium* spp., (bacteria), *Aspergillus* spp., *Gliocladium* spp., *Trichoderma* spp. and mycorrhizae (fungi) have been studied and used extensively. These biocontrol agents hold promise and tend to reduce the damage due to plant pathogens as well as dependence on the use of hazardous chemicals for plant disease management.

Among various biocontrol agents, fluorescent pseudomonads, equipped with multiple mechanisms for biocontrol of phytopathogens and plant growth promotion, are being used widely. They produce a wide variety of antibiotics, chitinolytic enzymes, growth promoting hormones, siderophores, HCN and catalase, and can solubilize phosphorus.

Cultivation of off-season vegetables is the main source of livelihood of small and marginal farmers in Garhwal Himalayas. However, under most farming conditions, the soil is acidic and poor in organic matter. Farmers have small (~0.5 ha) and scattered land holdings. Hence, intensive cultivation with reduced crop rotations and extensive monoculture result in reduced biodiversity that has led to high resident pathogen populations and increased crop losses due to diseases. Amongst the diseases, root rots are most common in different off-season vegetables. Pea (*Pisum sativum* L.) is cultivated twice and in some farming situations thrice a year. Root rot caused by *Fusarium solani* (Mart.) Sacc. f. sp. *pisi* (Jones) Snyd. & Hans. remains a major threat to successful cultivation of pea. The disease is particularly severe because of low soil fertility and acidity of soils. Inadequate rotation aggravates crop loss. Use of synthetic chemicals for management of disease is largely uneconomical and does not fit within the framework of ‘organic farming’, which is the state policy. Use of bioagents having biocontrol activity (BCA) and plant growth promoting (PGP) activity is a viable alternative to minimize use of synthetic chemicals and their hazardous effects, and to provide protection to the plants against resident pathogen populations.

To address the above issues and provide an environmentally sound alternative for disease management and plant growth promotion, we are establishing a repository of cold-tolerant isolates of fluorescent *Pseudomonas* by selecting putative candidates possessing desired BCA and PGP activities. Our long-term objective is to exploit candidate pseudomonads in the bio-intensive IPM programme that is underway in different farming situations in the region. So far, over 540 fluorescent *Pseudomonas* have been isolated from the rhizosphere of different annual and perennial crops from varied farming situations and characterized for phosphate solubilization, HCN production and siderophore production, besides being subjected to various other biochemical tests. Among these, four candidate strains, Pf-102, Pf-103, Pf-110 and Pf-173, were used in the present study to evaluate their potential for PGP in pea and BCA against *F. solani* f. sp. *pisi*, causing root rot disease.

Characteristics of the four *P. fluorescens* isolates have been given in Table 1 and 2. Bacterial strains were maintained on King’s medium B (KB)\(^1\). *F. solani* f. sp. *pisi*, the pea root rot isolate, was obtained from the stocks maintained in Plant Pathology Laboratory at the G.B. Pant University of Agriculture and Technology, Ranichauri. Pathogen was activated on potato dextrose agar (PDA) medium and multiplied on barnyard millet (*Echinochloa frumentaceae*) grains for soil inoculation. Grains were soaked in water overnight and steam-sterilized in autoclave glass bottles. Two discs (5 mm diameter) of fungal pathogen from one-week-old culture were transferred to bottles that were incubated at 25 ± 1°C. After two weeks, the grains covered with fungal growth were dried and ground to a fine powder. This inoculum was stored at 4°C until further use.

Table-based formulation of Pfs was used for seed treatment. Erlenmeyer flasks, each containing pre-sterilized 100 ml KB broth, were inoculated with each of the four test Pfs and incubated at 28 ± 2°C on an incubator shaker (150 rpm) until cell number reached approximately 10\(^9\) ml\(^{-1}\). Bacterial suspension was mixed with talc powder (1:2 w/v) containing 1% carboxy methyl cellulose to have a final CFU (colony forming unit) of 10\(^7\). The formulation was air-dried, packed in autoclaved polybags and stored at −20°C for further use.

Antagonistic behaviour of Pfs was evaluated by dual culture technique. Mycelial discs (5 mm diameter) from one-week-old cultures of *F. solani* were placed on PDA plates in the centre and Pfs were streaked at four corners. Inoculated plates were incubated at 28 ± 2°C for 5–7 days and the resulting zone of inhibition was measured.

Effect of secondary metabolites of Pfs on growth of the pathogen was evaluated in potato dextrose broth (PDB). Fresh culture filtrate (10, 20, 40, 50 ml) of Pfs was transferred to 250 ml conical flasks containing 90, 80, 60 and

<table>
<thead>
<tr>
<th>Strain</th>
<th>Origin</th>
<th>Catalase test</th>
<th>HCN production</th>
<th>Phosphate solubilization</th>
<th>Siderophore production</th>
<th>Antagonism against</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pf-102</td>
<td>Ragi</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Pf-103</td>
<td>Amaranath</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Pf-110</td>
<td>Amaranath</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pf-173</td>
<td>Mustard</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Detailed results on isolate characterization under publication elsewhere. +, Positive reaction; −, Negative reaction.
Table 2. Growth of P. fluorescens isolates used in the study at different temperatures and time intervals*

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Pf-102</th>
<th>Pf-103</th>
<th>Pf-110</th>
<th>Pf-173</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>– – + +</td>
<td>– – + +</td>
<td>– – + +</td>
<td>– – + +</td>
</tr>
<tr>
<td>6</td>
<td>– – + +</td>
<td>– – + +</td>
<td>– – + +</td>
<td>– – + +</td>
</tr>
<tr>
<td>8</td>
<td>– – + +</td>
<td>– – + +</td>
<td>– – + +</td>
<td>– – + +</td>
</tr>
<tr>
<td>10</td>
<td>– + + +</td>
<td>– + + +</td>
<td>– + + +</td>
<td>– + + +</td>
</tr>
<tr>
<td>15</td>
<td>– + + +</td>
<td>– + + +</td>
<td>– + + +</td>
<td>– + + +</td>
</tr>
<tr>
<td>20</td>
<td>– + + +</td>
<td>– + + +</td>
<td>– + + +</td>
<td>– + + +</td>
</tr>
<tr>
<td>25</td>
<td>– + + +</td>
<td>– + + +</td>
<td>– + + +</td>
<td>– + + +</td>
</tr>
</tbody>
</table>

*Detailed results on isolate characterization under publication elsewhere.
–, No growth; +, Less growth; ++, Full growth.

Table 3. Effect of culture filtrate of P. fluorescens isolates on growth of Fusarium solani f. sp. pisi in potato dextrose broth

<table>
<thead>
<tr>
<th>Culture filtrate (ml)</th>
<th>Weight of filter paper with dried mycelium of F. solani (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pf-102</td>
</tr>
<tr>
<td>10</td>
<td>0.68</td>
</tr>
<tr>
<td>20</td>
<td>0.65</td>
</tr>
<tr>
<td>40</td>
<td>0.65</td>
</tr>
<tr>
<td>50</td>
<td>0.60</td>
</tr>
<tr>
<td>Control-1</td>
<td>0.75</td>
</tr>
<tr>
<td>Control-2</td>
<td>0.50</td>
</tr>
<tr>
<td>CD (P = 0.05)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

All figures are average of three replications.
Control-1, Inoculated with two 5 mm mycelial discs of F. solani f. sp. pisi.
Control-2, Uninoculated PDB.

50 ml PDB respectively, and inoculated with two 5 mm mycelial discs from one-week old cultures of F. solani. Inoculated flasks were incubated at 28 ± 2°C in an incubator shaker (150 rpm). Flasks containing mycelial discs without culture filtrate served as control-1 and autoclaved PDB (without mycelial discs) served as control-2. After one week of incubation, culture broth was vacuum-filtered and mycelial mat harvested on filter paper. The filter papers were freeze-dried at –50°C for 24 h. Weight of filter papers together with mycelial mat indicated the extent of fungal growth in the broth.

PGP activity in pea (var. Arkel) and BCA activity of PFs against the target pathogen were evaluated in a greenhouse. Two sets of experiment (eight treatments, each with four replications) were maintained. In experiment I, unsterilized soil provided natural soil conditions to PFs and in experiment II, 250 mg pathogen inoculum was added to the top 1 kg soil in each pot to evaluate BCA of PFs. In both the experiments, seeds were treated (6 g/kg seed) by talc formulation of PFs. Ten seeds per pot were sown at a depth of 1 cm. Soils were moistened once a day. Germination was recorded every day after the first seed germinated and was continued till full germination was achieved in any of the treatments. Similarly, mortality was recorded daily. After 30 days the plants were uprooted, washed to remove adhering soil and subjected to observations related to plant growth characteristics, including per cent germination, root length, shoot length, seedling length, fresh weight, dry weight, vigour indices 1 and 2 and mortality.

Data were analysed by following analysis of variance (ANOVA) in CRD to calculate the significance by magnitude of the F value (P = 0.05).

All the four test strains, Pf-102, Pf-103, Pf-110 and Pf-173, successfully inhibited the growth of F. solani f. sp. pisi on KB plates (Table 1). Maximum inhibition was recorded at 5–7 days of incubation. Two types of fungal inhibition was recorded: (i) initial fungal inhibition but restoration of fungal growth on prolonged incubation, and (ii) no restoration of fungal growth resulting in mycelial degradation or lysis. Microscopic examination of both types of inhibition revealed fungistatic action of PFs in the first case, in which mycelial deformities were observed (Figure 1a, arrows), and lytic action in the second case (Figure 1b, arrows). Inhibition of the fungal growth by culture filtrate of PF was significant (60 to 100%) compared to control-1. As the quantity of culture filtrate in the broth increased, a marked decline in mycelial growth was recorded (Table 3). Mycelial growth was completely inhibited when the volume of culture filtrate increased to 50% in the broth.

All four PFs showed good PGP and BCA (Table 4). Significant enhancement in different plant growth parameters was recorded in both sets of experiments. Germinated pea seedlings exhibited enhanced root and shoot length in all the treatments. Maximum root length in Exp. I was recorded for Pf-173 (19.93 cm), while maximum shoot length (35.02 cm) was recorded in a combination of the four PFs. In Exp. II, maximum root length (17.54 cm) was recorded in Pf-103 treatment and highest shoot length (30.39 cm) was recorded for a combination of Pf-102 + Pf-103 + Pf-110. Similarly, seedling weight also increased significantly in both the experiments. A combination of all four test strains was superior to other treatments with respect to fresh and dry weights of pea seedlings and their vigour. Individually,
### Table 4. Effect of *P. fluorescens* isolates and their combinations on plant growth promotion and development of root rot disease in pea (var. Arkel)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination (%)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Seedling length (cm)</th>
<th>Fresh weight (g)</th>
<th>Dry weight (g)</th>
<th>Vigour index -1</th>
<th>Vigour index -2</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pf-102</td>
<td>90.00</td>
<td>70.00</td>
<td>18.9</td>
<td>15.50</td>
<td>25.45</td>
<td>21.85</td>
<td>44.35</td>
<td>37.35</td>
<td>1.95</td>
</tr>
<tr>
<td>Pf-103</td>
<td>92.50</td>
<td>85.00</td>
<td>19.61</td>
<td>17.54</td>
<td>30.45</td>
<td>27.85</td>
<td>50.05</td>
<td>45.39</td>
<td>2.42</td>
</tr>
<tr>
<td>Pf-110</td>
<td>95.00</td>
<td>92.50</td>
<td>18.23</td>
<td>14.81</td>
<td>31.00</td>
<td>28.03</td>
<td>49.23</td>
<td>42.84</td>
<td>1.90</td>
</tr>
<tr>
<td>Pf-173</td>
<td>97.50</td>
<td>87.50</td>
<td>19.93</td>
<td>15.38</td>
<td>32.63</td>
<td>28.03</td>
<td>52.56</td>
<td>43.41</td>
<td>2.32</td>
</tr>
<tr>
<td>Pf-102 + Pf-103</td>
<td>80.00</td>
<td>72.50</td>
<td>18.83</td>
<td>17.22</td>
<td>29.22</td>
<td>28.54</td>
<td>48.05</td>
<td>45.76</td>
<td>2.22</td>
</tr>
<tr>
<td>Pf-102 + Pf-103 + Pf-110</td>
<td>92.50</td>
<td>72.50</td>
<td>17.67</td>
<td>15.66</td>
<td>31.48</td>
<td>30.39</td>
<td>49.15</td>
<td>46.05</td>
<td>2.23</td>
</tr>
<tr>
<td>Pf-102 + Pf-103 + Pf-110 + Pf-173</td>
<td>87.50</td>
<td>87.50</td>
<td>19.80</td>
<td>16.89</td>
<td>35.02</td>
<td>28.40</td>
<td>54.82</td>
<td>45.29</td>
<td>3.19</td>
</tr>
</tbody>
</table>

All figures are average of four replications. Exp. I, Seeds sown in uninoculated soil; Exp. II, Seeds sown in soil inoculated with *F. solani* @ 250 mg per pot; Control, Seeds receiving no treatment; Vigour index -1 was calculated as % germination × seedling length; Vigour index -2 was calculated as % germination × dry weight.
PF-173 in Exp. I (Table 4) and PF-110 in Exp. II (Table 4) recorded highest vigour indices. Almost all treatments showed reduced mortality compared to control-1 and control-2. Treatment with a consortium of Pseudomonas recorded minimum mortality (Table 4).

Enhanced seedling length and seedling weight recorded in the present study was a result of growth promoting abilities of fluorescent Pseudomonas. The PGP ability of fluorescent Pseudomonas is a function of good root colonization and production of growth hormones like auxins, gibberellins and cytokinins\textsuperscript{13-15}. Increase in surface area covered by the root system and mineral solubilization ability of Pf's facilitate increased nutrient uptake, which may increase seedling biomass\textsuperscript{9,16}. Most of the treatments showed significant effect when compared to their corresponding controls in the two experiments. Per cent increment in different plant growth parameters was higher in treatments using Fusarium-inoculated soils (Exp. II), which was due to reduced germination (47.50%) and high mortality (53.33%) in pathogen-inoculated soil in control over which comparisons were made. Suppression of root rot disease clearly indicated the ability of Pf's and their combinations to antagonize F. solani f. sp. pisi and the subsequent reduction in disease development. Secretion of various secondary metabolites by Pseudomonas spp. has been well studied\textsuperscript{2,16}. These metabolites, including antibiotics, chitinolytic enzymes, HCN and siderophore have been found to be inhibitory against different phytopathogens, e.g. soil-borne fungal pathogens\textsuperscript{8,15,17,18}. In the present study, inhibition of F. solani f. sp. pisi in PDB suggested the presence of such metabolites in culture filtrate of Pf's. Good inhibition of pathogen in rhizospheric condition was achieved as Pf's were also equipped with the ability to produce HCN, catalase and siderophore, properties reported to antagonize fungal pathogens\textsuperscript{2,3-10}. Reduction in seedling mortality was maximum in treatments based on a consortium of the four Pf's. Presence or absence of pathogen in the soil can affect bacterial survival. In nature, some bacteria may actually need the pathogen to provide nutrition through nutrient leakage from host plant\textsuperscript{19}.

Generally, diverse populations of PGPRs are supposed to increase the PGP and BCA of the strains, as different strains possess different modes of action\textsuperscript{10,21}. This could be the reason that a combination of the four Pf's in most cases was better than treatments with individual strains, with respect to all planting value parameters. Application of a consortium of PGPR strains would more closely mimic the natural situation and might broaden the spectrum of action to enhance the efficacy and reliability of control\textsuperscript{22}.

It is reasonable to suggest that manipulation of composition of microorganisms in rhizosphere could be accomplished best with sustainable effects by introducing mixtures of compatible microorganisms. The findings of this study clearly indicate that cold-tolerant fluorescent Pseudomonas used here are potential candidates for BCA as well as PGP activities in pea in the region. Their field evaluation as well as applicability in others crops needs to be further studied. Chances of success of these Pf's would be greater, as they would be used in the same environment from where they were isolated, and would easily fit within the framework of organic farming as appropriate bioagents for plant growth and disease management.

RESEARCH COMMUNICATIONS


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Histochemical and biochemical studies of parasite-host interaction of Cassytha filiformis Linn. and Zizyphus jujuba Lamk.

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Parasite-host interaction of Cassytha filiformis and Zizyphus jujuba through histochemical studies revealed the presence of specialized glandular cells facilitating adhesion of parasite to the host and further specialization to obtain nutrients from phloem tissue of the host. Histoenzymological studies indicated the presence of high acid phosphatase activity of the parasite, which revealed the digestion of macromolecules/energy transfer and intercellular transport of parasite. Partial photosynthesis activity of parasite was noticed by accepting hydrogen released from photolysis of water through Hill reaction.

Keywords: Cassytha filiformis, haustoria, Hill reaction, parasite-host interaction, Zizyphus jujuba.

PARASITES are unusual plants, well adapted to their mode of life. More than 2500 species of higher plants are known to live parasitically on other plants. These parasitic plants produce flowers and seeds and belong to several widely separated botanical families. They vary greatly in their dependence on their host plants. For example, Viscum (mistletoes) have chlorophyll but no roots and therefore depend on their hosts only for water and minerals. Cuscuta (dodder) and Cassytha (amarbelii) have little chlorophyll and no true roots. Hence they depend on their hosts for water, food and minerals. The most common and serious parasites belong to the following botanical families and genera.

Cuscutaceae: Cuscuta (dodder); Scrophulariaceae: Striga (witch weed); Orobanchaceae: Orobanche (broom raperes); Cassythaceae: Cassytha (amarbeli); Loranthaceae: Elaeagnus, Korthalsella and Loranthus, Viscaceae: Arceuthobium (dwarf mistletoes); Phoradendron (American true mistletoes) and Viscum (European true mistletoes).

Cassytha filiformis Linn. Cassythaceae (Figure 1 a) is a twining parasitic, perennial angiosperm which adheres to the host by suckers (haustoria). The plant is characterized by leaves reduced to minute scales, small flower, hermaprodite, sessile, spicate, perianth tube short and globose, stamens six, ovary globose and fruit a drupe enclosed in the enlarged inflated perianth tube. During winter Cassytha seeds grow along with the seeds of the host plant (Zizyphus) in infested fields. During the growing season, the

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