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ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of fluorescent *Pseudomonas* spp. against *Pyricularia grisea, Rhizoctonia solani* and *Sclerotium rolfsii* causing blast, Sheath blight and foot rot diseases of finger millet (*Eleusine coracana* L.) crop in mid hills of Uttarakhand: *In vitro* study

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ABSTRACT
'Millets' is a collective term used to describe a number of small-grained cereal grasses. Millets are usually subdivided into large millets and small millets. The crop under the umbrella of small millet is finger millet (Eleusine coracana L.), being a hardy crop, it is also affected by many diseases among them blast, sheath blight and foot rot diseases caused by Pyricularia grisea (Perfect stage- Magnaporthe grisea), Rhizoctonia solani and Sclerotium rolfsii has been an increasing problem. Since then, these diseases have been causing enormous losses and also hampering the net productivity. Thus, adversely affecting the livelihood of marginal farmers who grow rainfed finger millet as staple food crops in hills of Uttarakhand. A study was conducted to evaluate the potential of fluorescent Pseudomonas isolates against blast, sheath blight and foot rot diseases of finger millet crop. An experiment was conducted in Division of Plant Pathology, College of Forestry, Ranichauri, New Tehri, to isolate, characterize and evaluate antagonistic efficacy of Pseudomonas spp. collected from different regions of Garhwal hills of Uttarakhand from rhizospheric soil of finger millet. Totally twenty fluorescent Pseudomonas isolates were isolated and only ten isolates were evaluated for antagonistic efficacy under in vitro condition against the three fungal pathogens namely P. grisea, R. solani and S. rolfsii. Among all the tested isolates, UUHF Psf-4 isolate proved to be effective than other isolates as showed maximum percent inhibition (72.30 % after 7 days of incubation period respectively) of P. grisea, whereas in case of Sclerotium rolfsii and Rhizoctonia solani (72.50 % and 68.75 % after 7 days of incubation period respectively) was measured, followed by UUHF-Psf-11 while minimum was recorded with the isolate UUHF Psf-10. The study reveals the potential of the isolate UUHF Psf-4 in controlling P. grisea, R. solani and S. rolfsii, causing blast, sheath blight and foot rot diseases in finger millet in mid hills of Uttarakhand.
Key Words: Blast, *Eleusine coracana*, Finger millet, Foot rot, *Pseudomonas,* Sheath blight

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INTRODUCTION

Small millets are small seeded annual cereal grasses used for food, feed and forage all over world particularly in tropical and certain parts of the warm temperate region of the world. The principle members of small millets group are finger millet (*Eleusine coracana* Gaertn.), barnyard millet (*Echinochloa frumentacea* (Roxb.) Link.), foxtail millet (*Setaria italica* Beauv.), proso millet (*Panicum milliaceum* Linn.), kodo millet (*Paspalum scrobiculatum* Linn.) and little millet (*Panicum miliare* Lam.) that are popular as 'nutricereals' owing to their rich calcium, iron, fibre content. All these small millets are cultivated in one or more states in India especially in rainfed and tribal areas. Among all the small millets, finger millet locally known as ragi, mandua, nagli, kapai, and marua occupies a special position in the hill agriculture of Uttarakhand occupying the largest area next only to rice. In India, the total area under finger millet cultivation in year 2012 was 11,93,700 hectare, with a production of 19,82,900 tons and productivity of about 16.61 quintals per hectare. Finger millet, locally known as mandua is the second major *Kharif* crop in the state of Uttarakhand. The coverage area of finger millet in Uttarakhand was 122,200 hectares with a production of 153,900 tons and a productivity of 13.72 quintal per hectare in year 2013-14 (Directorate of Economics and Statistics, Ministry of Agriculture. G.O.I, 2013-14). Though

millets rank sixth in world grain production after rice, wheat, maize, barley and sorghum, although they are usually considered as a subsistence product and more often than not looked upon as a poor man's crop [9]. The novel technologies in all areas of agriculture have improved agricultural production, but it affecting the environment. However, it is known to be effected by several disease *viz.,* blast, banded blight, smut, rust, foot rot and viral diseases [13].The recent challenge faced by modern agriculture is to achieve satisfactory control of plant disease in environment friendly manner [3.The search for effective alternative approaches to chemical control which have minimal deleterious effects, more ecofriendly and will contribute to the goal of sustainability in agriculture is needed [6]. Biological control is a potential alternative to chemically based disease control. Because of this increasing awareness of the problems associated with the chemically based disease control, biological control has received considerable attention during the last 40 years as a potential alternative [4].

Rhizosphere supports microbial populations exerting beneficial or detrimental effect on plant growth, which play a vital role in root health maintenance, nutrient uptake and environmental stress tolerance [1]. These beneficial microorganisms, including plant growth promoting rhizobacteria (PGPR) form a significant component of plant disease management strategies. Plant growth promoting bacteria (PGPR) are indigenous to soil as well as the plant rhizosphere and play a major role in the biocontrol of plant pathogens.

Fluorescent pseudomonads, a group of PGPR are the most studied ones. They help in soil health maintenance and are metabolically and functionally most diverse [10]. Fluorescent pseudomonads provide effective protection against bacterial and fungal pathogens, parasites and certain nematode infections [17]. Some strains have been recognized for a long time as biocontrol agents [12]. Present study is done to isolate, characterize and evaluate the antagonistic efficacy of native isolates of fluorescent pseudomonads.

MATERIALS AND METHODS

Isolation and identification of *Pseudomonas*

The soil samples were collected from the healthy finger millet plants grown in different regions of Garhwal hills (Table 1). Each sample was taken separately in polythene bags, tied with a rubber band and labeled. Soil samples were analyzed on the day of collection. One gram of soil was mixed thoroughly in 100 ml sterile water and processed to follow serial dilution plate techniqueand was spread on King's B (KB) medium to isolate *Pseudomonas* spp. One ml of last serial dilution *i.e.*, 10^{-8} was spread on King's B Medium [7] for proper isolation. The plates were incubated for 2 days at 28±2ºC and after incubation, pure culture was grown; colour of bacterial colony was initially yellow but turned yellow green as pigmentation were produced [2]. Colonies that developed on KB plates were observed under UV light on a transilluminator. The colonies fluorescing under UV light were picked up, purified and preserved in nutrient broth. The *Pseudomonas* spp. isolated from different regions were coded in Table 1.

Morphological study

Pure cultures of the selected isolates were streaked on King's B (KB) agar petri plates separately for colony development. The individual colonies were examined for shape, size, structure of colonies and pigmentation after 72 hrs (Fig. A).

Fungal pathogens

Three potential fungal pathogens (*Pyricularia grisea, Rhizoctonia solani* and *Sclerotium rolfsii*), involved in blast, sheath blight and foot-rot complex in finger millet crop, were obtained from the well-characterized culture stock being maintained in the Plant Pathology Laboratory, Hill Campus, Ranichauri (Fig. B and C).

Biochemical characterization of bacterial isolates

Biochemical tests specific for *Pseudomonas* spp., such as gelatin hydrolysis [14], the oxidase test [8], catalase [5], levan formation [11], starch hydrolysis (Lelliott et al.,1966) and ammonification (Lee and Kobyashi, 1989), were performed to distinguish fluorescent pseudomonads from rhizospheric populations of other bacteria.

Culture and maintenance

Medium consists of proteose peptone 20g, agar agar 20g, di potassium hydrogen phosphate (K_2HPO_4) 1.5g, magnesium sulphate ($MgSo_4$) 1.5g, glycerol 10ml and distilled water 1000 ml [7]**.** After mixing all the ingredients with distilled water, media was placed into a stainless steel pan and stirred with glass rod for proper mixing of all the ingredients. Now 200 ml medium was placed in each 500 ml capacity flasks. Flasks were tightly plugged with non-absorbent cotton plug and wrapped with butter paper and rubber band. Medium was autoclaved at 15 psi (121 ºC) for 30 min and cooled before pouring into Petri plates.

Another medium contain agar 20 g, dextrose 20 g, potato (peeled and sliced) 200 g, distilled water 1 L. 250 g of potato was peeled and cut into small and fine sliced pieces. Exactly 200g of potato pieces were weighed and placed into a stainless steel pan. 500 ml of water was added to potato pieces and boiled

gently for such a period until they are easily mashed by a glass rod. The decoction was filtered through muslin cloth and squeezed out all the liquid in a measuring cylinder and potato pieces were discarded. Now sufficient amount of water was added to make the volume 1000 ml. Now preweighed agar was added (20g) bit by bit to the boiling solution to dissolve it. At the same time dextrose (20g) was also added in boiling solution (melted with agar) and final volume made up to 1 L. It was poured @ about 200 ml in each of four conical flasks of 500 ml and 10 ml per culture tube to prepare the PDA slants. Both, flasks and culture tubes were tightly plugged with non-absorbent cotton and wrapped with butter paper and rubber bands. The culture tubes and flasks were placed vertically (mouths up) in wire baskets and then autoclaved at 15 psi (121 ºC) for 30 min. The bacteria, initially isolated in a pure culture on King's B media and sub cultured on PDA slants were allowed to grow at 28±2^{0}C temperature. The culture thus obtained was stored in refrigerator at 5^{0}C for further studies and was sub cultured periodically.

Antagonism of fluorescent pseudomonads

The antagonistic activity of fluorescent pseudomonads against *Pyricularia grisea, Rhizoctonia solani* and *Sclerotium rolfsii* were tested by dual culture technique. Bacterial isolates were streaked at one side of Petri dish (one cm away from the edge) containing PDA. 9 mm mycelial disc from seven days old PDA cultures of *P. grisea, R. solani* and *S. rolfsii* were placed at the opposite side of petri dishes perpendicular to the bacterial streak respectively and incubated at 27±2^{0}C for 7 days. Petri dishes inoculated with fungal discs alone served as control. Three replications were maintained for each isolate. Observations on width of inhibition zone and mycelial growth of test pathogen were recorded and per cent inhibition of pathogen growth was calculated by using the formula proposed by Vincent (1927).

Per cent inhibition (I) = C-T/C ×100

Where,

C- mycelial growth of pathogen in control

T- mycelial growth of pathogen in dual culture plate

RESULTS AND DISCUSSION

Isolation of fluorescent *Pseudomonas* spp.

A total of 20 native isolates of fluorescent *Pseudomonas* spp. were obtained from the rhizospheric soil samples of finger millet collected from different regions of Garhwal hills (Table 1) by using serial soil dilution technique.

Table 1: Description of host and location of rhizospheric soil samples used for isolation of *Pseudomonas* isolates for present study

S.No.	Host	Location		Isolates
		Village	Districts	
1	Finger millet	Phata	Rudraprayag	UUHF *Psf*-1
2	Finger millet	Phata	Rudraprayag	UUHF *Psf*-2
3	Finger millet	Phata	Rudraprayag	UUHF *Psf*-3
4	Finger millet	Phata	Rudraprayag	UUHF *Psf*-4
5	Finger millet	Lamgondi	Rudraprayag	UUHF *Psf*-5
6	Finger millet	Lamgondi	Rudraprayag	UUHF *Psf*-6
7	Finger millet	Naag	Rudraprayag	UUHF *Psf*-7
8	Finger millet	Naag	Rudraprayag	UUHF *Psf*-8
9	Finger millet	Naag	Rudraprayag	UUHF *Psf*-9
10	Finger millet	Naag	Rudraprayag	UUHF *Psf*-10
11	Finger millet	Khat	Rudraprayag	UUHF *Psf*-11
12	Finger millet	Khat	Rudraprayag	UUHF *Psf*-12
13	Finger millet	Khat	Rudraprayag	UUHF *Psf*-13
14	Finger millet	Korkhi	Rudraprayag	UUHF *Psf*-14
15	Finger millet	Korkhi	Rudraprayag	UUHF *Psf*-15
16	Finger millet	Daargi	New Tehri	UUHF *Psf*-16
17	Finger millet	Daargi	New Tehri	UUHF *Psf*-17
18	Finger millet	Gaja	New Tehri	UUHF *Psf*-18
19	Finger millet	Gaja	New Tehri	UUHF *Psf*-19
20	Finger millet	Gaja	New Tehri	UUHF *Psf*-20

Morphological characterization

The colony colour of eight isolates (UUHF *Psf*- 1, 2, 3, 6, 7, 9, 14 and 18) were pale yellow, dull yellowish and yellowish white with high, medium and weak fluorescens, respectively. Some isolates (UUHF *Psf*- 4, 10, 11, 12, 15, and 20) were round, non- spreading colony and rod shaped cells produced light green colour, while colonies were round with rod shaped cells. The fluorescens was high in the isolates UUHF

Psf-5, 8, 13, 16, 19 and 17 and the colony shape was irregular and spreading in nature having rod shaped cells (Table 2).

Biochemical characterization

In all, 20 bacterial isolates were collected from different regions of Garhwal hills of Uttarakhand. Among these, 15 were positive for catalase test, whereas 18 were negative for starch hydrolysis. A total of 16 isolates were found positive for gelatin hydrolysis and all isolates were positive for oxidase activity. A set of 15 isolates produced levan on sucrose-amended medium and were recorded positive for the trait. Of the 20 isolates that were subjected to the ammonification test, 15 were positive for this activity (data not shown).

Table 2: Morphological characteristics of native bacterial isolates

+ = Weak fluorescence, ++ = Medium fluorescence, +++ = High fluorescence.

S. No	Isolates	Colony Morphology			Cell shape	Fluorescence
		Shape	Color	Nature		
1	UUHF *Psf*-1	Irregular	Pale yellow	Spreading	Rod	++
2	UUHF *Psf*-2	Irregular	Dull yellowish	Non- spreading	Rod	+
3	UUHF *Psf*-3	Round	Pale yellow	Spreading	Rod	++
4	UUHF *Psf*-4	Round	Green	Non- spreading	Rod	+++
5	UUHF *Psf*-5	Irregular	Green	Spreading	Rod	++
6	UUHF *Psf*-6	Round	Yellowish white	Spreading	Rod	++
7	UUHF *Psf*-7	Irregular	Yellowish white	Spreading	Rod	+++
8	UUHF *Psf*-8	Irregular	Green	Spreading	Rod	+
9	UUHF *Psf*-9	Round	Dull yellowish	Spreading	Rod	+++
10	UUHF *Psf*-10	Round	Green	Non- spreading	Rod	+++
11	UUHF *Psf*-11	Round	Green	Non- spreading	Rod	+++
12	UUHF *Psf*-12	Round	Green	Non- spreading	Rod	+++
13	UUHF *Psf*-13	Irregular	Green	Spreading	Rod	+
14	UUHF *Psf*-14	Round	Dull yellowish	Spreading	Rod	++
15	UUHF *Psf*-15	Round	Green	Non- spreading	Rod	+++
16	UUHF *Psf*-16	Irregular	Green	Spreading	Rod	+
17	UUHF *Psf*-17	Irregular	Green	Spreading	Rod	+++
18	UUHF *Psf*-18	Irregular	Dull yellowish	Spreading	Rod	++
19	UUHF *Psf*-19	Irregular	Green	Spreading	Rod	+

Table 3: *In vitro* antagonistic ability of fluorescent *Pseudomonas* isolates against *Pyricularia grisea, Rhizoctonia solani* and *Sclerotium rolfsii*

S. No.	**Isolates**	***P. grisea***		***R. solani***		***S. rolfsii***	
		Mycelial growth (cm)*	**Mycelial growth inhibition (%)***	**Mycelial growth (cm)***	**Mycelial growth inhibition (%)***	**Mycelial growth (cm)***	**Mycelial growth inhibition (%)***
1	UUHF P*sf*-1	2.80	56.92	4.30	46.25	3.30	58.75
2	UUHF P*sf*-3	4.00	38.46	5.50	31.25	3.70	53.75
3	UUHF P*sf*-4	1.80	72.30	2.50	68.75	2.20	72.50
4	UUHF P*sf*-7	4.20	35.38	3.90	51.25	3.80	52.50
5	UUHF P*sf*-10	4.50	30.76	4.30	46.25	4.30	46.25
6	UUHF P*sf*-11	2.00	69.22	2.90	63.75	2.40	70.00
7	UUHF P*sf*-12	2.30	64.61	3.20	60.00	2.50	68.75
8	UUHF P*sf*-15	2.50	61.53	3.50	56.25	2.60	67.50
9	UUHF P*sf*-17	3.00	53.84	3.80	52.50	3.00	62.50
10	UUHF P*sf*-20	2.60	59.99	3.60	55.00	3.60	55.00
11	Control	6.50	-	8.00	-	8.00	-
	CV (%)	**2.897**	**2.971**	**3.645**	**3.902**	**2.662**	**2.158**
	CD at 5%	**0.161**	**2.483**	**0.255**	**3.191**	**0.161**	**2.018**

*Mycelial growth and per cent inhibition of the pathogen by fluorescent *Pseudomonas* isolates were calculated after 7 days of incubation period when the control plate was fully covered by the pathogen.

Dual culture technique

Among all the 20 rhizobacterial isolates, only ten (UUHF *Psf*-1, 3, 4, 7, 10, 11, 12, 15, 17 and 20) isolates were tested for their antagonistic efficacy through inhibiting the fungal plant pathogen mycelial growth namely *Sclerotium rolfsii*, *Rhizoctonia solani* and *Pyricularia grisea* causing foot rot, sheath blight and blast diseases in finger millet respectively, using dual culture assay. Actively growing four days old pure cultures of isolated pathogens were used for dual culture assay.The inhibition efficacy of the fluorescent *Pseudomonas* isolates was assessed on the basis of reduction in growth diameter of the fungal pathogens in the test plates in comparison to the control plates.

The results of the dual culture technique indicated that all the ten isolates inhibited the growth of the tested fungal pathogens significantly. When tested against the *Pyricularia grisea,* isolate UUHF *Psf*-4 was

found to be most effective in giving maximum per cent inhibition with 72.30 % efficacy followed by UUHF *Psf*-11 (69.22 % inhibition of pathogen), UUHF *Psf*-12 (64.61 % inhibition of pathogen) and UUHF *Psf*-15 (61.53 % inhibition of pathogen). While minimum, 30.76 % inhibition of pathogen was recorded with the isolate UUHF *Psf*-10.

Against *R. solani,* of the ten fluorescent *Pseudomonas* isolates, all isolates were found effective in inhibiting hyphal growth of the pathogen under *in vitro*. Isolate UUHF *Psf*-4 was found to be most effective in inhibiting pathogen growth with maximum percent inhibition (68.75 %), followed by UUHF *Psf*-11 (63.75 %), UUHF *Psf*-12 (60.00 %) and UUHF *Psf*-15 (56.25 %) respectively. Whereas in case of *Sclerotium rolfsii,* a maximum inhibition of 72.50 % was recorded by UUHF P*sf*-4 followed by UUHF *Psf*-11 (70.00 %), UUHF *Psf*-12 (68.75 %) and UUHF *Psf*-15 (67.50 %) respectively (Table 3).

Attempts were made to isolate and maintain different strains of *Pseudomonas* spp. from different agroecological zones, so that they can be used in future for stress management in various crops in varying agroecological situations. The isolate UUHF P*sf*-1 to UUHF P*sf*-4 were isolated from the rhizosphere of finger millet (*Eleusine coracana*) from a farmer field of the village Phata, isolates UUHF P*sf*-5and 6 from village Lamgondi, UUHF P*sf*-7 to 10 from Naag village, and UUHF P*sf*- 11 to 13 were isolated from village Khat, and the isolates UUHF P*sf*-14 and 15 from the village Korkhi of Rudraprayag district. The isolates UUHF P*sf*-16, 17 from Daargi village and UUHF P*sf*-18 and 19 and UUHF P*sf*-20 were isolated from a farmer field of village Gaja from New Tehri district. The results of the dual culture technique indicated that the UUHF P*sf*-4 isolate was able to inhibit growth of tested fungal pathogen significantly. Saravanan et al. [15] focused on the antagonistic potential of fluorescent *Pseudomonas in vitro,* and its inoculation effect on growth performance of *Lycopersicon esculentum* in *Fusarium oxysporum* and *Rhizoctonia solani* infested soil. Biochemical characteristics of fluorescent *Pseudomonas* showed that all ten isolates were positive to catalase, amylase, gelatinase and siderophore production. While three isolates (Pf5, Pf6 and Pf9) were oxidase positive, nine isolates (Pf1, Pf2, Pf3, Pf4, Pf6, Pf7, Pf8, Pf9, and Pf10) were tolerant to 6.5% NaCl. Isolates Pf5 and Pf6 were resistant to all the test antibiotics; in contrast, the remaining eight isolates responded differently to different antibiotics. Isolates Pf5 and Pf6 were antagonistic against 14 bacterial species, and two pathogenic fungi (*F. oxysporum* and *R. solani*). Treatment of plants with either *F. oxysporum*or *R. solani* drastically reduced the root and shoot length and dry weight of the plant. However, in the presence of fluorescent *Pseudomonas* the adverse effect of the pathogens on growth of *L. esculentum* was alleviated. Maximum inhibition was observed in *Sclerotium rolfsii* (63.15 %) followed by *Fusarium oxysporum* (61.85 %), *Rhizoctonia bataticola*(55.56 %) and *Rhizoctonia solani* (53.15 %).

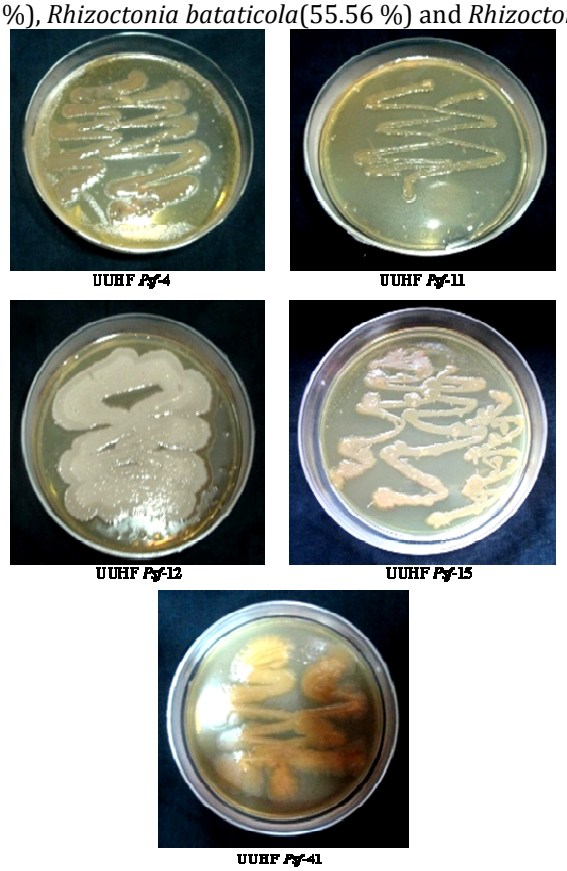

Fig 1A. Pure cultures of isolated *Pseudomonas* spp.

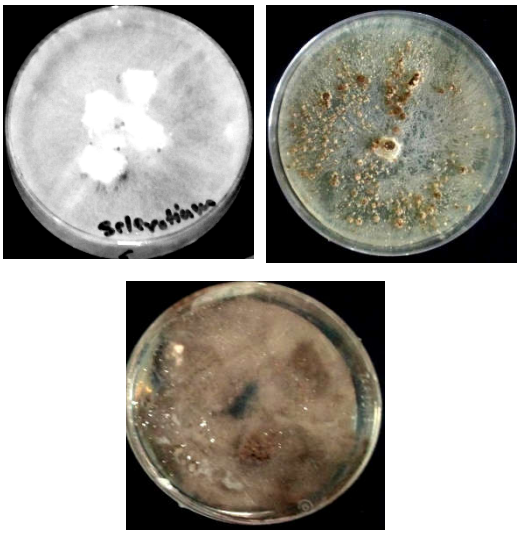

Fig 1C. Pure culture of Pathogens *viz., S. rolfsi, R. solani* and *P. grisea*

S. rolfsii causing foot rot *R. solani causing* sheath blight

P. grisea causing blast

Fig 1B. Major diseases of finger millet prevalent in Garhwal region of Uttarakhand hills.

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