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RESEARCH PAPER

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Feather degrading, phytostimulating, and biocontrol potential of native actinobacteria from North Eastern Indian Himalayan Region

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Twelve actinobacterial strains were isolated from tomato rhizospheric soil from Manipur, a state in North East Indian Himalayan Region and screened for keratinolytic and plant growth promoting traits. Nine promising isolates were identified as *Streptomyces* species using partial 16S rRNA gene sequencing. Among the seven isolates showing chicken feather degradation activity, three keratinolytic strains RCM-SSR-2, -6, and -12 were found to be the most efficient feather degrading strains achieving 90% feather weight loss within 48 h of incubation. They also showed maximum keratinase and soluble peptide production. Strain RCM-SSR-2, -5, -6, -8, and -11 showed positive results for all plant growth promoting traits tested. Maximum indole-3-acetic acid production was exhibited by RCM-SSR-6. Strain RCM-SSR-1, -2, -5, -6, -9, and -11 showed antagonistic activity against three important plant pathogens. Feather hydrolysate of RCM-SSR-6 was also evaluated for *in vitro* seed germination test using garden pea seeds. Higher concentration of feather protein hydrolysate (3 mg ml^{-1}) inhibited shoot and root length of the germinating embryo. However, lower concentration (0.01 mg ml^{-1}) of feather protein hydrolysate promoted seed germination. Among the 12 strains, four isolates namely RCM-SSR-1, -2, -5, and -6 were found to be promising as multi-traits plant growth promoting rhizobacteria for development of organic fertilizer, phytostimulator, and biocontrol agents.

KEYWORDS
actinobacteria, biocontrol, keratinolytic, phytostimulator, *Streptomyces*

1 | INTRODUCTION

The development of new organic products using natural waste materials and native microbes has become the focus of current microbiological research. During last few decades, many researchers have focused upon improvement in agronomic utilization of organic wastes [1]. Several organic wastes from

Abbreviations: CFM, chicken feather medium; IAA, indole-3-acetic acid; MSL, mean sea level; PGP, plant growth promoting; PGPR, plant growth promoting rhizobacteria; SCNA, starch casein nitrate agar; SCNB, starch casein nitrate broth; SDW, sterile distilled water.

animal origin, such as blood meal, hooves, horns, feathers, bones, and manure have been evaluated as a potential organic fertilizer [2]. Organic farming depends on the use of nitrogen-rich organic amendments that serve the dual purpose of improving plant growth and stimulating microbial activity in soil [3]. In this regard chicken feather waste is considered as naturally available good source of nitrogen. Ninety percent of feather weight is composed of pure protein known as keratin [4]. Keratin is very hard to degrade by common proteolytic enzymes such as trypsin, pepsin, etc. [5]. However, some keratinolytic microorganisms could degrade chicken feather. By use of efficient keratinolytic strains chicken feather waste could be transformed into nitrogen-rich feather meal. Feather meal thus produced will be an inexpensive and easily available potential nitrogenous fertilizer of organic origin.

Moreover, interest in use of plant growth promoting rhizobacteria (PGPR) as bio-control agents against plant pathogens and also for mining soil fertility for sustainable agriculture has increased tremendously. However, there are many challenges for using PGPR at commercial level. Most of the PGPRs show inconsistent performance in the field conditions other than their natural habitat [6]. Hence, there is an urgent need for screening of indigenous microbial strains suited to local conditions for sustainable crop production.

From an agro-microbiological point of view, keratinolytic microorganism, which is able to show both biocontrol and plant growth promoting activities, could offer a number of economic and environmental advantages over chemical-based fertilizer or pesticides. However, most of the studies on keratinolytic microorganisms have been focused on keratinase used for increasing the digestibility of feathers as an animal feed [7]. Hence, there is meager report on the isolation and characterization of keratinolytic and plant growth-promoting microorganism for agronomic utilization.

Moreover, use of keratinolytic PGPR as bioinoculants would benefit the plants in three ways; first it releases nitrogen into the soil through feather waste degradation, and secondly it colonizes plant root and promote plant growth by exerting plant growth promoting activities (indole-3-acetic acid production, phosphate solubilization, siderophore production, etc.), and lastly it protects plant from disease causing pathogens. The aim of the present study was to isolate and screen native actinobacteria from North East Indian Himalayan Region for feather degradation, plant growth promotion, and biocontrol activities.

2 | MATERIALS AND METHODS

2.1 | Isolation

Soil samples were collected from tomato rhizosphere in Manipur (25°05′N, 94°33′E, 770 m above msl). Samples were air dried for 2 weeks at room temperature. Then 1 g each of the air-dried sample was pretreated with 0.1 g $CaCO_3$. Isolation was done on starch casein nitrate agar (SCNA), pH 7.2 [8] by serial dilution plating (10^{-2}–10^{-6}). Morphologically distinct colonies were picked up and subcultured to obtain pure cultures. The purified cultures were preserved as agar slants (4 °C) and glycerol stocks (20% v/v, −20 °C) for further use.

2.2 | Identification of bacteria

Identification of bacterial isolates was performed by 16S rRNA gene sequence analysis. The 16S rRNA gene was amplified using the primers 27F (5-AGAGTTTGATCMTGGCTCAG-3) and 1492R (5-CGGTTACCTTTGTTACGACTT-3). The PCR mixture contained 12.5 µl 2× Master mix (GCC Biotech India), 1 µl of each primer (100 µM) and the final volume was made up to 25 µl with deionized water. PCR was carried out with initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 90 s and final extension at 72 °C for 10 min. The sequencing of amplified 16S rRNA gene was done by Macrogen Inc., Seoul, South Korea. The partial 16S rRNA gene sequence of the strain was identified using the EzTaxon-e server database [9] and NCBI GenBank databases. The sequences were aligned with the 16S rRNA gene sequences of other related species using CLUSTAL X v2.1. Phylogenetic analysis was performed using the software package MEGA v5.2 [10].

2.3 | Keratinolytic activity

Actinobacterial strains were screened for feather degradation in chicken feather medium (CFM) containing chicken feather, 1% (w/v); KH_2PO_4, 0.1% (w/v); K_2HPO_4, 0.3% (w/v) and $MgSO_4$, 0.02% (w/v), pH 7. Extent of feather degradation was studied by monitoring feather weight loss, soluble peptide, and keratinase production. Feather weight loss was measured according to Kshetri and Ningthoujam [11]. Soluble peptide content was determined according to Lowry method [12] using bovine serum albumin (BSA) as standard. For measuring keratinase activity according to Kshetri and Ningthoujam [11], 20 mg keratin azure was suspended in 4 ml of 50 mM phosphate buffer, pH 7. One ml of appropriately diluted enzyme was added and incubated at 55 °C in a water bath for 1 h. The reaction mixture was filtered through glass wool and absorbance was measured at 595 nm (Thermo Fisher Scientific, Genesys 10UV Spectrophotometer). One unit of keratinolytic activity was defined as the amount of enzyme that led to an increase in absorbance of 0.01 at 595 nm under standard assay conditions.

2.4 | Ammonia production

Ammonia production was screened in peptone water. Strains were inoculated in 10 ml peptone water (broth) and kept in a shaker (150 rpm, 30 °C) for 5 days; followed by addition of 0.5 ml of Nessler's reagent in each tube. Development of brown to yellow color indicated ammonia production [13].

2.5 | Indole-3-acetic acid (IAA) production

IAA production was determined according to the method of Bano and Musarrat [14]. Strains were inoculated in starch casein nitrate broth (SCNB) containing 2 mg ml^{-1} of L-tryptophan and kept incubated in a shaker (150 rpm, 30 °C, 5 days). The culture broths were centrifuged (8000*g*, 10 min) and 1 ml of the supernatant was mixed with 2 ml of Salkowski reagent. Reaction mixtures were kept for 20 min at room temperature. Appearance of pink color indicated IAA production. For the quantitative assay, the absorbance was measured at 530 nm, and the amount of IAA produced was calculated by comparing with the standard IAA curve.

2.6 | Siderophore production

Siderophore production was assayed according to You et al. [15] with some modifications. Agar plugs (6 mm diameter) of strains were inoculated on SCNA (without iron) amended with CAS-substrate and kept incubated at 30 °C for 7 days. Orange color halo zones surrounding the colonies were indicative of siderophore production.

2.7 | Phosphate solubilization

Solubilization of tricalcium phosphate was determined by spot inoculation of a loopful of the isolate in National Botanical Research Institute's phosphate growth medium containing bromophenol blue (NBRIP-BPB) as per the method given by Mehta and Nautiyal [16]. The strain fully grown on SCNA was spot inoculated in the NBRIP-BP medium and kept incubated at 30 °C for 5 days. Formation of halo zone around the colony indicated phosphate solubilization.

2.8 | Biocontrol activity

The actinobacterial strains were subjected to biocontrol assay against three important fungal pathogens namely, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Pyricularia oryzae* on oat meal agar plates by dual culture technique. Agar plugs (6 mm) of 5 days old *Streptomyces* cultures grown on SCNA were placed at the corners or streak horizontally on the oat meal agar plates leaving 1 cm from the margins. The plates were incubated at 30 °C for 24 h. Fungal plugs (6 mm) were then placed at the centers of the plates. Plates containing fungal plugs without the isolates were kept as controls. Then the plates were incubated at 30 °C for 5 days. Colony growth inhibition was calculated using the following formula:

$$\text{Percentage of colony growth inhibition} = [(C\text{-}T)/C] \times 100$$

where C represents the radial growth (mm) of the test pathogen in the control plates and T is the radial growth (mm) of the test pathogen in the test plates [17].

2.9 | *In vitro* seed germination test (Vigor index)

Bacterial strain was grown in feather medium for 72 h. Fermentation broth was filtered through a sieve (0.2 mm) to remove the undigested feather and feather hydrolysate was collected. Garden pea seeds were surface sterilized with 70% ethanol for 5 min followed by 0.2% sodium hypochlorite for 5 min and rinsed four times with sterile-distilled water (SDW). Sterilized seeds were soaked in different concentration of feather hydrolysate and kept under shaking conditions (150 rpm, 30 °C, 2 h). The seeds were then transferred to sterile plates containing wetted filter papers (10 seeds per plate). Pea seeds soaked in SDW were used as control. Plates were incubated at 28–30 °C and, after 5 days, the number of germinated seeds, root lengths, and shoot lengths were recorded and compared with the control. Three replications were taken per treatment and the experiments were repeated twice. Vigor index was calculated using the Baki and Anderson [18] formula:

$$\text{Vigour Index} = \text{Percent germination} \times \text{seedling length}$$

where seedling length = shoot length + root length.

2.10 | Statistical analysis

All the experiments were carried out in triplicate and the values given were the means ± SD values. Data obtained from the *in vitro* seed germination study was statistically analyzed using one-way ANOVA ($p \leq 0.05$) followed by Duncan's multiple range test.

3 | RESULTS

3.1 | Isolation and identification of actinobacteria

Twelve actinobacterial strains were isolated from tomato rhizospheric soil on SCNA medium. Of these, nine actinobacterial isolates were identified by partial 16S rRNA gene

sequence analysis. All the isolates were identified as *Streptomyces* species and partial 16S rRNA gene sequences were deposited in NCBI GenBank with the following accession numbers: KY659230–KY659238 (Table 1). Phylogenetic analysis based on 16S rRNA gene of the isolates with their closest *Streptomyces* species is presented in Fig. 1.

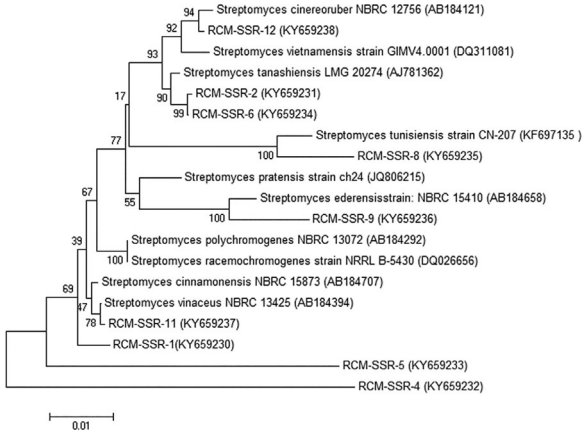

FIGURE 1 Neighbor-joining tree based on partial 16S rRNA gene sequences showing the relationships between actinobacterial strains and other closely related *Streptomyces* species. The tree was constructed with MEGA 5.2 using Neighbor-joining method. Bootstrap values indicated at nodes

3.2 | Screening for biodegradation of chicken feather waste

Seven isolates (RCM-SSR-1, -2, -4, -5, -6, -8, and -12) were found to degrade chicken feather waste. Strains RCM-SSR-2, -6, and -12 could achieve 90% feather weight loss with maximum enzyme and soluble protein production (Table 2).

3.3 | Screening of phytostimulating traits

The isolates were screened for PGP traits such as phosphate solubilization, production of ammonia, IAA, and siderophore. Of the 12 actinobacterial strains, 10 strains were positive for siderophore production, 7 strains for IAA production, and 8 strains for phosphate solubilization. All the 12 strains were found to be positive for ammonia production (Table 3). IAA production was observed in range of 10–38 $\mu g\ ml^{-1}$ (Fig. 2). RCM-SSR-6 showed highest IAA production ($36.3 \pm 2.1\ \mu g\ ml^{-1}$) followed by RCM-SSR-11 ($25 \pm 0.2\ \mu g\ ml^{-1}$), RCM-SSR-8 ($22.0 \pm 2.0\ \mu g\ ml^{-1}$), and RCM-SSR-5 ($21.5 \pm 0.8\ \mu g\ ml^{-1}$).

3.4 | Screening of biocontrol activity

The actinobacterial strains were screened for their biocontrol activity against major three fungal phytopathogens by dual culture method (Fig. 3). Among the strains, RCM-SSR-1, -2, -5, -6, -9, and -11 showed antagonistic activity against the three fungal pathogens viz. *Fusarium oxysporum*, *Rhizoctonia solani*, and *Pyricularia oryzae*. Strain RCM-SSR-5 and -11 showed more than 50% growth inhibitions in all the fungal phytopathogens (Table 4).

3.5 | Seed germination test

Feather hydrolysate produced by RCM-SSR-6 was evaluated for *in vitro* seed germination test using green garden pea seeds. Vigor index of seed germination was inhibited by high concentration feather protein hydolysate. However, when the seeds were treated with diluted feather hydrolysate (5× to 100×), vigor index was found to be increased. Maximum increase in root length, shoot length, and vigor index was observed with 30× dilution (0.1 mg protein ml^{-1}) (Table 5).

4 | DISCUSSION

In the present study, 12 actinobacterial strains were isolated from tomato rhizospheric soil on SCNA medium. SCNA

TABLE 1 Identification of actinobacterial species

Isolate code	Closest species	Similarity (%)	Accession no.
RCM-SSR-1	*Streptomyces virginiae*	97.59	KY659230
RCM-SSR-2	*Streptomyces tanashiensis*	98.25	KY659231
RCM-SSR-4	*Streptomyces polychromogenes*	92.24	KY659232
RCM-SSR-5	*Streptomyces pratensis*	92.90	KY659233
RCM-SSR-6	*Streptomyces tanashiensis*	99.63	KY659234
RCM-SSR-8	*Streptomyces tunisiensis*	97.25	KY659235
RCM-SSR-9	*Streptomyces ederensis*	97.51	KY659236
RCM-SSR-11	*Streptomyces vinaceus*	99.93	KY659237
RCM-SSR-12	*Streptomyces cinereoruber subsp. cinereoruber*	99.70	KY659238

TABLE 2 Screening of feather degradation by actinobacterial strains

Isolate code	Feather degradation	Feather weight loss (%)	Enzyme activity (U ml^{-1})	Soluble protein (mg ml^{-1})
RCM-SSR-1	+ve	78	41.2 ± 1.5^{b}	2.8 ± 0.27^{ab}
RCM-SSR-2	+ve	96	306 ± 11.3^{a}	3.0 ± 0.15^{a}
RCM-SSR-3	−ve	–	–	–
RCM-SSR-4	+ve	82	44.7 ± 10^{b}	2.5 ± 0.11^{b}
RCM-SSR-5	+ve	64	22.6 ± 2	1.1 ± 0.23
RCM-SSR-6	+ve	98	282.5 ± 9^{c}	3.0 ± 0.17^{a}
RCM-SSR-8	+ve	84	77 ± 5	2.2 ± 0.05
RCM-SSR-9	−ve	–	–	–
RCM-SSR-11	−ve	–	–	–
RCM-SSR-12	+ve	96	298 ± 4^{ac}	3.1 ± 0.09^{a}
RCM-SSR-13	−ve	–	–	–
RCM-SSR-14	−ve	–	–	–

Values having same alphabetical letter are not statistically significant at $p \leq 0.05$.

medium is a selective medium for actinobacteria especially *Streptomyces* species. Nine actinobacterial isolates exhibiting feather degradation or plant growth promoting (PGP) or biocontrol acivity were identified by partial 16S rRNA gene sequencing. All the isolates were identified as *Streptomyces* species which is the largest genus of actinobacteria and widely distributed in soil, water, and other environmental habitats [19]. They are well known for complex secondary metabolism and produces over two-thirds of the clinically useful antibiotics of natural origin. *Streptomyces* are also good decomposers via secreting many extracellular hydrolysates [20].

Of the 12 strains, 7 strains were found to degrade chicken feather waste. They can use feather waste as sole carbon and nitrogen source for growth. Among these keratinolytic strains RCM-SSR-2, -6, and -12 were found to be the most efficient feather degrading strains. They could degrade feather within 48 h of incubation (>90% feather weight loss). This is comparatively faster than the other *Streptomyces* species reported in literature. For instance, *Streptomyces exfoliates* CFS 1068 could achieve only 28% feather weight loss after 48 h incubation and for complete degradation (83% weight loss) it took 6–7 days incubation time [21]. Bockle and Muller [22] observed complete degradation of feather by *Streptomyces pactum* in 4 days. Soluble peptide production by the actinobacteria under study is observed in range of 1.1–3.1 mg g^{-1} feather and keratinase production was in the range of 40–306 U ml^{-1}. Maximum keratinase and soluble peptide production was also shown by strains RCM-SSR-2, -6, and -12.

Strain RCM-SSR-2, -5, -6, -8, and -11 showed positive results for all PGP traits tested. Phosphorus is one of the major

TABLE 3 Screening of actinobacterial strains for PGP traits

Isolate code	Siderophore production	IAA production	Ammonia production	Phosphate solubilization
RCM-SSR-1	+ve	−ve	+ve	+ve
RCM-SSR-2	+ve	+ve	+ve	+ve
RCM-SSR-3	+ve	+ve	+ve	−ve
RCM-SSR-4	+ve	+ve	+ve	−ve
RCM-SSR-5	+ve	+ve	+ve	+ve
RCM-SSR-6	+ve	+ve	+ve	+ve
RCM-SSR-8	+ve	+ve	+ve	+ve
RCM-SSR-9	+ve	−ve	+ve	−ve
RCM-SSR-11	+ve	+ve	+ve	+ve
RCM-SSR-12	+ve	−ve	+ve	+ve
RCM-SSR-13	−ve	−ve	+ve	−ve
RCM-SSR-14	−ve	−ve	+ve	+ve

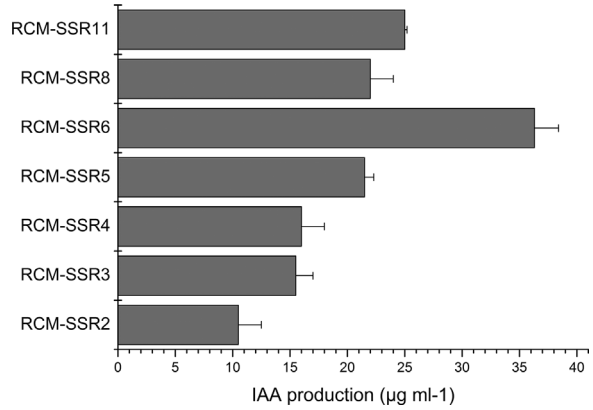

FIGURE 2 Quantitative estimation of IAA production by actinobacterial strains

plant nutrients for plant growth and development. Despite the abundance of phosphorus in the soil (400–1200 mg kg^{-1} of soil), only 0.1% of the total phosphates exists in a soluble form and is available for uptake by plants. Most of the soil phosphorus is in insoluble form and therefore, not available to support plant growth [23]. Plant growth promoting rhizobacteria have been reported to mobilize precipitated phosphorus to plants, representing a possible mechanism of plant growth promotion under field conditions [24]. Thus, solubilization and mineralization of phosphorus by phosphate solubilizing bacteria is an important trait in PGPR. In this present study, of the 12 actinobacterial strains tested, 8 strains were found to be positive for phosphate solubilization. The soils of North East Indian Himalayan Region, being acidic in nature, are found to be deficient in available phosphorus and this is one of the major limiting factors in crop production [25]. Hence, the native actinobacteria with phosphate solubilizing potential could alleviate this problem.

Ammonia is toxic to living organisms; it is the only gas present in sufficient concentrations in soil to inhibit soil fungi [26]. In this study, all the actinobacterial strains were found to be positive for ammonia production. Hence, presence of ammonia producing actinobacteria in the plant

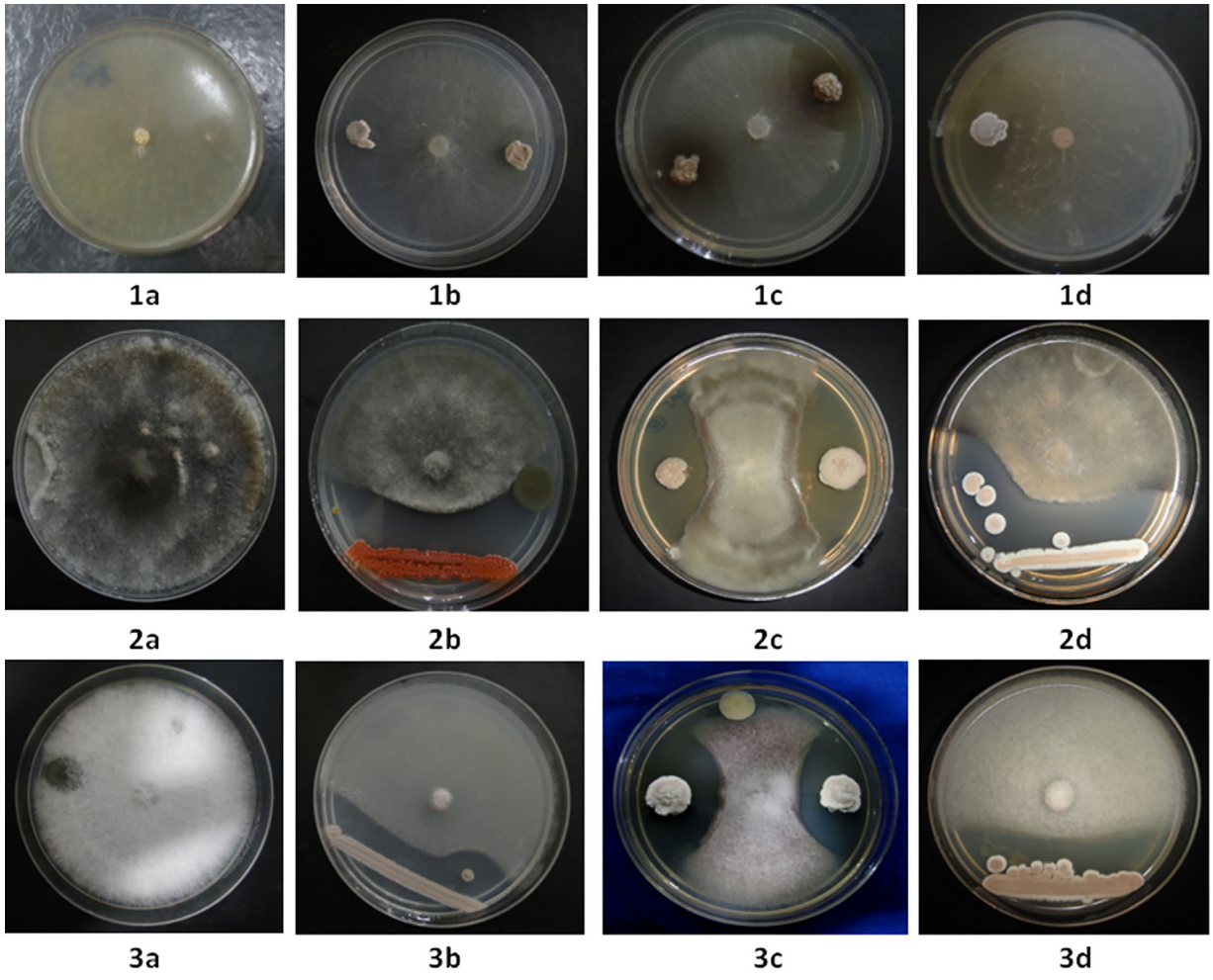

FIGURE 3 Antagonistic activity of actinobacteria against fungal phytopathogen. (1) Antagonistic activity of actinobacteria against *Rhizoctonia solani*: 1a: control, 1b: RCM-SSR-2, 1c: RCM-SSR-5, 1d: RCM-SSR-6. (2) Antagonistic activity of actinobacteria against *Pyricularia oryzae*: 2a: control, 2b: RCM-SSR-5, 2c: RCM-SSR-9, 2d: RCM-SSR-11. (3) Antagonistic activity of actinobacteria against *Fusarium oxysporum*: 3a: Control, 3b: RCM-SSR-1, 3c: RCM-SSR-5, 3d: RCM-SSR-11

TABLE 4 Screening of actinobacterial strains for antagonistic activity against fungal phytopathogens

	Colony growth inhibition (%)		
Isolate code	***Fusarium oxysporum***	***Pyricularia oryzae***	***Rhizoctonia solani***
RCM-SSR-1	55	48	46
RCM-SSR-2	50	38	60
RCM-SSR-3	–	–	–
RCM-SSR-4	37	52	–
RCM-SSR-5	67	60	52
RCM-SSR-6	62	46	54
RCM-SSR-8	–	–	–
RCM-SSR-9	54	60	61
RCM-SSR-11	62	60	55
RCM-SSR-12	–	–	–
RCM-SSR-13	–	–	–
RCM-SSR-14	–	–	–

rhizosphere can provide ammonia to the host plants to fulfill their nutritional need. However, over production of ammonia serve as a prompting factor for the virulence of opportunistic plant pathogens [27].

IAA is a well-known phytohormone responsible for root initiation, cell division, and cell enlargement [28]. Among the 12 *Streptomyces* strains, 7 strains were found to be positive for IAA production. IAA production was observed in range of 10–38 μg ml^{-1}. Khamna et al. [29] has reported IAA production by *Streptomyces* isolates in the range of 11–144 μg ml^{-1} when the production medium was supplemented with 2 mg ml^{-1} of tryptophan.

Siderophores are small, iron-chelating compounds secreted by soil microorganisms. They may enhance plant growth by increasing availability of Fe near the root or by inhibiting the colonization of roots by phytopathogen and making iron unavailable to them [27,30]. Hence, production of siderophore is an important factor for phytopathogen antagonism and developing growth of the plant [31]. In our study, of the 12 actinobacterial isolates, 83.34% strains (RCM-SSR-1, -2, -3, -4, -5, -6, -8, -9, -11, and -12) could produce siderophore. Production of siderophore by *Streptomyces* spp. was also observed by Khamna et al. [29] in *Streptomyces* CMU-SK 126 and by Tamreihao et al. [32] in *Streptomyces corchorusii* UCR3-16.

Pathogenic microorganisms affecting different plant species are a major and chronic threat to food production and ecosystem stability worldwide. However, the massive use of chemical pesticides cause several negative effects i.e., development of pathogen resistance to the applied agents, and pose serious risks to the environment and human health [33]. Therefore, the use of native bacteria having biocontrol potential for agricultural crops holds great promise for a sustainable and ecofriendly farming system. Hence, the

TABLE 5 *In vitro* seed germination test of garden pea seed using RCM-SSR-6 feather hydrolysate

Treatment	Soluble protein content (mg ml^{-1})	Root length (cm)	Shoot length (cm)	Vigor index
Control	0	3.43 ± 0.5^{a}	0.40 ± 0.1^{a}	343.07 ± 50^{a}
Undiluted	3	2.17 ± 0.2^{b}	0.62 ± 0.2^{ab}	217.3 ± 22^{b}
2×	1.5	2.47 ± 0.6^{ab}	0.83 ± 0.1^{bc}	247.5 ± 61^{ab}
5×	0.6	4.57 ± 0.5^{c}	1.4 ± 0.4^{d}	458.7 ± 45^{c}
10×	0.3	5.07 ± 0.1^{c}	1.48 ± 0.2^{d}	508.2 ± 14^{c}
15×	0.2	6.58 ± 0.1^{d}	1.58 ± 0.7^{de}	666.2 ± 1.2^{d}
20×	0.15	6.43 ± 0.2^{d}	1.7 ± 0.1^{de}	645.1 ± 24^{d}
30×	0.1	6.75 ± 0.3^{d}	2 ± 0.1^{e}	677.3 ± 35^{d}
50×	0.06	4.70 ± 0.3^{c}	1.24 ± 0.1^{cd}	471.5 ± 27^{c}
100×	0.03	4.63 ± 0.2^{c}	1.22 ± 0.1^{cd}	464.4 ± 19^{c}

Values having same alphabetical letter are not statistically significant at $p \leq 0.05$.

actinobacterial strains were screened for their biocontrol activity against major three fungal phytopathogens. Among the strains, RCM-SSR-1, -2, -5, -6, -9, and -11 showed antagonistic activity against the three fungal pathogens. Strain RCM-SSR-5 and -11 were found to be more antagonistic as compared to other strains.

Feather hydrolysate produced by RCM-SSR-6 was also evaluated for *in vitro* seed germination test using green garden pea seeds. Seeds treated with undiluted feather hydrolysate (3 mg protein ml^{-1}) showed low vigor index as compared to control. Inhibitory effect of feather hydrolysate on seed germination may be attributed to concentration effect. For example Rietsema et al. [34] reported that high concentration of IAA inhibits growth of datura embryo while lower concentration of IAA stimulates. Thus, it is essential to determine the optimal concentration of feather hydrolysate for using as seed germination stimulator. In this present study, maximum increase in vigor index was observed with 30× dilution (0.1 mg protein ml^{-1}).

Many keratinolytic *Streptomyces* spp. have been reported in literature [35–38]. However, there are sporadic reports on *Streptomyces* spp. having keratinolytic, phytostimulating, and biocontrol activity together. This present study revealed that actinobacteria isolated from tomato rhizosphere in North East Indian Himalayan Region are potential microbial inoculants because of their intensified phytostimulating traits such as phosphate solubilization, ammonia production, IAA production, and siderophore production. Moreover, these strains were also found to be promising for both feather degradation and biocontrol activity.

Four actinobacterial strains viz. RCM-SSR-1, -2, -5, and -6 reported in this study were identified as multi-traits PGPR and can be considered as promising candidates for development of organic fertilizers or biocontrol agents and can also be exploited for the production of agroactive compounds like IAA. Further studies on interactions among these promising strains on keratinolytic, phytostimulating, and biocontrol activity also holds a great promise for a more effective and commercially viable consortium of bioinoculants for sustainable crop production.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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