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# Diversity and plant growth-promoting potential of actinomycetes associated with the rhizosphere of *Arnebia euchroma* from Himachal Pradesh (India)

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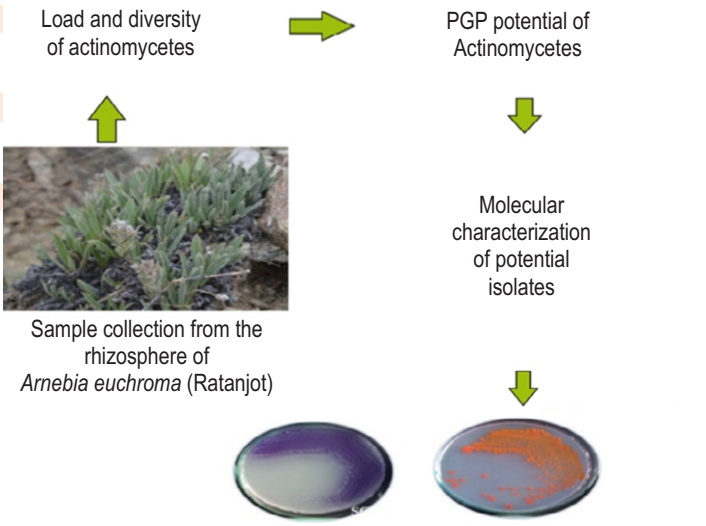
## Abstract

**Aim:** The present study aimed to explicate the diversity and plant growth promoting (PGP) potential of actinomycetes present in the rhizosphere of an endangered medicinal plant, *Arnebia euchroma*.

**Methodology:** Rhizospheric soil samples of *A. euchroma* collected from trans-Himalayan region of Himachal Pradesh were processed for elucidating actinomycetes diversity and load by employing Standard Plate Count Technique. All recovered isolates were screened for their PGP potential using standard protocols. The potential strains were identified through 16SrRNA ribotyping and were deposited in the National Culture Collection Centre, NCMR, Pune, (India).

**Results:** The rhizosphere of *A. euchroma* harboured a great deal of actinomycetes diversity (33 diverse morphotypes). Starch casein agar was best for isolating the actinomycetes. The same actinomycete isolate showed variations in their morphological features including pigments production on different isolation media. They exhibited multifarious plant growth-promoting activities like P-solubilization, phytase activity, N- fixation, siderophore production etc. The potential isolates were identified as *Streptomyces silaceus* CA7 (MK836019) and *Streptomyces rectiviolaceus* NA8 (MK836018).

**Interpretation:** The actinomycetes evinced a considerable plant growth promoting potential that might be helpful in the adaptation and perpetuation of *A. euchroma* under prevailing harsh environmental conditions of Himachal Pradesh.




**Key words:** Actinomycetes, *Arnebia euchroma*, Medicinal plant, PGP, Rhizosphere

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## Introduction

India, particularly the Trans-Himalayan region, is a varietal emporium of medicinal plant species that have been a major part of India's rich medicinal plant heritage (Devi and Kaundal, 2017). Medicinal plants are regarded as the fountainhead of traditional medicines worldwide. More than 50 per cent of traditionally used medicinal plants in India come from the Himalayan region (Uniyal *et al.,* 2002; Verma and Tewari, 2016). Owing to the variations in altitude and topography, Himachal Pradesh, located in the lap of the Himalayas, exhibits varied climatic conditions, which make this state ideal for thriving of a wide diversity of medicinal plant species (Singh and Thakur, 2014). This biodiversity of medicinal plants and its sustainable utilization by local people for various activities (traditional healthcare, cultural and religious purposes) help in sustaining their health, medicinal, spiritual and other needs (Gautam *et al.,* 2011). However, owing to the ruthless and unscientific exploitation, many important medicinal plant species are becoming rare and some of them have become critically endangered (Pandey *et al.,* 2005).

*Arnebia euchroma* (Ratanjot) represents a classical example of a critically endangered plant found in the trans-Himalayan region of Himachal Pradesh. Though mainly harvested for its roots, almost all parts of the plant are being used to make medicines, food, dyes and beverages. Of the five species of *Arnebia* in total, only three have been reported in Lahaul and Spiti - the cold desert of trans-Himalayan region ((Aswal and Mehrotra, 1994; Sharma *et al.,* 2011). *A. euchroma* (syn. *Macrotomia perennis* Boiss), commonly referred to as Dimok or Khamed in the native language, and popularly known by the trade name Ratanjot, is one of the three species that are being exploited commercially (Singh *et al.,* 2012). It has now been included in the list of critically endangered plants of Himachal Pradesh according to the latest International Union for Conservation of Nature (IUCN) categorization (Manjkhola *et al.,* 2005; Singh *et al.,* 2012). *A. euchroma* belongs to family Boraginaceae, is a well- known traditional perennial, erect, caespitose and hairy herb with many stems arising from the axils of its basal leaves and known to be distributed in dry regions of Asia and Northern Africa (Liu *et al.,* 2012).

It has sweet and bitter tastes and exhibits cold property while acting on the liver and heart channels. It regulates blood circulation and counteracts toxicity that is associated with measles, inflammation and sores. The roots contain a copious purple dye - a good source of red naphthoquinone pigment has anti-microbial, anti-inflammatory, anti-tumour and contraceptive properties. Beside these medicinal properties, the metabolites have commercial importance as natural colorants in food, cosmetics, textiles and exhibit various pharmaceutical properties (Kumar *et al.,* 2011). It is a usual practice in the Lahaul and Spiti valley to sell and use its roots as edible dye and medicine (Sharma *et al.,* 2011). Local doctors (known as *Amchis* in the western Himalayas) use its root extract in different indigenous medicines, prescribed for cough and blood purification. Actinomycetes constitute a significant component of rhizospheric microbial communities associated with medicinal plants. Medicinal plants' rhizosphere is one of the best sources of actinomycetes isolation because addition of root exudates and litters of plants make it an enrichment environment that serves as a nutritive source for the growth and biological activity of microorganisms including actinomycetes.

They are Gram-positive, filamentous bacteria that exhibit multifarious plant growth-promoting traits and stimulate plant growth by various direct and indirect mechanisms (Monteiro *et al.,* 2017). Actinomycetes directly stimulate growth by solubilizing insoluble nutrients like phosphates, producting growth hormones, nitrogen fixation and indirectly by antagonizing pathogenic organisms or by the producting siderophores, lytic enzymes, antibiotics, fluorescent pigments and cyanides etc (Kamal and Sharma, 2014). Therefore, the aim study was to assess the diversity and plant growth-promoting (PGP) potential of actinomycetes existing in the rhizosphere of a medicinal plant- *A. euchroma* (Ratanjot).

## Materials and Methods

**Study area and sample collection:** Four different sites *viz.,* Chango and Nako in Kinnaur district and Gue and Kibber in Lahaul & Spiti district of Himachal Pradesh were selected for the collection of rhizospheric soil samples of *A. euchroma* (Ratanjot), a medicinal plant.

**Load, diversity and growth behaviour of actinomycetes:** Standard Plate Count technique (Wollum, 1982) was employed to determine actinomycetes load on four different media *viz.,* Nutrient Agar, Actinomycetes Isolation Agar, Starch Casein Agar and Kenknight Medium. The results were expressed in terms of log CFU $g^{-1}$. Different morphotypes of actinomycetes which appeared on Nutrient Agar, Actinomycetes Isolation Agar, Starch Casein Agar, and Kenknight Medium were selected in order to elucidate their diversity. To observe the difference in the growth behaviour, same actinomycete isolates were streaked and grown on different selected media.

**Screening of actinomycetes for multifarious plant growth-promoting attributes:** Qualitative screening of actinomycetes for P-solubilization was done by spot inoculating them on Pikovskaya's agar plates. The formation of a clear halo zone around the colonies indicated P-solubilization (Pikovskaya, 1948). Quantitative estimation of inorganic P- solubilization was done employing Vanado-molybdate-yellow colour method from the standard curve of $KH_2PO_4$. The P- solubilizing index was calculated. All actinomycetes were spot inoculated on phytase specific medium followed by incubation at 28 ± 2°C for 7-15 days to detect the phytase activity qualitatively. Formation of a clear halo zone around the colonies indicated positive phytase activity. The phytase activity was quantified using phytate as a standard (Quan *et al.,* 2001). Chrome Azurol Sulphonate (CAS) agar plates

were spot inoculated with actinomycetes and incubated at 28±2°C for 30 days. The yellow-orange halo zones around the colonies demonstrated siderophores production. The CAS-shuttle assay (Schwyn and Neilands, 1987) was employed to quantify siderophore units. All actinomycetes were streaked on Nutrient Agar amended with 4.4 g $l^{-1}$ glycine. A Whatman filter paper No. 1 soaked in 2% $Na_2CO_3$ prepared in 0.5% picric acid solution was placed on the top of the plate accompanied by sealing and incubation of plate at 28 ± 2°C for 15-30 days. The change in the colour of filter paper from orange to brown indicated HCN production (Baker and Schippers, 1987). Actinomycetes were grown in peptone broth for 3-7 days at 28 ± 2°C followed by addition of 1 ml of Nessler's reagent. The production of varying intensity of yellow (++) to brown (++++) colour in the test tubes indicated ammonia production (Dye, 1962). All the actinomycetes isolates were streaked on Jensen's medium (N-deficient medium) to examine their nitrogen-fixing ability (Jensen, 1987).

**Determination of lytic enzymes production by actinomycetes:** Qualitatively, chitinase activity was estimated by spot inoculating actinomycetes on Colloidal Chitin Agar plates. Formation of clearance zone around the colonies indicated positive chitinase activity (Rodriguez *et al.,* 1993). Standard curve of glucose was used for quantitative estimation through dinitrosalicyclic acid method. Qualitative estimation of protease activity was performed by spot inoculating actinomycetes on skim milk agar plates. Formation of a clear halo zone around the colony indicated positive proteolytic activity. Caseinolytic assay method was used to quantify the protease activity from the standard curve of tyrosine (Dunn et al., 1997). Carboxymethyl cellulose agar plates were spot inoculated with actinomycetes and incubated at 28 ± 2°C for 7 days.

The plates were then flooded with Gram's iodine. A decolourized halo zone around the colony indicated the Carboxymethyl cellulose degrading activity (Kasana *et al.,* 2008). The standard curve of D-glucose was used for quantification through DNSA method. The pectinolytic activity of actinomycetes was checked by spot inoculating them on Pectinase screening medium plates followed by incubation at 28 ± 2°C for 6-7 days. The formation of a clear zone around the colony indicated positive pectinolytic activity. The pectinolytic activity was quantified in terms of the amount of glucose released in the reaction mixture using D-glucose as standard (Miller, 1959). All actinomycete isolates were spot inoculated on tributyrin agar medium and incubated at 28 ± 2°C for 7 days. The formation of a clear zone around the colony indicated positive lipase activity. Based on olive oil hydrolysis, the lipase activity was quantified titrimetrically using phenolphthalein as an indicator (Lawrence, 1967).

**Identification and characterization of potential isolates:** The potential isolates were identified and characterized as per the Bergey's Manual of Systematic Bacteriology (Vos *et al.,* 2009) and deposited at the National Centre for Microbial Resources, Pune.

**Statistical Analysis:** The data analysis was carried out using online Statistical software (Sheoran *et al.,* 1998). The experiments were conducted in triplicates using Completely Randomised Design and the error variance obtained from (ANOVA) was used to estimate standard error and critical difference ($CD_{p=0.05}$).

## Results and Discussion

In total, 33 different morphotypes of actinomycetes were obtained from the rhizosphere of *A. euchroma* during the course of this investigation. At Nako, the maximum percentage of morphotypes was recorded (45.4%), followed by Chango (21.2%), Gue (18.1%) and Kibber (15.1%). Actinomycetes possess an unusual metabolic potential which enables them to survive under a variety of harsh environmental conditions like aridity and low water activity, generally, prevailing in the cold temperate zone (Nako, Chango, Gue and Kibber) of Himachal Pradesh. Secondly, their ability to produce motile spores facilitates their dispersal and survival in these arid regions (Buedenbender *et al.,* 2017) and may be the likely reason for varied diversity at these locations.

Furthermore, significant difference ($P<0.05$) was observed in the population density of actinomycetes among different sites and on different isolation media. The maximum actinomycetes load (Log CFU $g^{-1}$) was observed at Chango (1.44), followed by Nako (1.40), Gue (1.38) and Kibber (1.33). Likewise, their maximum load was recorded on Starch Casein Agar (1.48), followed by Actinomycetes Isolation Agar (1.41), Nutrient Agar (1.29) and Kenknight Medium (0.88) proving Starch

**Table 1:** Total viable count of actinomycetes in the rhizosphere of *Arnebia euchroma* (Ratanjot) obtained from different sites on different media

| Districts | Sites | Isolation media (Log CFU $g^{-1}$) | | | | |
|---|---|---|---|---|---|---|
| | | NA* | KM** | SCA*** | AIA**** | Mean |
| Kinnaur | Chango | 1.47 | 1.14 | 1.51 | 1.67 | 1.44 |
| | Nako | 1.25 | 1.48 | 1.46 | 1.50 | 1.40 |
| Lahaul and Spiti | Gue | 1.27 | 1.49 | 1.44 | 1.17 | 1.38 |
| | Kibber | 1.20 | 1.30 | 1.53 | 1.30 | 1.33 |
| | Mean | 1.29 | 0.88 | 1.48 | 1.41 | |
| CD (0.05) M* : 0.017 ; S* : 0.017; S×M: 0.034 | | | | | | |

*- Nutrient Agar; **- Kenknight and Munaier's Medium; ***- Starch Casein Agar; ****- Actinomycetes Isolation Agar; M*= Type of media S*=Sampling sites

**Table 2:** Phosphate solubilizing index shown by actinomycetes obtained from the rhizosphere of *Arnebia euchroma*

| Isolate | Phosphate solubilization ($\mu g\ ml^{-1}$) | Phosphate solubilization index (SI)** | Pikovskaya's broth | |
|---|---|---|---|---|
| | | | Initial pH | Final pH |
| NA1 | 47.33[b] | 1.6 | 7.0 | 5.72 |
| NA4 | 20.77[e] | 1.3 | 7.0 | 6.42 |
| SCANI | 6.44[h] | 4.0 | 7.0 | 6.76 |
| KMN2 | 29.20[c] | 1.6 | 7.0 | 5.40 |
| NA8 | 53.66[a] | 2.0 | 7.0 | 5.70 |
| CA6 | 15.11[f] | 4.2 | 7.0 | 6.53 |
| CA7 | 13.88[g] | 4.6 | 7.0 | 6.50 |
| NA10 | 23.77[d] | 1.6 | 7.0 | 6.34 |
| CD(0.05) | 0.28 | | | |

Each value represents mean of three replicates. According to One way ANOVA, significant differences are indicated by different letters. Same letters represent that their values are statistically at par.

**Table 3:** Qualitative and quantitative estimation of phytase activity exhibited by actinomycetes obtained from the rhizosphere of *Arnebia euchroma*

| Isolate | Halo Zone Diameter (HZD) (mm) | Phytase activity ($U\ ml^{-1}$) |
|---|---|---|
| NA1 | 10 | 45.77[i] |
| SCANI | 11 | 114.12[f] |
| SCAK1 | 10 | 31.55[j] |
| NAN2 | 12 | 87.22[h] |
| NA8 | 13 | 126.33[b] |
| CA5 | 11 | 115.66[e] |
| CA6 | 13 | 124.88[d] |
| CA7 | 13 | 134.44[a] |
| NAN2 | 12 | 89.22[g] |
| NA8 | 13 | 125.33[c] |
| NA10 | 10 | 15.33[k] |
| CD (0.05) | | 0.42 |

Each value represents mean of three replicates. According to One way ANOVA, significant differences are indicated by different letters. Same letters represent that their values are statistically at par.

Casein Agar to be the best medium for their isolation (Table 1). The interaction between different sites and media was statistically significant, indicating that both types of media and sites had significantly affected the actinomycetes population. These results evinced that the rhizosphere of *A. euchroma* anchorages a quite good population density of actinomycetes which may be attributed to the deposition of various root exudates like sugars, amino acids, organic acids, aromatics, polysaccharides, proteins and various other secondary metabolites in the rhizosphere (Abd Elgawad *et. al*., 2020).

However, the overall population of actinomycetes was low in comparison to the actinomycetes obtained from the rhizosphere of other medicinal plants like *Kochia indica* (Yakoob et al., 2013), *Ocimum sanctum*, *Catharanthus roseus*, *Aloe vera* and *Coleus forskholii* (Karthikeyan and Sakthivel, 2012) because there may be some selective pressure exhibited by the root exudates of *A. euchroma*, which permitted only selective types of organisms to thrive in the rhizospheric soil. Interestingly, variation in the morphological features and pigment production of same actinomycete isolate on different media was observed in the present study. The same isolate was able to produce pigment on one medium, while it failed to do so on another medium which could be ascribed to the difference in the composition and pH of isolation media. Our findings are in line with the previous reports which confirmed that medium composition and pH influence the pigment production by actinomycetes (Sanchez-Marroquin and Zapata, 1954).

The present study also throws light on the PGP potential of actinomycetes. In phosphate solubilizing test, out of 33, only 8 isolates were able to solubilize tri-calcium phosphate in agar plate assay (Fig. 1) with phosphate solubilization index ranging from 1.3 to 4.6. The highest index (4.6) was obtained for the isolate CA7 whereas NA4 registered the lowest index (1.3). Many species of rhizobacteria have demonstrated their ability to solubilize inorganic phosphate in agar plate assay (Devi and Sowndaram, 2014). The P-solubilization index (SI) of

**Table 4:** Qualitative and quantitative estimation of siderophore production by actinomycetes obtained from the rhizosphere of *Arnebia euchroma*

| Isolates | Halo Zone Diameter (mm) | % siderophore units |
|---|---|---|
| NA2 | 5 | 7.67[g] |
| NA4 | 5 | 7.48[h] |
| NA5 | 4 | 6.13[k] |
| GA5 | 4 | 6.21[k] |
| GA6 | 9 | 16.25[b] |
| GA7 | 4 | 7.85[f] |
| CA4 | 5 | 7.30[i] |
| SCAN1 | 4 | 5.46[l] |
| SCAK5 | 9 | 17.24[a] |
| SCAC3 | 6 | 7.24[j] |
| CA5 | 4 | 9.41[e] |
| CA7 | 7 | 12.35[c] |
| NA9 | 4 | 6.18[k] |
| Na7 | 6 | 10.23[d] |
| NA6 | 5 | 6.72[j] |
| CD(0.05) | | 0.15 |

Each value represents mean of three replicates. According to One way ANOVA, significant differences are indicated by different letters. Same letters represent that their values are statistically at par.

*Streptomyces* spp. recovered from the rhizosphere of wheat (*Triticum aestivum*) ranged between 2.20 to 2.33 (Anwar *et al.,* 2016). Further, significant difference (P<0.05) was observed in the tri-calcium phosphate solubilisation in Pikovskaya's broth among the isolates (Table 2). The maximum tri-calcium phosphate solubilisation (53.66 µg $ml^{-1}$) was recorded for NA8 whereas the lowest (6.44 µg $ml^{-1}$) was observed for SCAN1. Variation in P-solubilization results could be ascribed to the fact that P-solubilization, which is measured in terms of diameter of halo zone, mainly depends upon nature of the phosphatic compounds released, organisms used, quantity and different rate of organic acids produced by microorganisms into the surrounding medium (Vessey, 2003).

A decrease in the pH of Pikovskaya's broth was also observed during P-solubilization, which is attributed to the development of low molecular organic acids such as gluconic, α-ketogluconic, glycolic, oxalic, lactic, acetic, formic, malonic and succinic acids that lower the pH of the external medium and help dissolve the insoluble P at low pH (Vyas and Gulati, 2009). In the present study, out of 33 isolates, eleven actinomycetes were selected as efficient phytase producers (Fig. 1) qualitatively. Quantification results reveal a statistically significant difference in the pytase activity ranging from 15.33 to 134.44 U $ml^{-1}$ among the isolates (Table 3). Isolate CA7 was found to exhibit maximum phytase activity (134.44 U $ml^{-1}$) while the minimum phytase activity (15.33 U $ml^{-1}$) was shown by NA10. This variation in phytase activity could be ascribed to the fact that phytase activity varies with respect to the type of actinomycete strains used and media composition. Aly *et al.* (2015) observed that phytase activity values exhibited by *Streptomyces* spp. recovered from decaying wood samples ranged from 0.08-7.90 U $ml^{1}$. In siderophore test, out of 33, only 15 isolates were positive showing orange halo around their respective colonies (Fig. 1). Significant difference (P<0.05) was observed in the per cent siderophore units among the isolates ranging from 6.13 to 17.24 per cent (Table 4). Isolates SCAK5 and SCAN1 showed significantly the highest (17.24 %) and the lowest siderophore production (5.46% SU), respectively However, Khamna *et al.* (2009) observed comparatively higher siderophore production [16.19 (catechols) -54.9 (hydroxamate) µg $ml^{-1}$] than that reported in the present investigation in *Streptomyces* spp. isolated from rhizospheric soil of *Curcuma mangga*. The low siderophore production by actinomycetes could be ascribed to the competition that actinomycetes face for iron supply with other bacteria and fungi in the rhizosphere (Tokala *et al.,* 2002).

Furthermore, all actinomycetes showed the ability to fix nitrogen in N-deficient medium (Fig. 1) as they showed luxuriant growth on Jensen's medium. However, out of 33, only one isolate i.e. GA6 showed HCN production with the orange-brown colouring of filter paper impregnated with sodium carbonate in picric acid (Fig. 1). In terms of ammonia production, only 2 isolates, i.e. CA7 and NA5 produced brown colour indicating strong ammonia production. Nevertheless, 5 isolates *viz.,* GA1, CA5, CA6, NAN2 and SCAK3 produced yellow-brown colour indicating moderate ammonia production. The remaining isolates exhibited a light yellow colour which indicated weak ammonia production (Fig.1). These results indicate that the rhizosphere of *A. euchroma* anchorages a good proportion of nitrogen fixers likely to be helpful in the perpetuation of this plant under prevailing harsh environmental conditions of the trans-Himalayan region of Himachal Pradesh as nitrogen is an essential nutrient for improved growth and yield in plants. These findings are supported by Kaur *et al.* (2013), who observed that *Streptomyces* spp. isolated from different plants exhibited reasonable N- fixing ability.

**Table 5:** Extracellular production of lytic enzymes by actinomycete isolates obtained from the rhizosphere of *Arnebia euchroma*

| Isolate | Chitinase activity ($U\ ml^{-1}$) | Protease activity ($U\ ml^{-1}$) | Cellulase activity ($U\ ml^{-1}$) | Pectinase activity ($U\ ml^{-1}$) | Lipase activity ($IU\ ml^{-1}$) |
|---|---|---|---|---|---|
| Na1 | $3.52^{b}$ | - | $26.96^{c}$ | $19.33^{c}$ | $1240^{c}$ |
| NA2 | $4.36^{b}$ | - | $28.92^{b}$ | $9.00^{g}$ | - |
| Na3 | $3.53^{b}$ | - | $27.74^{b}$ | $21.66^{b}$ | - |
| Na4 | $3.76^{b}$ | - | $26.82^{c}$ | $8.33^{h}$ | $1200^{c}$ |
| Na5 | $5.03^{b}$ | - | $31.21^{a}$ | $12.66^{e}$ | $2040^{a}$ |
| GA1 | $3.42^{c}$ | $7.40^{g}$ | $27.12^{c}$ | $20.33^{b}$ | $1000^{d}$ |
| Ga2 | $2.86^{c}$ | $13.42^{d}$ | $27.51^{c}$ | $6.66^{i}$ | $1120^{c}$ |
| Ga4 | $3.31^{c}$ | $21.68^{b}$ | $27.56^{c}$ | $19.34^{c}$ | $1240^{c}$ |
| Ga5 | $3.26^{c}$ | $8.58^{f}$ | $28.41^{b}$ | $7.66^{h}$ | $1280^{c}$ |
| GA6 | - | $6.24^{h}$ | $24.18^{d}$ | $1.60^{k}$ | $1160^{c}$ |
| GA7 | $0.53^{d}$ | $12.66^{d}$ | $26.72^{c}$ | $15.00^{d}$ | $1240^{c}$ |
| Ca1 | $0.42^{d}$ | - | $25.94^{c}$ | $16.00^{d}$ | - |
| CA3 | | | $26.44^{c}$ | $4.32^{j}$ | $1400^{b}$ |
| CA4 | $3.83^{b}$ | - | $25.12^{d}$ | $16.00^{d}$ | - |
| Ka1 | $3.93^{b}$ | - | - | - | - |
| KA2 | $2.73^{c}$ | - | $26.58^{c}$ | - | - |
| SCANI | $5.26^{a}$ | - | $26.62^{c}$ | $4.66^{j}$ | $1160^{c}$ |
| SCAK1 | $4.56^{b}$ | $7.20^{g}$ | $25.64^{d}$ | $2.30^{k}$ | $1040^{c}$ |
| KMN2 | $6.46^{a}$ | - | $26.78^{c}$ | $2.00^{k}$ | $1120^{c}$ |
| SCAN4 | $3.43^{c}$ | - | $24.50^{d}$ | $7.62^{h}$ | $1520^{b}$ |
| SCAK5 | $2.46^{c}$ | $5.21^{i}$ | $24.52^{d}$ | $7.81^{h}$ | $1000^{e}$ |
| SCAC3 | $4.43^{b}$ | $1.25^{j}$ | $28.82^{b}$ | $6.67^{i}$ | $1120^{c}$ |
| SCAK3 | $3.46^{c}$ | - | $25.72^{d}$ | $7.33^{h}$ | $1120^{c}$ |
| KMN1 | $6.71^{a}$ | $17.20^{c}$ | $29.36^{b}$ | $16.12^{d}$ | - |
| NAN2 | $3.83^{b}$ | - | $25.12^{d}$ | $10.00^{g}$ | $1000^{e}$ |
| NA8 | $4.93^{b}$ | - | $24.92^{d}$ | $11.66^{f}$ | $1000^{e}$ |
| CA5 | $6.52^{a}$ | - | $28.36^{b}$ | $6.62^{i}$ | $1240^{c}$ |
| Ca6 | $5.76^{a}$ | -- | $27.52^{c}$ | $10.02^{g}$ | $1160^{c}$ |
| CA7 | $4.53^{b}$ | - | $25.28^{d}$ | $13.12^{e}$ | $1160^{c}$ |
| Na7 | $5.26^{a}$ | $28.13^{a}$ | $27.48^{c}$ | - | - |
| Na9 | - | $22.10^{b}$ | $27.55^{c}$ | - | - |
| Na10 | $4.86^{b}$ | $1.22^{j}$ | $26.72^{c}$ | $2.61^{k}$ | $1000^{e}$ |
| Na6 | $6.81^{a}$ | $11.15^{e}$ | $27.12^{c}$ | $48.33^{a}$ | $1000^{e}$ |
| C.D (0.05) | 1.56 | 0.99 | 1.64 | 1.42 | 174.66 |

Each value represents mean of three replicates. According to One way ANOVA, significant differences are indicated by different letters. Same letters represent that their values are statistically at par.

Ahmed *et al.* (2014) reported that HCN production shown by actinomycetes helped in disease suppression hence is an important mechanism of bioprotection. Ammonia is also considered to be one of the growth-promoting and antimicrobial substances produced by microorganisms. The production of ammonia along with HCN in the growth medium by *Streptomyces* contributed towards disease suppression among plants (Anwar *et al.,* 2016). In the present study, most of the isolates were able to produce hydrolytic enzymes like chitinase, protease, cellulase, pectinase, lipase and some of them showed combined activities. Collectively, 96.96, 90.90, 84.84, 72.72 and 42.42 per cent of the total isolates exhibited cellulase, chitinase, pectinase, lipase and protease activities, respectively. Apparently, actinomycetes present in the rhizosphere of *A. euchroma* may serve as an imperative resource for screening valuable lytic enzymes that could be due to natural selection of microbes to thrive in the competing environment (Arjit *et al.,* 2012). Since the environmental conditions are too harsh in the trans- Himalayan region of Himachal Pradesh, this region can be considered as unreliable from substrate availability point of view for microbial metabolism including actinomycetes. Hence, those actinomycetes which produce a wide spectrum of extracellular lytic ezymes are the ones with the greatest possibilities for successful adaptation to survive there. To thrive in these ecosystems, the actinomycetes should be able to uptake several substrates as nutrients (Tropeano *et al.,* 2013)

The perusal of data depicted in Table 5 reveal a significant difference ($P<0.05$) among actinomycetes in their ability to produce lytic enzymes extracellularly. In terms of chitinase activity, the isolates NA6 and CA1 showed maximum (6.81 $U\ ml^{-1}$) and minimum chitinase activity (0.42$U\ ml^{-1}$). The maximum

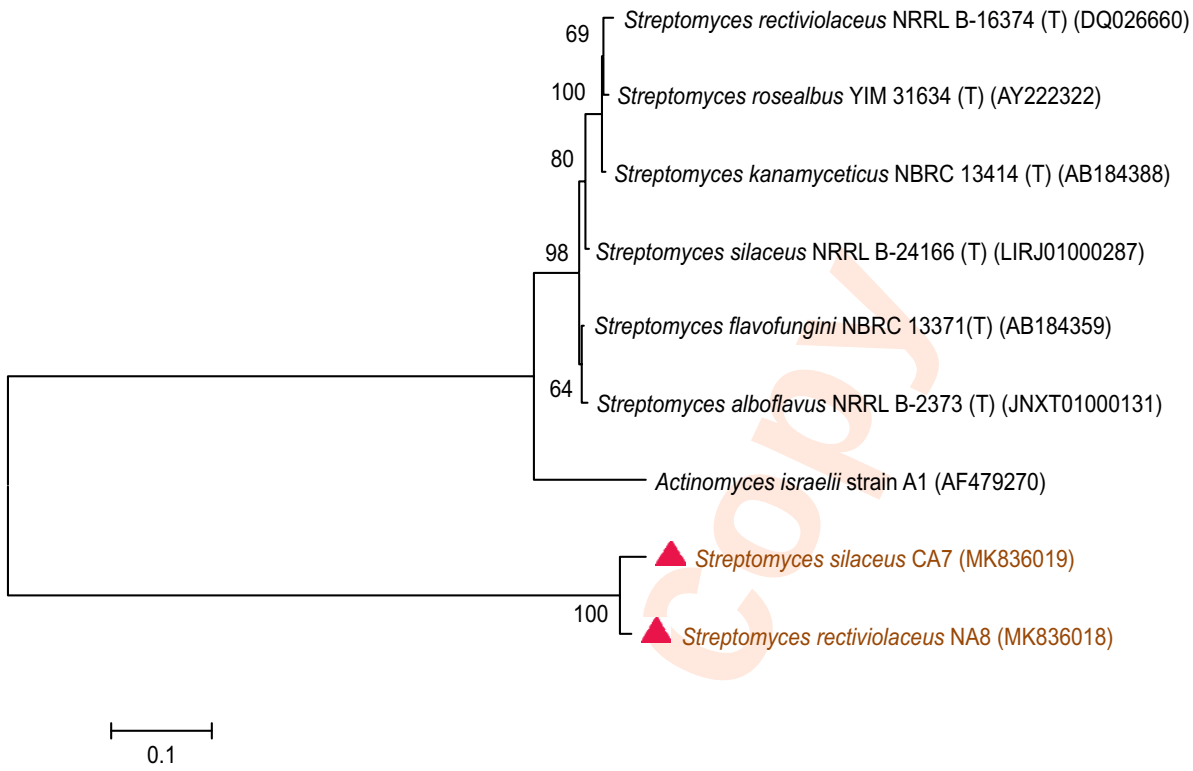


**Fig. 1:** Phylogenetic tree showing evolutionary relationship of ▲potential PGP actinomycetes isolated from the rhizosphere of *Arnebia euchroma* and its related taxa constructed using MEGA 7.0 software by neighbour-joining method.

protease activity (28.13 U $ml^{-1}$) was recorded for isolate NA7. whereas, the minimum protease activity (1.22 U $ml^{-1}$) was observed in NA10. Furthermore, the isolates NA5 and GA6 displayed the maximum (31.21 U $ml^{-1}$) and minimum (24.18 U $ml^{-1}$) cellulase activities. Among all the isolates, NA5 showed the highest lipase activity (2040 IU $ml^{-1}$). The isolates NA6 and GA6 displayed the maximum and minimum pectinase activities, of 48.33 U $ml^{-1}$ & 1.60 U $ml^{-1}$, respectively. Microbes can directly suppress the growth and activities of pathogens by secreting lytic enzymes like glucanases, proteases, chitinases, lipases etc., involved in lysis of fungal cell wall (Neeraja *et al.,* 2010). These enzymes either digest or deform the components of fungal pathogen's cell wall, which is an important eco-friendly mechanism to control soil-borne pathogens (Aeron *et al.,* 2011; Fasusi and Babalola, 2021). The field of microbial systematic has been revolutionized by the most frequently used marker -16S rRNA gene (Morra, 2002) because its extensive distribution and highly conserved nature make it appropriate for determining the phylogenetic relationship among the bacteria. Based on PGP potential, two potential actinomycete isolates, *i.e.,* NA8 and CA7 were identified with respect to their morphological, physiological, biochemical and molecular characteristics as *Streptomyces rectiviolaceus* NA8 and *Streptomyces silaceus* CA7, respectively.

Their 16SrRNA sequences were submitted to NCBI, GenBank, USA and were assigned with accession numbers MK836018 (NA8) and MK 836019 (Ca7). Phylogenetic tree of isolates NA8 and CA7 with respect to other actinomycetes is presented in Fig. 1. Yilmaz *et al.* (2008) identified actinomycetes isolated from the rhizosphere of local endemic plants of Turkey as *Streptomyces lydicus, S. rochei, S. microflavus, S. griseoflavus, S. albidoflavus* and *S. violaceus* using 16S rRNA gene sequencing. Microorganisms are generally maintained by culture collection centres, which are set up with the prime objective of conserving microbial diversity and valuable germplasm. The main goal of these culture collections is to act as a depository, supplying authentic microbial strains to researchers and to provide strains for aiding teaching. Sometimes, these authentic cultures also help in identification of unknown strains. National Centre for Cell Science, National Centre for Microbial Resources, Pune, (India) is one of such National culture depositories, where these potential actinomycetes strains *viz., Streptomyces silaceus* CA7 and *Streptomyces rectiviolaceus* NA8 were deposited and eventually authenticated through the assignment of deposition accession numbers MCC 4170 and MCC 4262, respectively, by the depository.

In can be concluded that the rhizosphere of *A. euchroma* harbours a great deal of actinomycetes diversity. Further, these actinomycetes exhibit multifarious plant growth-promoting

activities like P- solubilization, phytase activity, N- fixation, siderophore, ammonia and HCN production etc., that may be helpful in plant's adaptation and perpetuation besides their impact on growth.


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## Add-on Information

**Authors' contribution: S. Devi:** Designed the study& wrote the paper, **P. Sharma, A. Rana, J. Pal:** Performed research, **A. Kumari:** Analysed data.

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