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Exploring actinomycete diversity and growth potential in replant sites of apple orchards in Shimla District, Himachal Pradesh

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Abstract
Plant Growth Promoting Rhizobacteria (PGPR) are recognized as vital biological agents for enhancing plant growth, with Actinomycetes being a prominent group among them. The study aimed to isolate and screen diversity of actinomycetes from replant site of apple rhizosphere as growth promoter of apple *in vitro*. Six actinomycetes isolates have been cultured from apple rhizosphere on Kenknight media. All the actinomycetes isolates underwent screening for plant growth promoting activitites including Phosphate solubilization, siderophores, ammonia, HCN and Protease production. Based on their superior plant growth-promoting attributes, the A3 isolate was prioritized for further analysis via 16S rRNA sequencing. The analysis revealed a high degree of homology between the A3 isolate and *Streptomyces bluensis* (with a similarity of ≥98%). Our finding underscore a potent A3 actinomycetes isolate as a beneficial contributor to addressing replant issues in apple cultivation and promoting sustainable agricultural practices..

Keywords: Replant problem, actinomycetes, plant growth promoting rhizobacteria, 16SrRNA

1. Introduction
Apple (*Malus domestica* Borkh.) is predominantly cultivated in the north western Himalayan region of India, which includes the states of Jammu and Kashmir, Himachal Pradesh, Uttarakhand, the north eastern hilly states, and the South Nilgiri hills. In 2024, the apple harvest in Himachal Pradesh was estimated to be 580.296 tons. The productivity drops from the previous figure of 672.343 ton for 2023with no corresponding increase in productivity. Apple orchards planted in early sixties exhibits symptoms of declining yield and quality as these fruit trees have reached the conclusion of their economic life span. This decline may be due to rising population, extreme environmental factors and land resources are diminishing. With limited land availability and need for crop for variations in mountainous regions, orchardists are compelled to replace old apple orchard sites with new apple trees, resulting in numerous diseases that significantly decreases the crop production and quality. Replant disease is a prevalent challenge in apple cultivation, characterized by the diminished growth of fruit trees upon replanting in soil that previously hosted the same or closely related species. (Traquair, 1984) [37].

Replant disease in apple is marked by severe growth suppression, with many trees on the affected site exhibiting stunted development, which can lead to the death of young trees. Symptoms include severe growth suppression, rosetted leaves, dwarf internodes, stunted root systems, diseased or discolored roots, fewer lateral roots, root hairs and reduced productivity (Klaus, 1939) [18]. Replant disease is affected by both biotic and abiotic factors. Biotic causes include bacteria, fungi, actinomycetes, nematodes, and their interactions. Actinomycetes are notably implicated in this disease. Fungal genera associated with the condition include *Thielaviopsis*, *Pythium*, *Rosellinia*, *Rhizoctonia Phytophthora*, *Cylindrocarpon*, *Alternaria Fusarium*and *Penicillium* (Benizri *et al*. 2005) [5]. Abiotic factors contributing to replant disease encompass phytotoxins, nutrient imbalances, inappropriate pH levels, soil structure and drainage issues, and moisture extremes (Utkhede and Smith, 1994) [38].

Plant growth promoting rhizobacteria (PGPR) are the rhizospheric microorganims which colonizes rhizosphere aggressively and facilitate plant growth directly and indirectly. These microorganisms improves enhances nutrient availability in soil, which promotes plant growth (Zineb *et al*. 2022) [45] and they also supply phytohormones, and induce systemic resistance induction against phytopathogens. In the past few years, there has been a notable rise in the usage of Actinobacteria in farming methodologies, due to their great capabilities as PGPR and their widespread presence in plants (Yadav *et al*. 2018) [42]. Actinomycetes are Gram-positive, filamentous bacteria that showcase their diverse multitudinous plant growth-promoting attributes and highlighted as most promising candidates of biofertilizer agents (Devi *et al*. 2021) [9]. Several actinomycetes genera, including *Actinoplanes*, *Streptomyces* and *Micromonospora* have been widely studied for increasing agricultural crops productivity. Among these, *Streptomyces* the most investigated genera in respect to the plant growth promoting activity (Wahyudi *et al*. 2019) [41]. Despite their potential, the diversity of actinomycetes in the apple rhizosphere in India remains underexplored. However, various actinomycetes species have been utilized for plant growth promotion (PGP) and biocontrol of plant pathogens in agriculture. Phukan *et al*. (2012) [28] documented that actinomycetes improves the growth promotion of several crops, including tea., rice (Gopalakrishnan *et al*. 2014) [14], wheat (Hamdali *et al*. 2008) [15], tomato (El-Tarabily *et al*. 2008) [12]. Therefore, the objective of my research was to investigate the diversity and plant growth-promoting (PGP) capabilities of actinobacteria found in the rhizospheric soil of apple trees. This investigation holds significant implications for managing replant problems and enhancing apple growth.

2. Materials and Methods

Study site: Two different replant sites were selected for sample collection in Shimla district of Himachal Pradesh. *viz*., Maggots and Rauni

2.1 Collection of sample

One composite soil sample was made from by mixing five rhizospheric soil samples taken from five different plant. Three composite samples were similarly gathered from different sub sites within two different replant sites in Shimla district. Total twelve samples were meticulously collected from the two replant sites of Maggots and Rauni (Shimla district) and transported to the laboratory in polyethene bags and subsequently kept at 4 ºC.

2.2 Isolation, diversity and growth behaviour of actinomycetes

One gram of composite mixed soil was processed following serial dilution technique. The total actinomycetes count were isolated using standard plate count method using Glycerol yeast extract agar (GYA) and Kenknight media (KeM). The colonies were purified by streaking and further observations of colony morphological characterization such as form, elevation, margin, and colour of each isolate.

2.3 Screening of actinomycetes for multifarious plant growth promoting attributes

The screening of actinomycetes isolates for multiple plant growth promoting traits *viz*., P-solubilization, siderophore, HCN and ammonia production, lytic enzymes production. To assess the phosphate solubilization potential of actinomycetes isolates, Pikovskaya's agar were inoculated with cell free culture supernatants of isolates by well plate method. Results were estimated for yellowish color halo around the well (Pikovskaya, 1948) [29]. The production of siderophores was estimated by plate assay methodon chrome azurol-S (CAS) media (Schwyn and Neilands, 1987) [33]. In this method the formation of yellow/orange zone produced around the well can be measured in mm dia after incubation at 28±2 ºC for 48 h.

All actinomycetes isolates were assess for hydrogen cyanide (HCN) production by streaking on King's B medium containing 1.4 g/l glycine. Filter paper strips (Whatman No. 1) were immersed in solutions containing 0.5% picric acid and 2% sodium carbonate. Subsequently, these treated strips were positioned on the lids of individual Petri plates. The plates were sealed using parafilm and then incubated at a temperature of 28±2 ºC for a duration of 4-5 days. Observation for a change in coloration of the filter paper from yellow (-) to light brown to orange brown (++ or++++) (Bakker and Schippers, 1987) [3]. The quantitative estimation of actinomycetes sp. was conducted by inoculating them into 5ml of peptone water in test tubes (Lata and Saxena, 2003) [21]. After incubation at 28 ±2 ºC for 4 day, Nessler's reagent (1ml) was added. Ammonia production was assessed based on color development. A very light brown coloration marked as (+) denoted slight ammonia production, whereas a gradient from light brown (++) to orange-brown (++++) indicated varying levels of ammonia production, with darker hues representing larger amounts.

All actinomycetes sp. were assessed on skim milk agar plates for proteolytic activity by well plate assay (Kaur *et al*. 1989) [16]. at 28 ± 2 ºC for 48 h. Proteolytic activity was measured by clear zones formed around the well in mm after incubation at 28 ± 2 ºC for 48 h. All the actinomycetes sp. were tested *in-vitro* for their biocontrol potential against five fungal pathogens *viz*., *Dematophora necatrix, Phytophthora cactorum*, *Pythium ultimum*, *Fusarium oxysporum* and *Rhizoctonia solani* by well plate assay method (Vincent, 1947) [39] using dual culture technique. An indicator fungus was introduced onto pre-poured Malt Extract Agar (MEA) plates using a sterile well cutter and an inoculating loop, positioned on a designated side of the plates. On the opposite side of the MEA plates, wells were created using a sterile well borer. 100 µl of a 72-hour-old cell-free culture supernatant from each test bacterial strain was dispensed into individual wells on the plates, with a diameter of 7 mm. Subsequently, the plates were incubated at 28 ± 2 ºC for a duration ranging from 3 to 7 days. During this incubation period, observations were made to detect any inhibition of mycelial growth surrounding the wells.

Antifungal activity was calculated as percent inhibition shown by fungal mycelia

$$\% I = \frac{C-Z}{C} \times 100$$

Where,
C=Growth of mycelia in control
Z=Growth of mycelia in treatment

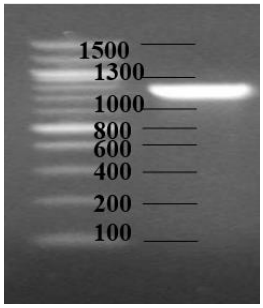

Plate 1: Agarose gel electrophoresis of amplified genomic DNA of actinomycetes isolate

2.4 Molecular characterization using 16S gene amplification

Actinomycetes isolates showing great PGP activities was characterized and purified by the method of Kumar *et al*. (2010) [19] and Sambrook *et al*. (1989) [32] with primers FP-1 (AGAGTTTGATCCTGGCTCAG-3′) and RP-1 (TACGGCTACCTTGTTACGACTT-3′). The purity of PCR product was assessed on 1% agarose gel. Sequencing was done using a 96-capillary 3730xl DNA analyzer (Hitachi). Sequences were aligned using CLUSTAL_X, version 1.81 algorithm (Thompson *et al*. 1997) [35]. MEGA software was used for phylogenetic and molecular evolutionary analyses by the neighbor-joining method (Saitou and Nei, 1987) [31].

2.4.1 Statistical Analysis: Data analysis was performed using statistical software available online, following the methodology outlined by Sheoran *et al*. (1998) [34]. The experiments were conducted in triplicate, employing a Completely Randomized Design (CRD). Error variance derived from the analysis of variance (ANOVA) was utilized to determine both the standard error and the critical difference (CD0.05).

3. Results and Discussion

3.1 Isolation and screening of actinomycetes isolates from apple rhizosphere

Actinomycetes, primarily residing in soil environments, represent one of the most prevalent groups of microorganisms found across diverse natural habitats (Oskay *et al*. 2004) [27]. Actinomycetes were isolated from rhizospheric soil samples using medium *viz*., Ken Knight agar (KeM) and Glycerol yeast extract agar (GYA). Samples were collected from normal and replant sites located in Maggots and Rauni (Shimla district) for comparative analysis. The highest actinomycetes population was recorded on KenKnight agar medium as compared to Glycerol yeast extract agar (Table 1 and Fig 1). The maximum actinomycetes count (19-22×10^3) was recorded from rhizospheric soil sample collected from normal site of Maggots (Shimla district) and the actinomycetes population ranged from 19-26×10^3 cfu/g soil in normal site of Rauni on KenKnight agar medium. Whereas in case of replant site of Maggots, the actinomycetes population ranged from 7-17×10^3 cfu/gsoil on KenKnight agar medium. The maximum population was ranged from of 8-12×15^3 cfu/g soil sample from replant site of Rauni on Kenknight agar medium.

Similarly, the maximum actinomycetes count (7-10×10^3) was recorded from normal site of Maggots (Shimla district) and the actinomycetes population ranged from 7-10×10^3 cfu/g soil in normal site of Rauni on glycerol yeast extract agar medium. Whereas in case of replant site of Maggots, the actinomycetes population ranged from 2-7×10^3 cfu/g soil on glycerol yeast extract agar medium. The highest count was ranged in of 3-6×10^3 cfu/g soil sample from replant site of Rauni on glycerol yeast extract agar medium. The population density of actinomycetes in normal sites of apple of both areas i.e. Maggots and Rauni (Shimla district) was more than their respective population in replants sites of apple. Similar results were observed by Tian *et al*. (2004) [36].

Total six actinomycetes isolates from rhizospheric soil of apple were screened for production of different plant growth promoting substances. All the isolates displayed Gram-positive characteristics and featured a filamentous morphology. They presented a circular shape with a distinct convex elevation. Notably, all the actinomycetes exhibited pigmentation, ranging from whitish to greyish-white, and displayed entire margins when cultured on KenKnight agar plates. (Table 2). All the six actinomycetes isolates *viz*., A1, A2, A3, A4, A5 and A6 were screened outfor multifarious plant growth promoting traits *viz*., phosphate solubilisation, siderophore, protease, HCN, ammonia production and antagonism against pathogenic fungi *viz*., *Dematophora necatrix*, *Fusarium oxysporum*, *Phytophthora cactorum and Pythium ultimum.*

Bacteria can enhance phosphorus (P) availability to plants via production of phosphatise enzyme, which releases free phosphorus which is present in organic matter and organic acids / chelating compounds that lower the pH in the rhizosphere (Rashid *et al*. 2004) [30]; Chen *et al*. 2006) [7]. All the screened isolates of actinomycetes produced phosphate solubilising activity (Table 3 and Fig 2). Results revealed that five isolates exhibits phosphate solubilisation in the range of 19 to 23 mm dia on Pikovakaya's agar plate except one. The highest result was showcase by isolate A3 i.e.23 mm. One isolate A4 did not show phosphate solubilizing activity. The present study throw light on the importance of actinomycetes in the solubilization of phosphorus, thereby increasing the concentration of soluble phosphate, which enhances plant growth. Anwar *et al*. (2016) [2] screened actinomycetes isolates for phosphate solubilisation using tricalcium phosphate as a sole source of phosphorus. The highest phosphate solubilization was observed in *Streptomyces* sp. WA-1 (72.13 mg/100 ml. Actinomycetes isolated from wheat rhizosphere shows substantial phosphate activity (Chukwuneme *et al*. 2020) [8].

Out of six actinomycetes isolates, five were found to produce siderophores on chrome azurol-S (CAS) agar plates with a production range of 10 to 18 mm. The highest siderophore production was exhibited by A3 isolate i.e. 18 mm, followed by A4 (15mm). The lowest production was exhibits by A1 isolate i.e.10 mm and only one isolate A3 was positive for HCN production. Ammonia production also play a significant role in the suppression of plant disease by HCN production. Khamna *et al*. (2009) [17] revealed that *Streptomyces* CMU-SK 126 strain showcase significant amount of siderophore production isolated from the rhizospheric of *Curcuma mangga.* Ahmed *et al*. (2014) [1] concluded actinomycetes aided in disease suppression by HCN production, thus serving as an important aspect of bioprotection. Furthermore, ammonia is considered as one of the growth-boosting and antimicrobial compounds generated by microbes. Ammonia and HCN production by *Streptomyces* contributed to disease suppression in plants (Anwar *et al.* 2016) [2]. In our study, ammonia production

were shown by only two isolates A2 and A4 (++). Results was exhibited by all the actinomycetes isolates ranged from 19 to 23 mm dia of clear zone (Table 3). Microorganisms can inhibit metabolic activities of pathogens by secreting lytic enzymes like lipases, chitinases, proteases, glucanases, that directly facilitate the breakdown of fungal cell walls (Neeraja *et al*. 2010) [26]. All the bacterized plants treated with *Actinomycetes* isolates were enhances the growth of tomato seedlings' by significant plant growth promoting activities (Hazem and Camele, 2022) [16].

Biocontrol is regarded as an alternative and ecofriendly practices. Numerous studies have investigated that actinobacteria exhibits antifungal activities by secreting compounds by isolating from rhizosphere of different plants such as sagebrush (*Artemisia tridentata*) (Basil *et al*. 2004) [4] and *Vitis vinifera L*(Loqman *et al*. 2009) [23]. In past years, capabilities of plant-growth promoting *Streptomyces* sp. has been revealed for inhibiting pathogen invasion and improving crop productivity (Dias *et al*. 2017; Vurukonda *et al*. 2018) [10, 40].

Antifungal activity were screened for different against plant pathogen. Against *Dematophora necatrix* all the isolates exhibits acitivity ranged from 23.0% to 46.1% inhibition. The highest activity was showcase by isolate A3 i.e. 46.1% inhibition whereas the minimum was shown by isolate A5 i.e. 23.0% inhibition. Two isolates A4 and A6 did not showed inhibition against *Dematophora necatrix* plant pathogen (Table 4 and Fig 3). All other isolates exhibits activity against *Fusarium oxysporum* and *Phytophthora cactorum* ranged from 20.0 to 44.0% Inhibition and 16.66 to 46.6% Inhibition respectively. The maximum percent inhibition of 44.0% and 46.6% was shown by isolates A5 and A3 against *Fusarium oxysporum* and *Phytophthora cactorum* respectively. Only four isolates we showed antifungal activity against *Pythium ultimum* plant pathogen. The maximum activity was shown by isolate A2 i.e. 32.7% inhibition whereas minimum was shown by isolate A5i.e. 12.7% inhibition. Two isolates A1 and A4 did not showed percent inhibition against *Pythium ultimum.* Our results align with the prior reports such as the work by Lee *et al*. (2012) [22], which demonstrated the ability of *Streptomyces cavourensis* SY224 to prevent *Colletotrichum gloeosporioides* infection through the production of hydrolytic enzymes. Antifungal efficacy of *Trichoderma harzianum* QTYC77 was attributed to the secretion of chitinase and β-1,3-glucanase targeting *F. oxysporum* f. sp. *cucumerinum* (Zhang S. *et al*. 2020) [45]. Zhang *et al*. (2021) [44] concluded that *Streptomyces* BITDG-11 could potentially be developed as a multifunctional biopesticide and as well as bioinoculant to enhance plant growth.

Table 1: Isolation and enumeration of actinomycetes from apple rhizosphere in normal and replant sites of Maggots and Rauni of Shimla district (H.P.)

Sites	Composite soil samples	Total actinomycetes count					
		KeM		cfu/gm	GYA		cfu/
		10^{-2}	10^{-4}	($\times10^3$)	10^{-2}	10^{-4}	gm ($\times10^3$)
Maggots	Mn1	22	4	22×10^3	10	1	10×10^3
	Mn2	19	5	19×10^3	7	2	7×10^3
	Mn3	21	6	21×10^3	9	1	9×10^3
Rauni	Rn1	26	2	26×10^3	10	3	10×10^3
	Rn2	23	3	23×10^3	9	1	9×10^3
	Rn3	19	1	19×10^3	7	2	7×10^3
Maggots	Mr1	14	1	14×10^3	7	1	7×10^3
	Mr2	7	2	7×10^3	3	1	3×10^3
	Mr3	17	1	17×10^3	2	1	2×10^3
Rauni	Rr1	10	1	10×10^3	5	2	5×10^3
	Rr2	8	2	8×10^3	3	1	3×10^3
	Mn1	12	1	12×10^3	6	1	6×10^3

*10^{-2} dilutions were used to calculate total actinomycetes counts as (cfu/g) of rhizospheric soil

Table 2: Morphological characteristics of actinomycetes isolated from rhizosphere of apple in replant sites of Shimla district

Replant Sites	Actinomycetes isolates	Gram staining	Colony colour	Colony characterstics		
				Form	Elevation	Margin
Maggots	A1	+	Whitish	Circular	Convex	Entire
	A2	+	Greyish white	Circular	Convex	Entire
	A3	+	Whitish	Circular	Convex	Entire
Rauni	A4	+	Greyish white	Circular	Convex	Entire
	A5	+	Whitish	Circular	Convex	Entire
	A6	+	Whitish	Circular	Convex	Entire

Table 3: Characterization of actinomycetes for *in-vitro* production of multifarious plant growth promoting activities

Actinomycetes isolates	PGP activities (mm dia)				
	Phosphate solubilization	Siderophores	Protease	HCN	Ammonia
A1	21	10	22	-	-
A2	19	11	20	-	++
A3	23	18	19	+	-
A4	-	15	21	-	++
A5	22	13	23	-	-
A6	21	-	20	-	-
$CD_{0.05}$	0.74	0.44	0.10	-	-

Table 4: Antagonistic activity of actinomycetes sp. against plant fungal pathogens

Actinomycetes isolates	Antifungal activity(mm dia & % Inhibition)							
	Percent inhibition of fungal pathogens							
	Dematophora necatrix		*Fusarium oxysporum*		*Phytophthora cactorum*		*Pythium ultimum*	
	mm	% I	mm	% I	mm	% I	mm	% I
A1	45	30.7	30	40.0	45	25.0	0	0.00
A2	39	40.0	0	0.00	36	40.0	307	32.7
A3	35	46.1	37	26.0	32	46.6	42	23.6
A4	0	0.00	40	20.0	50	16.6	0	0.00
A5	50	23.0	28	44.0	0	0.00	48	12.7
A6	0	0.00	33	34.0	42	30.0	40	27.2
$CD_{0.05}$		1.64		1.65		1.65		1.65

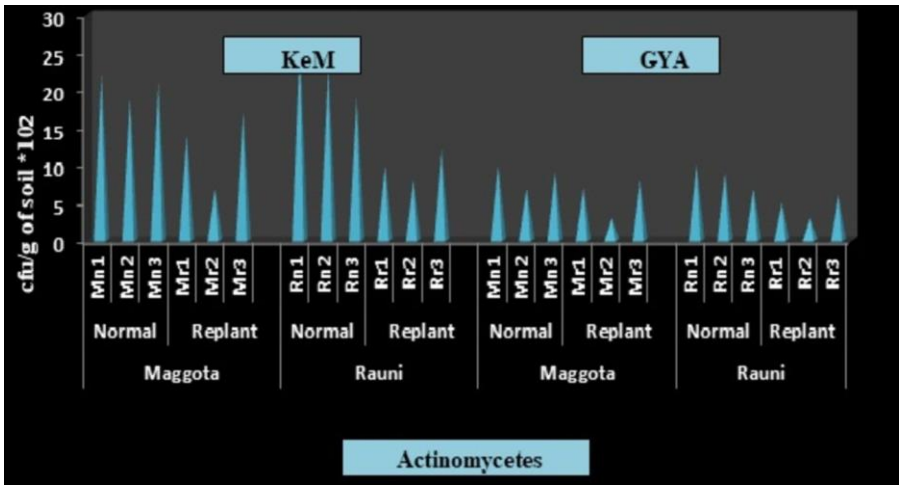

Fig 1: Comparison of total actinomycetes in normal and replant sites of Maggot and Rauni of Shimla dist.

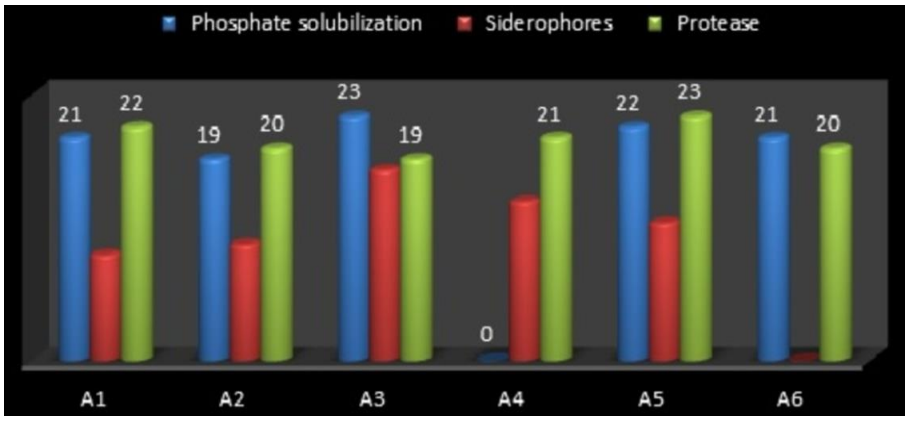

Fig 2: Comparison of different plant growth promoting attributes exhibits by actinomycetes isolates

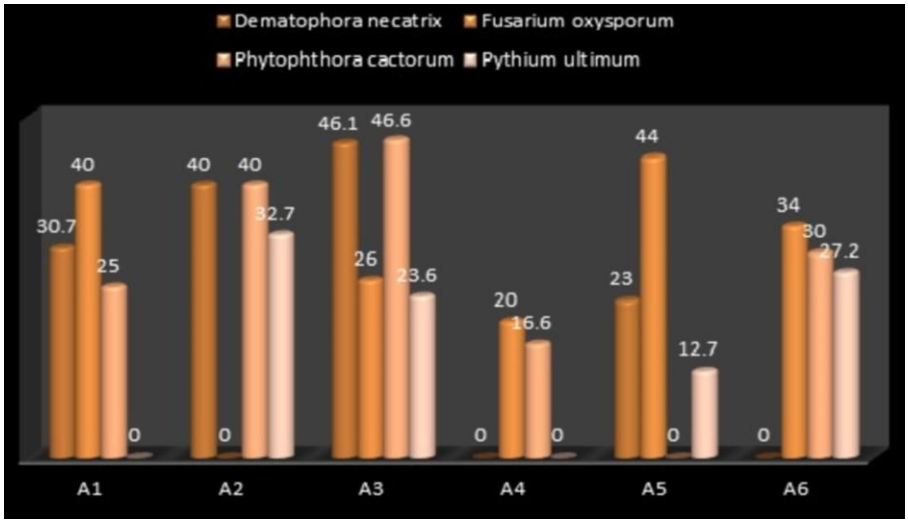

Fig 3: Antifungal activity of actinomycetes isolates against plant pathogens

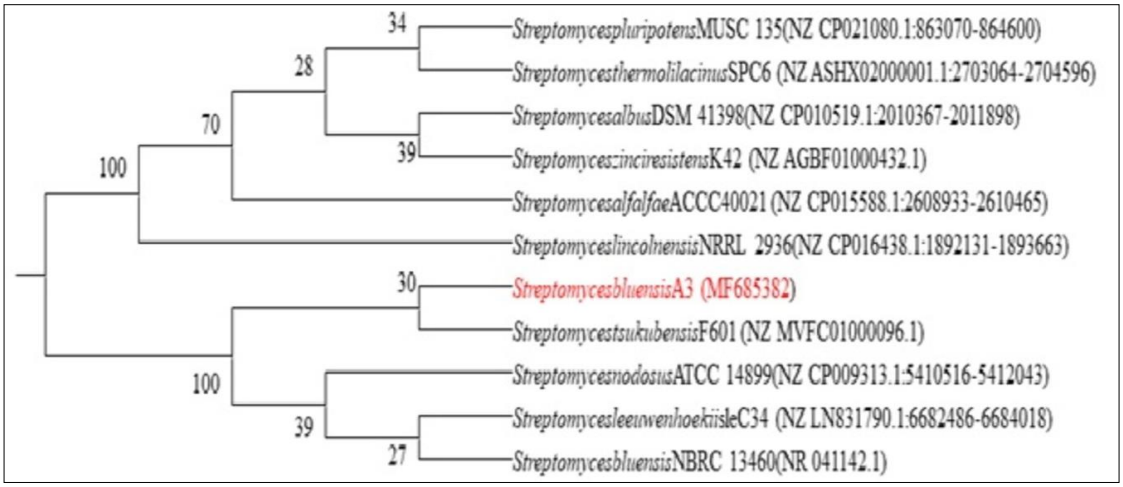

Fig 4: Evolutionary relationship of actinomycetes isolates A3 and its related taxa constructed using neighbor-joining method

3.2 16S rRNA gene sequencing of actinomycetes isolates

Molecular methodologies serves as a pivotal tools for the examination of microorganisms from biological substances. These approaches provide an platform or evaluating a diverse array of substances in a singular assay (Field and Wills, 1998) [13]. Genomic DNA from one actinomycetes isolates *viz*., A3was isolated by some modification in the method given by Kumar *et al*. (2010) [19] and the DNA was determinerd via agarose gel electrophoresis (using 1.0% agarose) (Plate 1). The genomic DNA of only one potential isolate A3was directly sentto xceleris private limited for further amplification and sequencing. Similarity searches in NCBI BLAST database revealed that the sequences were of predominantly actinomycetes origin.

The realm of microbial systematic has undergone a transformation propelled by the most frequently adopted marker -16SrRNA gene (Morra, 2002) [24] because its widespread occurrence and remarkable conservation render it suitable for elucidating the phylogenetic connections among bacteria. In light of their potential for plant growth promotion (PGP), a promising actinomycete isolate A3 was identified. A similarity search of the sequence for isolate A3 revealed a 98% match with the existing sequence of *Streptomyces bluensis* strain NBRC 13460 (accession no. NR_041142.1). Phylogenetic tree of *Streptomyces bluensis* as inferred by the neighbour joining method has been presented in Fig4. On submission of partial sequence of A3 to GenBank database (NCBI) the accession no. assigned was MF685382. Yilmaz *et al*. (2008) [43] conducted a study where they characterized actinomycetes isolated from the rhizosphere of indigenous plants of Turkey Through sequencing, they identified as *Streptomyces lydicus, S. rochei, S. microflavus, S. griseoflavus, S. albidoflavus* and *S. violaceus*

4. Conclusion

The rhizosphere of *Malus domestica*, boast a rich diversity of actinomycetes. Further, these actinomycetes showcase a multifarious plant growth-promoting activities like P-solubilization, siderophore, ammonia and HCN production etc. that may be beneficial in management of replant problem in apple. These diverse activities demonstrate significant potential for effectively addressing the replant problem in apple orchards by enabling competition with plant pathogens through the secretion of various compounds.

5. Acknowledgments

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6. References

1. Ahmed EA, Hassan EA, Tobgy KMK, Ramadan EM. Evaluation of rhizobacteria of some medicinal plants for plant growth promotion and biological control. Ann Agric Sci. 2014;59:273-280.
2. Anwar S, Ali B, Sajid I. Screening of rhizospheric actinomycetes for various *in-vitro* and *in-vivo* plant growth promoting (PGP) traits and for agro active compounds. Front Microbiol. 2016;7:1334.
3. Baker AW, Schippers B. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation. Soil Biol Biochem. 1987;12:57-60.
4. Basil AJ, Janice L, Heather S, Smith MK, Crawford DL. Studies on the microbial populations of the rhizosphere of big sagebrush (*Artemisia tridentata*). J Ind Microbiol Biotechnol. 2004;31:278-88.
5. Benizri E, Piutti S, Verger S, Pagès L, Vercambre G, Poessel JL. Replant diseases: bacterial community structure and diversity in peach rhizosphere as determined by metabolic and genetic fingerprinting. Soil Biol Biochem. 2005;37:1738-1746.
6. Boukhatem FZ, Merabet C, Tsaki H. Plant growth promoting actinobacteria, the most promising candidates as bioinoculants? Front Agron. 2022;4:1-19.
7. Chen Y, Rekha P, Arun A, Shen F, Lai WA, Young C. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol. 2006;34:33-41.
8. Chukwuneme CF, Babalola OO, Kutu FR, Ojuederie OB. Characterization of actinomycetes isolates for plant growth promoting traits and their effects on drought tolerance in maize. J Plant Interact. 2020;15(1):93-105.
9. Devi S, Sharma P, Rana A, Pal J, Kumari A. Diversity and plant growth-promoting potential of actinomycetes associated with the rhizosphere of *Arnebia euchroma* from Himachal Pradesh (India). J Environ Biol. 2021;42:964-72.
10. Dias MP, Bastos MS, Xavier VB, Cassel E, Astarita LV, Santarém ER. Plant growth and resistance promoted by *Streptomyces* spp. in tomato. Plant Physiol Biochem. 2017;118:479-93.

11. Elshafie HS, Camele I. Rhizospheric actinomycetes revealed antifungal and plant-growth-promoting activities under controlled environment. Plants. 2022;11(14):1872.
12. El-Tarabily KA. Promotion of tomato (Lycopersicon esculentum Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing *Streptomycete* actinomycetes. Plant Soil. 2008;308:161-174.
13. Field D, Wills C. Abundant microsatellite polymorphism in *Saccharomyces cerevisiae*, and the different distributions of microsatellites in eight prokaryotes and *S. cerevisiae*, result from strong mutation pressures and a variety of selective forces. Proc Natl Acad Sci U S A. 1998;95:1647-1652.
14. Gopalakrishnan S, Vadlamudi S, Bandikinda P. Evaluation of *Streptomyces* strains isolated from herbal vermicompost for their plant growth-promotion traits in rice. Microbiol Res. 2014;169:40-48.
15. Hamdali H, Hafidi M, Virolle MJ, Ouhdouch Y. Rock phosphate-solubilizing actinomycetes: screening for plant growth-promoting activities. World J Microbiol Biotechnol. 2008;24:2565-2575.
16. Hazam ES, Camele I. Rhizospheric actinomycetes revealed antifungal and plant-growth-promoting activities under controlled environment. Plants. 2022;11(14):1872.
17. Kaur M, Gupta M, Tripathi KAK, Gupta KG. Lytic effect of *Pseudomonas aeruginosa* elastase on gram positive and gram negative bacteria. Indian J Microbiol. 1989;34:855-9.
18. Khamna S, Yokota A, Lumyong S. Actinomycetes isolated from medicinal plant rhizosphere soils: Diversity and screening of antifungal compounds, indole-3-acetic acid and siderophore production. World J Microbiol Biotechnol. 2009;25:649-655.
19. Klaus H. Das Problem der Bodenmüdigkeit unter Berücksichtigung des Obstbaus. Landw Jahrb. 1939;89:413-459.
20. Kumar V, Bharti A, Gusain O, Bisht GS. An improved method for isolation of genomic DNA from filamentous actinomycetes. J Sci. Eng. Technol. Mgt. 2010;2(2)
21. Lata, Saxena AK. Characterization of plant growth promoting rhizobacteria. In: Saxena AK, editor. Training manual on Biofertilizer Technology. Delhi: IARI; c2003. p. 24-5.
22. Lee SY, Tindwa H, Lee YS, Naing KW, Hong SH, Nam Y, *et al*. Biocontrol of anthracnose in pepper using chitinase, beta-1,3 glucanase, and 2-furancarboxaldehyde produced by *Streptomyces cavourensis* SY224. World J Microbiol Biotechnol. 2012;28:391-396.
23. Loqman S, Barka EA, Clement C, Ouhdouch Y. Antagonistic actinomycetes from Moroccan soil to control the grapevine gray mold. World J Microbiol Biotechnol. 2009;25:81-91.
24. Morra MJ. Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*). Apple Environ Microbiol. 2002;68:2161-2171.
25. Neeraja C, Anil K, Purushotham P, Suma K, Sharma P, Moerschbacher BM. Biotechnological approaches to develop bacterial chitinases as a bioshield against fungal diseases. Crit Rev Biotechnol. 2010;30:231-241.
26. Oskay M, Tamer AÜ, Azeri C. Antibacterial activity of some actinomycetes isolated from farming soils of Turkey. Afr J Biotechnol. 2004;3:441-446.
27. Phukan I, Madhab M, Bordoloi M. Exploitation of PGP microbes of tea for improvement of plant growth and pest suppression: a novel approach. Two Bud. 2012;59:69-74.
28. Pikovskaya RE. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Microbiologia. 1948;17:362-370.
29. Rashid M, Khalil S, Ayub N, Alam S, Latif F. Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under *in vitro* conditions. Pak J Biol. Sci. 2004;7:187-96.
30. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4(4):406-425.
31. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; c1989.
32. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. Anal Biochem. 1987;28:751-759.
33. Sheoran OP, Tonk DS, Kaushik LS, Hasija RC, Pannu RS. Statistical software package for agricultural research workers. In: Hooda DS, Hasija RC, editors. Recent Advances In Information Theory, Statistics And Computer Applications. Hisar India: Department of Mathematics Statistics, CCS HAU; c1998. p. 139-43.
34. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997;25(24):4876-4882.
35. Tian M, Huitema E, Da Cunha L, Torto-Alalibo T, Kamoun S. A Kazal-like extracellular serine protease inhibitor from *Phytophthora infestans* targets the tomato pathogenesis-related protease P69B. J Biol Chem. 2004;279:26370-26377.
36. Traquair JA. Etiology and control of orchard replant problems: A review. Can J Plant Pathol. 1984;6:54-62.
37. Utkhede RS, Smith EM. Biotic and abiotic causes of replant problems of fruit trees. Acta Hort. 1994;363:25-31.
38. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1974;159:239-241.
39. Vurukonda S, Giovanardi D, Stefani E. Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. Int J Mol Sci. 2018;19:952.
40. Wahyudi AT, Priyanto JA, Afrista R, Kurniati D, Astuti RI, Akhdiya A. Plant growth promoting activity of actinomycetes isolated from soybean rhizosphere. Online J Biol. Sci. 2019;19(1):1-8.
41. Yadav AN, Verma P, Kumar S, Kumar V, Kumar M, Kumari Sugitha TC. Actinobacteria from rhizosphere. In: Elsevier, editor. New and future developments in microbial biotechnology and bioengineering; c2018. p. 13-41.
42. Yilmaz EI, Yavuz M, Kizil M. Molecular characterization of rhizospheric soil streptomycetes isolated from indigenous Turkish plants and their antimicrobial activity. World J Microbiol Biotechnol. 2008;24:1461-1470.

43. Zhang L, Zhang H, Huang Y, Peng J, Xie J, Wang W. Isolation and Evaluation of rhizosphere actinomycetes with potential application for biocontrolling *Fusarium* Wilt of Banana Caused by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4. Front Microbiol. 2021;12:763038.
44. Zhang S, Sun F, Liu L, Bao L, Fang W, Yin C, *et al*. Dragonfly-associated *Trichoderma harzianum* QTYC77 is not only a potential biological control agent of *Fusarium oxysporum* f. sp. *cucumerinum* but also a source of new antibacterial agents. J Agric Food Chem. 2020;141:161-177.
45. Zineb FB, Merabet C, Tsaki H. Plant growth promoting actinobacteria, the most promising candidates as bioinoculants? Front Agron. 2022;4:1-19.