



Screening and Identification of Cellulolytic *Streptomyces viridochromogenes* Strain LPA75 in Cotton Crop Compost from Beed, Maharashtra

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ABSTRACT: Cellulose is the most abundant carbohydrate in nature and the main structural component of plant cell walls. Cellulases, enzymes that break down cellulose, hold significant industrial potential. This study successfully isolated and identified a cellulolytic strain, *S. viridochromogenes* LPA75, from cotton agricultural waste compost (CAWC) in Beed, Maharashtra. The strain exhibited strong cellulase activity, making it a promising candidate for degrading cellulose-rich agricultural waste. Morphological, biochemical, and molecular characterization, including 16S rRNA gene sequencing, confirmed its identity. *S. viridochromogenes* LPA75 shows potential in sustainable organic agriculture, enhancing crop productivity and soil health in cotton-growing regions. The investigation not only highlights its enzymatic abilities but also the value of bacterial diversity in agro-waste ecosystems. After optimization, *S. viridochromogenes* LPA75 could be scaled up for eco-friendly waste recycling and nutrient management, reducing reliance on chemicals. This study presents a breakthrough in bio-organic agricultural strategies, offering a green approach to improve soil fertility and productivity, paving the way for future research into its industrial-scale applications.

Keywords: Cellulose, Actinobacteria, 16S rRNA gene sequencing, Polyphasic approach, *S. viridochromogenes* LPA75.

INTRODUCTION

Cotton farming produces a huge number of agricultural wastes, a type of lignocellulosic biomass. These lignocellulosic wastes are environment-intrusive as they are difficult to dispose of and decompose very slowly. On the other hand, such waste has untapped potential as a renewable resource through the action of microorganisms that break down its cellulose content. Among these microorganisms, actinomycetes stand out as Gram-positive bacteria with a filamentous structure. These microorganisms are found to have high capacities for decomposing complex organic materials that include cellulose. These bacteria normally inhabit soil and produce different types of extracellular enzymes, especially cellulases, that break down cellulose into simpler sugars (Sharma *et al.*, 2014; Putri and Setiawan 2019). Isolation and investigation of cellulolytic actinomycetes from cotton waste compost will deepen our understanding of microbial processes during composting but open opportunities to sustainable biotechnological applications such as production of

biofuels and biochemicals. Recent research has focused on the study of natural composts and organic waste materials to identify new actinomycete strains with high cellulolytic potency. Actinomycetes show metabolic flexibility, making them quite viable for bioconversion processes in various diverse environments. Most of their enzymatic systems are relatively more stable and more suited to the industrial conditions compared to fungal or other bacterial systems, thereby giving them the edge for industrial applications (Dashtban *et al.*, 2009; Rein *et al.*, 2016). The cellulolytic actinomycetes are of growing interest due to the current need for sustainable waste management arrangements from cotton agricultural waste compost. Cotton wastes, including stems, leaves, and hulls of cottonseed, constitute a bulk of biomass that could be exploited for biotechnological applications. The conventional modes of disposal like burning and landfilling adversely affects the environment by contribution to greenhouse effects and soil degradation. However, the use of cotton waste as a substrate in the production of industrially important enzymes such as cellulases presents an

attractive alternative that also aligns with the principles of the 'circular economy' (Maki *et al.*, 2011). Actinomycetes, in particular members of the genera *Streptomyces* and *Micromonospora*, are well documented for their ability to produce a wide array of extracellular enzymes that also include cellulases (Carro *et al.*, 2018). These gut microbes have shown to be quite efficient at degrading cellulose, the most abundant organic polymer on Earth, into simple sugars that can be fermented further for production of bioethanol, production of paper, and treatment of waste, among other uses (Immanuel *et al.*, 2006). The screening of actinomycetes from composted cotton waste may provide newer cellulolytic organisms with improved enzymatic activities and stability under extreme conditions, which gives desirable traits for industrial applications (Silva *et al.*, 2022). Environmental factors, like temperature, pH, and moisture content, can drastically impact the population dynamics of microbes in a compost (Sambusiti *et al.*, 2012). Compost containing waste cotton is thus abundant in lignocellulosic material, which encourages a highly selective environment for the proliferation of cellulolytic microorganisms (Chukwuma *et al.*, 2016). The composition of actinomycetes populations in such cotton waste composts and its cellulolytic potential have not yet been understood and needs more research. This research work is majorly focused on the isolation and identification of cellulolytic actinomycetes from cotton agricultural waste compost, targeting high cellulase activity strains. The study enriches our developing knowledge of microbial diversity in compost ecosystems and provides a basis for their further exploitation in industrial processes by examining the cellulolytic enzyme profiles of isolated actinomycetes *Streptomyces viridochromogenes* (*S. viridochromogenes*) strain LPA75 (Ohmiya *et al.*, 1977; Chein *et al.*, 2022). Such insights acquired from the present study may further lead to better ways of managing cotton waste sustainably by turning what is otherwise viewed as a problematic by-product into an important resource through microbial intervention (Bettache *et al.*, 2018).

MATERIAL AND METHODS

Sample Collection. Cotton agricultural compost samples were collected in a sterile sampling bag (HiMedia, India) from various (n=8) locations of Beed district, Maharashtra, India. All collected samples were air-dried in a shaded area at room temperature and transported to the microbiology laboratory of Vaishnavi Mahavidyalaya, Wadwani, Beed.

Screening and Isolation of Actinobacteria. The air-dried compost samples were sieved through a 2.0 mm width sterile mesh to remove stones and plant debris. Cellulolytic microorganisms were isolated using the dilution and plating method on suitable selective media. Briefly, total of 10 grams of sample was mixed into 90 mL of sterile distilled water and then 1 mL of sample was taken and suspended in 9 mL of sterile distilled water to obtain desired serial dilutions of 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵ and 10⁻⁶. 0.1 mL samples were taken from

the dilutions of 10⁻², 10⁻³ and 10⁻⁴ and spread on Starch Casein Agar (SCA) medium and incubated at 30°C for 14 days. Purification of actinobacterial isolates was done on SCA medium. The potential test of cellulose degrading isolates was carried out using a method by Donso *et al.* (2022); McDonald, *et al.* (2012). The isolates were grown on 1% Carboxymethyl Cellulose (CMC) medium and incubated at 30°C temperature for 7 days. The grown isolates were doused in 0.1% Congo Red solution and left for 15 minutes. The isolates were then rinsed thrice with 1% NaCl solution to remove Congo red solution. The isolates were re-incubated at 30°C for 72 hours for the formation of a clear zone. Gram's iodine was utilized for screening the cellulase-producing strains (Kasana *et al.*, 2008; Maravi and Kumar 2020; Bhagat and Kokitkar 2021). The strains representing potent cellulolytic activity were selected for compatibility test for formulation of consortium (Fig. 1).

Compatibility Test for consortium formulation. To verify the possibility of antagonism among the isolated strains of cellulose-degrading strains, selected isolates were used for compatibility tests as per previously developed protocol with suitable modifications. Briefly, a cross-streak method was used for the inoculation of cellulose-degrading strains on Nutrient Agar (NA) medium and incubated at 30°C for 3 days. If the strains represent growth at the intersection of the streaks, there is no antagonism; if the intersection represents no growth, there is antagonism. The strain with higher compatibility with other microbial strains with higher cellulose degradation activities were selected for further process.

Identification of cellulose degrading strains. The potential strain was identified by morphological and biochemical tests. Bacterial identification was done by 16S rRNA gene sequencing followed by phylogenetic tree analysis was used for identification.

Morphological and cultural characteristics. Actinobacterial isolates grown on SCA medium were characterized macroscopically and microscopically. Macroscopic characterization included colony morphology in the form of elevation, aerial mycelium color, substrate mycelium color and pigmentation whereas microscopic characterization was performed by Gram staining. Microscopic characterization aimed to observe the shape of the mycelium using a light microscope with 1000x magnification. Actinobacteria isolates grown on SCA were used to observe their cultural characteristics.

Biochemical characterization. Biochemical characterization was done based on different biochemical tests which include Indole test, Methyl Red test, Voges-Proskauer test, Citrate utilization test, Catalase test, Oxidase test, Sugar fermentation test, Urease test, Nitrate test, Gelatin hydrolysis, Starch hydrolysis and Casein hydrolysis.

16S rRNA gene sequencing and phylogenetic analysis
The molecular identification of the cellulase producing strain was carried out using 16s rRNA gene sequencing at National Centre for Microbial Research, Pune. The 16S rRNA query sequence was analyzed using the NCBI BLAST similarity search tool. Multiple sequence

alignment was performed using the MEGA program. The aligned sequences were refined to remove alignment noise. Finally, phylogenetic analysis was

conducted with the MEGA-X program (Saitou and Nei 1987).

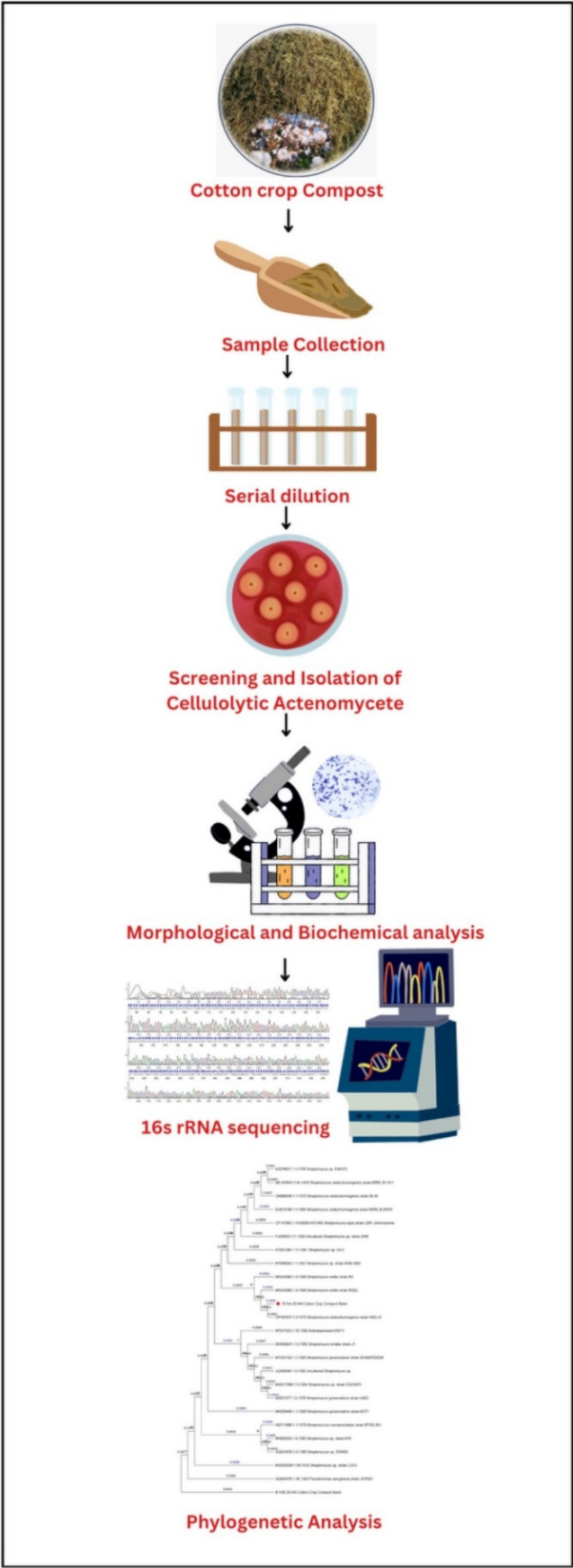


Fig. 1. Flowchart of *S. viridochromogenes* Isolation from Cotton Crop Compost.

RESULTS AND DISCUSSION

Results

Screening and Isolation. Through the screening on SCA, 71 out of 78 microbial strains exhibited zones of clearance around the colony, indicating their cellulolytic potential. On the basis of data generated, strains BEED LC A6, BEED LC A17, BEED LC A25 and BEED LC A75 showed potent CMCase activity. However, only strain BEED LC A75 represented compatibility with other strains under testing during the compatibility test. Therefore, in present study strain

BEED LC A75 was selected for further investigations strains (Maravi and Kumar 2020; Bhagat and Kokitkar 2021).

Morphological, cultural and biochemical characteristics Microscopic examination showed that the isolate was Gram-positive bacteria. Biochemical tests confirmed these isolates as *Streptomyces* species. The morphological (Table 1) and biochemical characterization (Table 2) confirmed the presence of *S. viridochromogenes*.

Table 1: Colony characterization of Isolate.

Isolate name	Colony Characteristics				Filament	Spores	Motility	Aerial mycelium	Gram Staining	Growth at 28°C
	Color	Size	Shape	Opacity						
LCA75	Off-white	small	Round	Opaque	+	+	-	+	G+	Grown

G+ = Gram positive, + = Positive/ Present, - = Negative/ Absent

Table 2: Biochemical characterization of Isolate.

Biochemical Test	
Pigment	-
Catalase	+
Oxidase	+
Urease	+
H ₂ S Production	+
Nitrate Reduction	+
Methyl Red- (MR)	+
Voges-Proskaur (VP)	+
Citrate utilization	+
Hydrolysis	
Starch	+
Utilization of Sugar	
D- Glucose	+
Sucrose	+
D- Mannitol	+
Fructose	+
Nitrogen source Utilization	
D- Alanine	+
D- Alanine	+

+ = positive reaction, - = negative reaction

Phylogenetic analysis. Identification of isolates Comparison of 16S rRNA gene sequences of isolate LPA75 (1778nt), with sequences of close *Streptomyces* species deposited in databases indicated that these isolates belong to genus *Streptomyces*. Rooted phylogenetic trees based on neighbour joining method, indicated that these were included in distinct clades in their respective trees 99% similarity with *S. viridochromogenes* strain KhEc-6 (Fig. 2).

Discussion. The isolation and characterization of *S. viridochromogenes* from CAWC highlights the potential for bio-based sustainable bioconversion and adds to the growing body of research emphasizing the role of actinomycetes in organic agriculture. This study focused on isolating and identifying cellulase-producing bacteria from CAWC samples, aiming to understand their potential for degrading cellulose, a vital polysaccharide in plant cell walls. To isolate cellulase-producing bacteria, CMC was used as a substrate. The presence of clear zones around colonies indicated cellulase activity, which aligns with established screening techniques. The use of Congo red and Gram's iodine for detecting cellulase production

was consistent with previous studies, confirming their reliability as indicators of enzyme activity (Kasana *et al.*, 2008; Maravi and Kumar 2020; Bhagat and Kokitkar 2021). The microbial degradation of cellulosic materials involves various microbial enzymes. Microorganisms from environments rich in cellulose are promising sources for discovering these enzymes. In this study, CMC was found to be an effective substrate, likely due to its simpler structure compared to more complex cellulosic materials. The strain, isolated from the CAWC in Beed, Maharashtra, exhibited substantial cellulolytic activity, highlighting its capabilities in cellulose degradation and subsequent usefulness in biofertilizer production (Ejaz *et al.*, 2021; Arelli *et al.*, 2021). Similar to investigation done by past recent studies, *Streptomyces* species are known to secrete range of extracellular enzymes (Sousa *et al.*, 2016), particularly, cellulases, that play a game changing role in the degradation of complex polysaccharides molecules like cellulose and lignocellulose into simpler sugars, thereby improving fertility and productivity of agricultural soil (Obeng *et al.*, 2017). Results obtained from present study align with the work of El-Naggar *et*

al. (2011), where *S. viridochromogenes* exhibited considerable amount of cellulolytic enzyme production, justifying its efficiency in breaking down of complex lignocellulosic biomass in agro-waste compost. The identification of the strain was confirmed through 16S rRNA gene sequencing, a gold standard method frequently employed in bacterial taxonomy due to its high accuracy in species-level bacterial identification (Mehnaz *et al.*, 2001). Further phenotypic characterization revealed that *S. viridochromogenes* not only degrades complex cellulose but also demonstrated antimicrobial properties, making it a dual-functional organism for both composting and plant protection (Korn-Wendisch and Kutzner 1992). The high cellulolytic activity of this strain can be attributed to its ability to produce a synergistic mixture of endoglucanases and β -glucosidases, similar to what has been reported by previous studies (Ghio *et al.*, 2020; Fernandes *et al.*, 2022) and others in *Streptomyces* research. This finding is particularly significant given the abundance of cellulose in cotton agricultural waste, and the need for efficient microbial decomposers in large-scale composting systems. Notably, such composting practices could offer eco-friendly alternatives to chemical fertilizers while reducing

agricultural waste and improving soil health (Ho *et al.*, 2022). Moreover, the isolation of *S. viridochromogenes* from CAWC in Beed, Maharashtra, contributes to the understanding of regional microbial diversity and its potential industrial applications in sustainable agriculture. The strain's robustness under varying environmental conditions further underscores its adaptability, an attribute that enhances its applicability in diverse agro-climatic zones (Schütz *et al.*, 2018). This work creates new approaches for future investigations that could explore genetic engineering approaches to accelerate the cellulolytic potential particularly in *S. viridochromogenes* or optimize its applications in bioconversion industries, aiming for grate productivity in composting processes and biofuel production (Kiruba *et al.*, 2022). Given the global focus on sustainable agriculture, the integration of such biotechnological advances could pave the way for more environmentally friendly practices in waste management and crop cultivation. Ultimately, Present study underlined the huge prospectives of actinomycetes in CAWC recycling and focusing the crucial role of microbial diversity in attaining a circular bioeconomy.

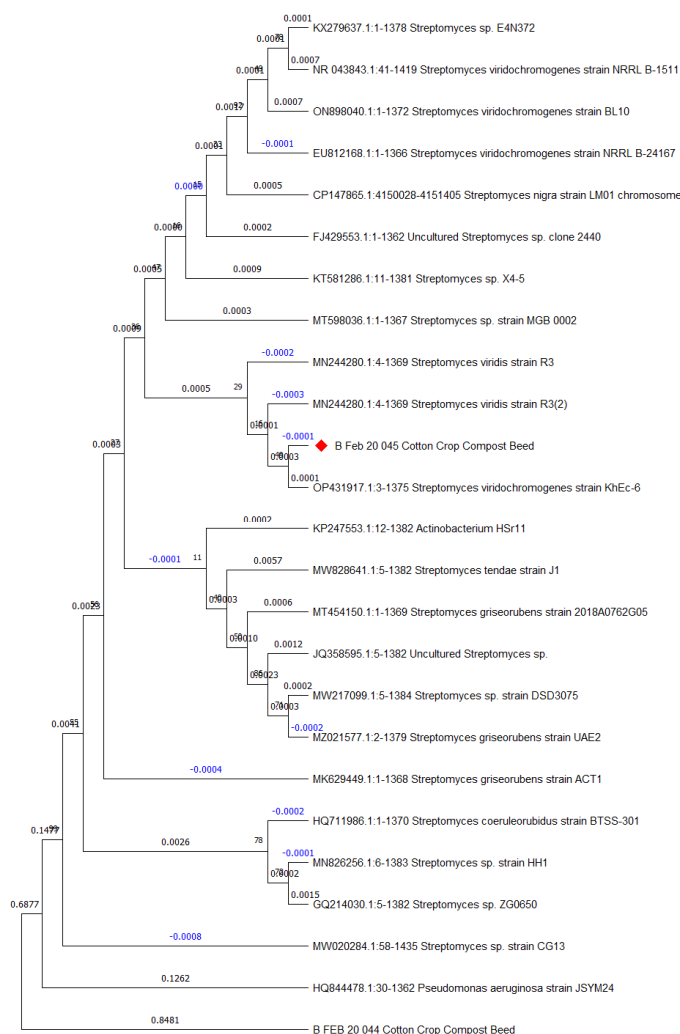


Fig. 2. Phylogenetic analysis of *S. viridochromogenes* isolated in present study.

CONCLUSIONS

The successful isolation and characterization of *S. viridochromogenes* strain LPA75 from cotton crop compost in Malpuri Village of Beed, represent/potential of agricultural waste as a promising reservoir of industrially important enzymes, particularly CAWC which is rich in complex cellulose. This strain's cellulolytic capacities hold considerable promise for sustainable agriculture, particularly in accelerating composting efficiency and facilitating the decomposition of agricultural biomass. The identification of strain LPA75 adds to the growing body of evidence underlined the role of Streptomyces species in agro-biotechnological applications. Globally, increasingly prioritizing eco-friendly and sustainable practices, the findings of this study could serve as a cornerstone for innovative approaches to waste management and agricultural productivity. Future studies could explore optimizing the enzymatic activity of this strain LPA75 and its applications in different agro-economic processes. Further work needs to be done for purification of cellulase enzyme by column chromatography, determining the enzyme activity and protein content in purified culture extracts followed by peptide profiling by using Mass spectrometry. Thus, the discovery of *S. viridochromogenes* strain LPA75 represents a significant step toward achieving a more sustainable and productive agricultural ecosystem.

FUTURE SCOPE

The strain *S. viridochromogenes* LPA75 can be optimized for its enzymatic activity and applied in large-scale composting, biofertilizer production, and sustainable waste management strategies. This strain also has the potential to improve soil fertility and agricultural productivity, offering an eco-friendly alternative to chemical fertilizers. It can also contribute to enhancing the circular bioeconomy by promoting the recycling of agricultural waste into useful resources.

Author contributions. PPD and LNC Conceptualized the present work, LNC Designed and conduct all the experiments under the supervision of PPD, AVD provide intellectual support during laboratory work, MDS, LNV and AVD analyzed the data, LNV and AVD wrote the manuscript under supervision of MDS and PPD. All authors reviewed the manuscript.

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Conflict of Interest. None.

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