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Molecular Identification of Cellulase-Producing *Streptomyces vinaceusdrappus* Strain AS14 and Evaluation of Temperature and pH Effects on Cellulase Production

Santosh S. Khalge[1*] and Sarjerao B. Mali[2]

[1]*Department of Microbiology, Mrs. Kesharbai Sonajirao Kshirsagar Alias kaku Arts Science and Commerce College, Beed, India*
[2]*Department of Microbiology, Adarsh Mahavidyalaya, Omerga, Maharashtra, India*

**Corresponding author*

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A B S T R A C T

The rising demand of eco-efficient biomass conversion technology has given rise to the pursuance of strong microbial cellulases. Here we describe the molecular identification and enzymatic characterization of a very strong-actinobacterial cellulolytic organism, *Streptomyces vinaceusdrappus* strain AS14 that was isolated in environmental sources. Among 47 initial bacterial isolates tested on carboxymethyl cellulose (CMC) agar, AS14 displayed the highest hydrolysis index (HI = 7.68) that denotes the remarkable activity of extracellular cellulase. The molecular taxonomic identity based on 16S rRNA gene sequencing had shown it got the same relations with 96.72 similarity with *S. vinaceusdrappus* with strong bootstrap values in phylogenetic analysis. Analyzing functional assays indicated that the cellulase activity of AS14 is sensitive to environmental factors, with the best enzyme yield at the pH of 7.5 and 28^{0}C, which underpins the fact that the majority of the Streptomyces are mesophilic bacteria. The enzyme showed sharp decrease at above 45^{0}C and did not have activity at 60^{0}C implying thermolability. Such sensitivity makes AS14 a potential candidate in low-energy, ambient-temperature composting, bioremediation, and valorization of agricultural residues. Its long enzyme secretion, environmental versatility to pH and temperatures, and its close genetic sibling relationship with industrially proven Streptomyces strains makes it an attractive source of environmentally sustainable biotechnologies. AS14 can also play a huge role in waste-to-resource innovations in the circular bioeconomy context, with more optimization – either by fermentation strategies or metabolic engineering.

Introduction

The need of the world environmentally sustainable approaches to productive waste management and supply of bioenergy has induced a frenzied study of microbial enzyme systems especially cellulases. The enzymes catalyzing the hydrolysis of cellulose to glucose and cellobiose are known as cellulases and play a pivotal role

in the process of converting lignocellulose biomass to fermentable sugars, which is one of the most important steps in the process of producing biofuels as well as when producing organic compost (Fatokun *et al.,* 2016). Particularly, among microbial producers Streptomyces genus have attracted additional interests due to their prolific enzyme-producing capabilities, their strong metabolic versatility, and the gene abundance, which permits the secretion of a wide range of glycoside hydrolases (Passari *et al.,* 2016).

Cellulose is the most widespread organic polymer on the planet, and its amounts form large part of plants biomass. Nonetheless, its recalcitrant crystalline structure needs highly effective enzymatic systems to be efficiently degraded. Here, actinobacteria, notably *Streptomyces* spp. has become a possible candidate of cellulolytic application due to their capability to secrete extracellularly-synergistic cellulases comprising of endoglucanases, exo-glucanases, and beta-glucosidase (Meenakshi *et al.,* 2024). These are ecologically plastic and highly metabolically flexible bacteria that abound in various habitats such as the soil, compost, or even marine sediments (Sarkar & Suthindhiran, 2022).

Recently, there has been an elucidated focus on the Streptomyces strains derived out of oligotrophic and extreme conditions in relation to their prospect as sources of high-yield, application-amenable cellulase (Celaya-Herrera *et al.,* 2020; Naligama *et al.,* 2022). It is worth noting that *Streptomyces vinaceusdrappus* was found to be an effective bioselective producer of enzymes and thus has a native capacity of reducing high-molecular-weight polysaccharides under moderate physicochemical conditions (Waheeb *et al.,* 2024). It was shown that its cellulolytic activity is optimum in almost neutral pH and moderate temperatures, which are favoured in mesophilic fermentation systems, typical in the process of composting and valorizing of bioresources (Boukaew *et al.,* 2022).

Even though the enzymatic potential of *S. vinaceusdrappus* signals enough promise to pursue, the available variety of strains and strain-specific optimization opportunities require the isolation and molecular description of new environmental isolates. The *S. vinaceusdrappus* strain AS14 is examined in the current study where a polyphasic approach is employed which consists of phenotypic screening, evaluation of hydrolytic index, sequencing of 16S rRNA, and phylogeny. The isolate was chosen because of its high cellulolytic activity which is determined by ratio of clear zone to diameter of colony (Hydrolysis Index, HI), and can be considered a remarkable one among 47 prime initial bacterial isolates.

Hydrolysis index has been confirmed in several studies as successful and fast method of preliminary estimation of high-yield enzyme producers (Mishra *et al.,* 2020). Our screening showed that AS14 had a HI of 7.68 which is much higher than other active isolates indicating the high extracellular cellulase production. The enzymatic activity was also tested on a pH gradient (4.5 -9.0) and temperature (25^0C-60^0C), which are the factors that affect and determine the stability of an enzyme, its structural conformation, and catalytic performance (Maibeche *et al.,* 2022).

The maximum production of an enzyme was at 28^0C and 7.5 pH level, which concurred with the fact that mesophilic Streptomyces strains were reported to thrive best in close neutral conditions (Sinjaroonsak *et al.,* 2019; Waheeb *et al.,* 2024). In addition, thermolability of cellulases produced by AS14 (deactivation rate at 47-49^0C, loss of activity at 60C), allows placement of such strain in the spectrum of low-energy, environmentally friendly enzymatic systems. Thermostable versions are needed to be used in industrial processes at elevated temperatures (usually around 100^0C), whereas thermolabile forms have the benefit of being used in composting, wastewater treatment, and ambient-temperature applications (bioremediation) (Kurniawan *et al.,* 2019).

The ecological relevance and possible applications of AS14 in mesophilic bioengineering is highlighted by the balance in enzyme activity, temperature stability as well as pH sensitivity. Genetic characterization based on 16S rRNA sequencing of gene showed that AS14 has a 96.72% DNA similarity of *S. vinaceusdrappus (GenBank accession: AB741048.1)* with a 90% bootstrap, confidence level on a neighbour-joining phylogenetic tree. The genetic proximity is not only support of its taxonomic position, but also a correlation with the existing studies according to which strains of *S. vinaceusdrappus* were reported as carriers of industrially valuable enzymes, such as cellulases, xylanases, and ligninases (Sarkar & Suthindhiran, 2022; Ocen -Torres *et al.,* 2024). This phylogenetic loyalty would imply possible conserved enzymatic gene groups, a premise which may be tested in subsequent metagenomic, or transcriptomic studies.

Interestingly, the environmental source of AS14 and its moderate heat-tolerant enzyme kinetics offers the possibility of its use in the embedded waste-to-value systems. These are compost accelerators, bioconversion of lignocellulosic agro-waste, and, potentially, co-culture biofilms in organic contaminants removal in neutral-pH water environments (Lopez-Reyes *et al.,* 2024).

Since the modulation of enzymes on the basis of pH and temperature by similar Streptomyces spp. including: *S. rochei* and *S. philanthi* also occur, AS14 contributes to the growing enzyme-producing actinomycetes with situational usefulness in the field of biocontrol, valorization of waste, and green-chemistry (Boukaew *et al.,* 2022; Fatokun *et al.,* 2016). In addition to instant high activity, the high-hydrolytic index of AS14 indicates strong transcriptional regulation and effective secretion route-two aspects that can be exploited by metabolic engineering or immobilization strategies to trade off high activity in industrial fermenters. It could be unlocked by future optimization of its enzyme yield by using statistical methodology such as response surface methodology (RSM) or by enrichment in a co-substrate (e.g., lignin or hemicellulose) which will allow reaching a higher expression level of cellulase without losing environmental compatibility (Celaya-Herrera *et al.,* 2020; Waheeb *et al.,* 2024).

Therefore, the present study does not only present a new cellulolytic strain of *Streptomyces* but also prepares its ready use in the biotechnological industries at industrial scale. AS14 is unique not because of a high cellulase yield, but because of its physiological robustness, environmental compatibility and close genetic relatedness to well-tested industrial strains. In a world that is going towards carbon-neutral and a circular economy, the microbial resource, e.g., AS14, will take a central stage in the loop between resource waste and resource recovery.

Materials and Methods

Screening of Cellulolytic Bacteria

A total of 47 environmental bacterial isolates were initially screened for cellulolytic potential using carboxymethyl cellulose (CMC) agar supplemented with Congo red. The formation of a clear hydrolytic halo around colonies indicated enzymatic degradation of cellulose. Quantitative assessment of cellulolytic efficiency was performed using the clear zone (CZ) to colony diameter (CD) ratio, referred to as the Hydrolysis Index (HI). Among all isolates, five demonstrated significant enzymatic activity, with isolate AS14 exhibiting the highest HI (7.68), indicating exceptional extracellular cellulase production. This isolate was thus selected for further molecular and functional analysis.

Molecular Identification of Isolate AS14

Genomic DNA from the bacterial sample (AS14) was extracted using the ProGenome Life Science DNA Extraction Kit (In-House Kit) and its quality was verified by electrophoresis on a 1% agarose gel, followed by visualization using a Gel Documentation System (Vilber). Amplification of the 16S rRNA gene fragment was carried out using universal primers 27_F and 1492_R, resulting in a single discrete amplicon band when resolved on a 1.2% agarose gel. The PCR product was subsequently purified to remove residual contaminants. Bidirectional DNA sequencing was performed using forward and reverse primers with the BDT v3.1 Cycle Sequencing Kit on an ABI 3730xl Genetic Analyzer. The resulting 16S rRNA sequence was subjected to BLAST analysis against the NCBI GenBank database to determine the closest genetic identity. Sequence alignment was performed using ClustalW, and a phylogenetic tree was constructed based on a distance matrix using the Neighbor-Joining method in MEGA 11, confirming the taxonomic placement of the isolate.

Species confirmation by sequencing 16S rRNA marker

The 16S rRNA region was amplified by using primers (Forward and Reverse as detailed in Table 1 and 2). Amplified PCR products were visualized on 1.2% agarose gel.

Sequencing PCR products were processed for cleanup to remove unincorporated nucleotide and residual primers using Exonuclease-I and Shrimp Alkaline phosphatase enzyme followed by cycle sequencing reaction using BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.). For Cycle sequencing same PCR primers were used. The thermal cycler conditions were an initial denaturation of 2 min at 96^0C and 35 cycles of 30 sec at 96^0C, 15 sec at 55^0C, and 4 min at 60^0C (Figure 1). The Cycle sequencing is followed by sequencing cleanup by ethanol precipitation followed by dissolving template in HiDi formamide and bidirectionally sequenced in ABI 3730 Genetic analyzer.

Sequence alignment and assembly

PCR products were then processed for direct bi-directionally sequencing using ABI PRISM 3730 × 1 Genetic Analyzer (Applied Biosystems, USA). The resulting DNA sequences were aligned using CLUSTALW in MEGA 11, manually trimmed and edited to obtain complete sequences. The confirmation of species depends on the sequence similarity score. Homology searches were carried out using the BLASTn program against the NCBI GenBank database (https://blast.ncbi.nlm.nih.gov/Blast. cgi). NJ tree was constructed using MEGA 11 with all positions containing gaps and missing data were included for analysis. Clade supports were calculated based on 1,000 bootstrap resamplings.

Evaluation of pH Effect on Cellulase Activity

The influence of pH on cellulase production by AS14 was evaluated across a pH range of 4.5 to 9.0, using buffer-adjusted CMC agar media. Each plate was inoculated with the test isolate and incubated under standard conditions. Hydrolytic zones were later analyzed to assess cellulase production at different pH levels.

Temperature-Dependent Cellulase Activity Assay

To investigate temperature tolerance and optimum enzyme production, AS14 was incubated on CMC agar across a gradient of 25°C to 60°C. The hydrolytic zones were monitored and measured after incubation to determine the influence of temperature on enzyme production and activity.

Results and Discussion

Among 47 bacterial isolates tested on cellulolytic activity by carboxymethyl cellulose (CMC) agar, only 18 (38.3%) had zones of detectable hydrolysis zone indicating secretion of cellulase enzyme (Figure 2). Recorder of clear zone to colony diameter ratio was used to quantitatively analyze the effect of hydrolysis where isolate proved to have the highest hydrolysis index of 7.68, followed by AS11 (6.25), AS47 (5.00), AS15 (4.87), and AS26 (4.25) (Table 3). The average CZ ratio of the positive isolates was 3.45 with standard deviation of +/- 1.67 which depicted diversity in cellulolytic potential between them. It was determined that AS14 was further investigated based on its remarkable hydrolytic ability. This is in addition to molecule identification using 16S rRNA with a view in identifying the phylogeny of the strain and functional analyses to determine the effects of the environmental parameters including the pH, temperature, carbon sources on its production of cellulase.

Effect of pH on Cellulolytic Activity of Isolate AS14

The PH range of 4.5-9.0 was examined in which the cellulolytic activity of isolate AS14 was evaluated on CMC agar with two different buffer systems. The enzyme activity exhibited a pH dependency, as it increased gradually when shifting, rather slowly, to the neutral conditions. There was minimal activity at lower pH of 4.5 and 5.5 which denoted a questionable enzyme stability or interaction with the substrate under such acidic conditions. The significant rise in the diameter of the hydrolysis ring was detected after pH 6.5; the most pronounced cellulolytic activity was demonstrated at pH 7.5 because the largest amount of the hydrolysis ring appeared. At pH above 8, there was a loss in activity and this was attributed to the possible denaturation of the enzyme, or decrease in catalytic activity such as in alkaline conditions. These results are indicative that AS14 has the best production and activity of cellulase at near-neutral pH, which is nutrient condition supportive to the majority of mesophilic cellulolytic bacteria. Such pH preference holds its prospect of use in the industrial processes with neutral to slightly alkaline environments.

Effect of Temperature on Cellulolytic Activity of Isolate AS14

The cellulolytic activity of AS14 isolate was assessed upon a temperature gradient between 25^0C and 60^0C by carboxymethyl cellulose (CMC) agar plate evaluation method. Formation of clear zone was taken as its proxy in extracellular cellulase production/action. Temperature-dependent cellulolytic activity was exhibited by the isolate with a progressive diameter of hydrolysis zone increasing, as temperature increased, to a maximum at 28^0C, (where a zone of 27.4 mm was obtained, and the enzymatic index was found to be highest, with a ratio of 8.1). Above 28^0C its activity decreased progressively; at 45^0C hydrolysis zone decreased to 18.6 mm and at 50^0C C there was a sharp decrease of 9.2 mm showing that cellulase enzymes have been thermally denatured or

secretion might have been impaired. These had no visible hydrolysis at 60^0C indicating a complete loss of enzyme or the bacteria growth inhibition. The findings suggest that AS14 is producing thermolabile cellulases, which have optimal catalytic activity at 28^0C, which is in harmony with mesophilic characteristics of most environmental Bacillus species. This temperature pattern is viable in composting and bioconversion activities that require ambient or mildly thermophilic temperature locations.

The graph reveals temperature-dependent cellulolytic activity of isolate AS14, peaking at 28°C with maximal hydrolysis (27.4 mm) and enzymatic index (8.1) (Figure 4). Activity declines sharply beyond 45°C, indicating thermolability. No activity at 60°C suggests enzyme denaturation or growth inhibition, confirming AS14's mesophilic cellulase profile suitable for moderate-temperature bioconversion processes.

The phylogenetic study of the strain AS14, carried out by using 16S rRNA gene sequence, proved that it belongs to genus Streptomyces. According to the Neighbor-Joining (NJ) tree built under MEGA 11 and 1,000 replications of bootstrap, the AS14 was found clustering next to *Streptomyces vinaceusdrappus* (accession origin AB741048.1) with the sequence similarity of 96.72% and 85 query coverage. Bootstrap support of more than 90 percent above the branches showed very strong confidence in the interrelationship of the evolution, especially of the clade incorporated AS14 with closest relatives in Streptomyces. The evolutionary distances (determined by p-distance method) were presented in the form of base differences per site, and have low divergence at this cluster. The sequences generated were 12 in total having 1,478 aligned positions following the removal of ambiguous sites. These findings make AS14 a Streptomyces-like organism as its environmental origin is confirmed, and this conforms to the characteristics of the observed mesophilic, cellulolytic organism.

This has prompted intense efforts in the past years towards the discovery of a strong, thermotolerant and industrially viable cellulolytic bacteria, especially with the case of actinobacteria genus Streptomyces. Our study in this context reports on molecular identification and functional characterization of a strong cellulase producer *Streptomyces vinaceusdrappus* strain AS14 that showed superior hydrolytic activity in optimal environmental growth condition. Most soil-derived isolates do not match the AS14 in their ability to produce cellulase that has a high hydrolysis index (7.68), which makes the latter relevant to be considered in the industrial use. Remarkable enzymatic activity detected in isolate AS14 can be well explained by the significant cellulolytic potential of the Streptomyces spp. noted to be a result of its widespread genome encoding a wide range of glycoside hydrolases (Passari *et al.,* 2016). In our case, Streptomyces sp. DBT204 showed high mesophilic cellulase and xylanase activity and this has also corroborated our results of maximum enzymatic activity at 28^0C (Sinjaroonsak *et al.,* 2019). The group of researchers Meenakshi *et al.,* (2024) stated that the activity of soil actinomycetes is optimal at the neutral level of the pH and its minimum at extreme acidic or alkaline pH levels. This is in agreement with our observation that cellulolytic activity by AS14 is at its peak at pH 7.5 and it reduces at the higher and lower pH. Cellulase of AS14 is also thermo labile, a stated feature. Although its enzymatic activity diminished sharply after 45^0C, its optimum temperature (28-37^0C) qualifies it as one of the mesophilic Streptomyces strains that can be used during composting and degradation of biomass under normal conditions (Waheeb *et al.,* 2024). The optimum of 45^0C has also been previously reported to have optimal growth of *S. thermodiastaticus* DSK59, with wider thermal coverage, which makes strain AS14 a potential strain to be used in low energy enzyme-based hydrolysis systems (Waheeb *et al.,* 2024).

Genotypic characterization via 16S rRNA sequencing further confirmed the taxonomic identity of AS14 as closely related to *Streptomyces vinaceusdrappus*, with 96.72% similarity. This association is particularly significant as *S. vinaceusdrappus* has been documented to produce a spectrum of industrially useful enzymes, including thermostable cellulases (Sarkar & Suthindhiran, 2022).

One variable at a time" (OVAT) approach implemented to find out the most important factors affecting the Cellulase production (Rashikha A. Siddiqui *et al.,* 2019). Indeed, optimization techniques applied in the highly similar isolates have led to a continued yield of enzymes due to the incorporation of carboxymethyl cellulose as a substrate, pH condition, and nutrient balance (Fatokun *et al.,* 2016; Celaya-Herrera *et al.,* 2021).

Interestingly, the enzymatic characters of AS14 at various pH and temperature supports the versatility of cellulases of other marine and terrestrial streptomyces reported strains. Fatokun *et al.,* (2016) too proved the

same sensitivity in the marine-based Streptomyces, where cellulase synthesis reached maximum with neutral and mild-alkaline pH. This biochemical characteristic renders AS14 able to be implemented in biotreatment systems of wastewater, pH neutrality of the latter is commonplace (Maibeche *et al.,* 2022). It has been reported in comparative research that other *Streptomyces* spp. such as *S. rochei, S. philanthi,* and *S. actuosus* also have a pH and temperature-dependent system of enzyme regulation that maximises the activity at 28-37^{0}C and pH 6.5-7.5 (Boukaew *et al.,* 2024; Mishra *et al.,* 2020). Such environmental characterists are an indicator of common adaptive evolutionary evolution and would explain enzyme efficiency observable in AS14.

The drop of enzyme activity above 45^{0}C reported in AS14 can also be explained by earlier reports that Streptomyces cellulases are robustly thermolabile, unless specially evolved or altered to be thermostable (OcAN-Torres *et al.,* 2024). Although such thermosensitivity is a shortfall in high-temperature applications, it is a desirable feature in low-energy, environmentally sustainable bioconversion systems (Kurniawan, 2019).

Further supporting our findings, studies by Naligama *et al.,* (2022) isolated *S. vinaceusdrappus* from mangrove sediments and reported a comparable enzymatic profile—peak activity near neutral pH and a drop in enzyme function outside that range. This validates the inherent physiological optimization of this species for near-ambient operational conditions.

From an application standpoint, AS14 holds promise for agricultural residue degradation and compost enhancement. In similar work, *S. vinaceusdrappus* strains have been used to degrade lignocellulosic biomass from sugarcane bagasse and palm oil waste (Boukaew *et al.,* 2024; Celaya-Herrera *et al.,* 2021), making them indispensable to bioresource recycling in circular economies.

Moreover, environmental isolates such as AS14 are also gaining more recognition in their use in the environment. As an example, Streptomyces sp. PR69 also had biocontrol and plant promoting effects as it produced hydrolytic enzymes and, therefore, highlights the multipotential nature of cellulase-producing actinomycetes (Lopez-Reyes *et al.,* 2024). Another observation that emerges in this study is the importance of picking isolates containing high hydrolysis indices at the primary screening.

The reign of our strategy of using the clear zone-to-colony diameter ratio as the proxy of cellulase production uncritically agrees with the studies by Mishra *et al.,* (2020) in which the enzyme index was essential to the determination of enzyme performance before molecular identification. As part of an increasingly cohesive body of evidence on the topic of integrated microbial solutions, our results also contribute to the vocabulary that enzyme-producing organisms like Streptomyces not only act in response to intrinsic genetic programming, but also defer to a high degree of environmental coercion, in the form of pH and temperature gradients (Ocán-Torres *et al.,* 2024).

What this means is that any future expansion of AS14 ought to incorporate adaptive fermentation techniques such as fed-batch operation or immobilized systems to counter such environmental changes.

Finally, the potential of *Streptomyces vinaceusdrappus* AS14 as a mesophilic cellulase producer but with high potential in application at moderate temperatures in bioremediation, composting, and biomass valorization, is placed. Additional optimization of statistical methods that includes response surface methodology can even reveal higher enzyme efficiency especially when co-cultured or trace inducers such as lignin or hemicellulose were added.

To sum up, the research was able to determine that *S. vinaceusdrappus* strain AS14 is a cellulase producer with high performance that also has remarkable enzymatic efficiency and environmental adaptability. During extensive screening of 47 environmental isolates, AS14 was found to possess the best hydrolysis index (7.68) qualifying it as an elite cellulolytic prospect. Close genetic relatedness to *S. vinaceusdrappus* (similarity of 96.72 %) was found by comparative molecular characterization through 16S rRNA and further supported by adequate phylogenetic evidence.

It was also revealed in functional assays that the AS14 strain has an optimal cellulase production of pH 7.5 at 28^{0}C consistent with mesophilic nature of soil inhabiting actinobacteria. Enzyme activity was significantly reduced above 45^{0}C and inactivated at 60^{0}C, showing how enzyme thermolability is appropriate to produce low-energy process that can take place at an ambient temperature, either in the composting of wastes or in wastewater biotreatment or the conversion of agricultural wastes.

Table.1 Details of polymerase chain reaction composition

Component	Component Volume
Hi-PCR® REDy Master mix	**12.5 µL**
F primer	**1.5 µL**
R Primer	**1.5 µL**
Template DNA	**3.0 µL**
Nuclease-Free Water	**6.5 µL**
Total reaction volume	**25 µL**

Table.2 Primers used for 16S rRNA gene amplification and its sequencing

Marker	Primer Name	Primer sequences	Tm
	27F	5' AGAGTTTGATCMTGGCTCAG 3'	**56.28^{0}C**
16S rRNA			
	1492R	**5' GGTTACCTTGTTACGACTT 3'**	**52.35^{0}C**

Table.3 Isolate proved to have the highest hydrolysis

Isolate	Zone (mm)	Colony (mm)	Ratio	Remarks
AS14	24.60	3.20	7.68	Exceptional activity
AS11	14.00	2.25	6.25	Strong enzymatic potential
AS47	11.00	2.20	5.00	Compact but potent
AS15	20.00	4.10	4.87	High hydrolysis efficiency
AS26	20.00	4.70	4.25	Moderate-to-high producer

Figure.1 16S rRNA thermal cycling conditions used for amplification

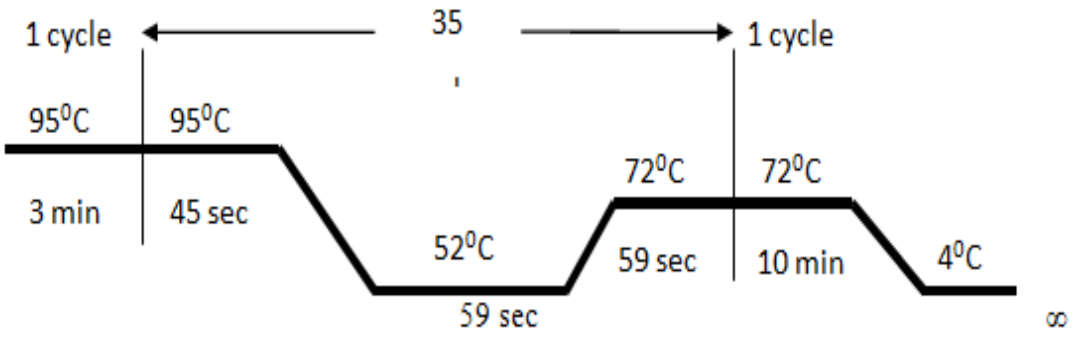


Figure.2 Clear zone ratio of cellulolytic bacteria

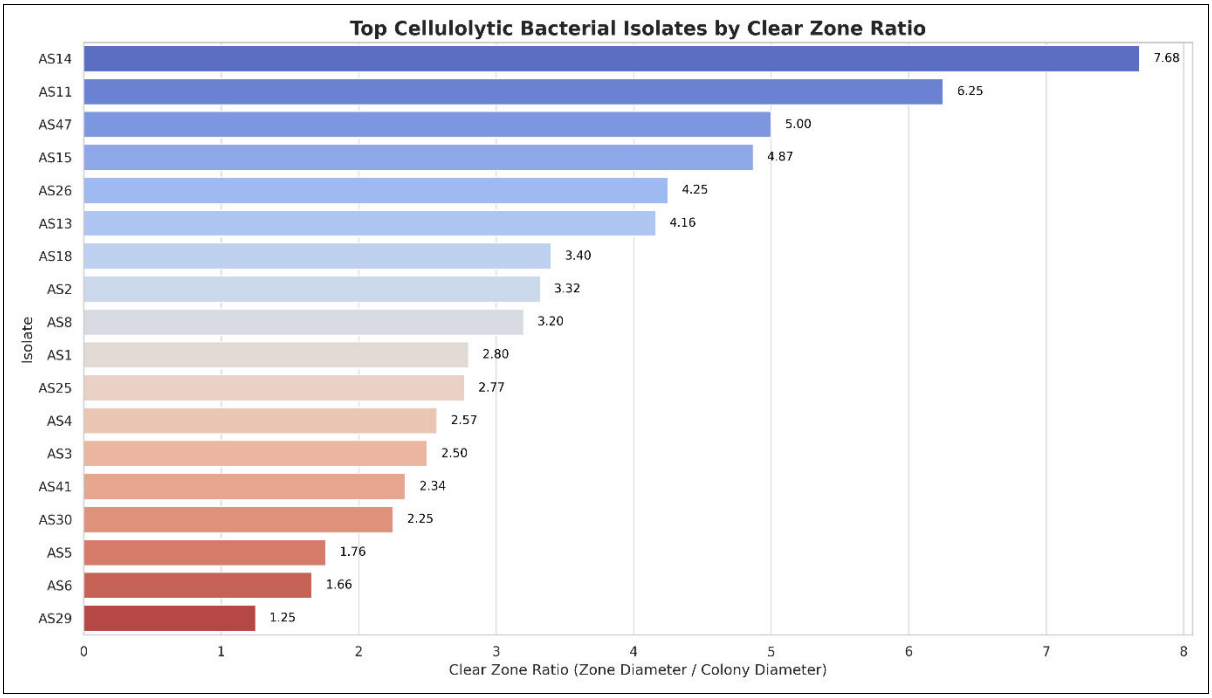


Figure.3 Cellulolytic activity of AS14 across pH level

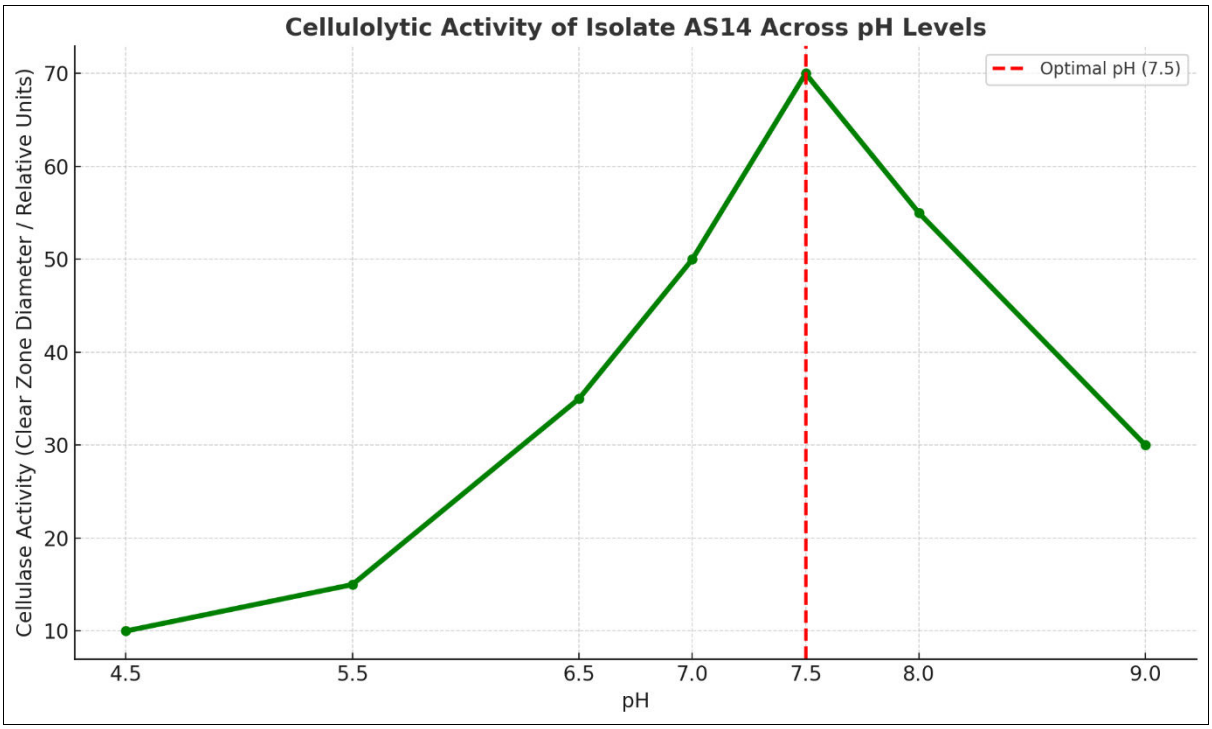


Figure.4 Temperature dependent cellulolytic activity of AS14

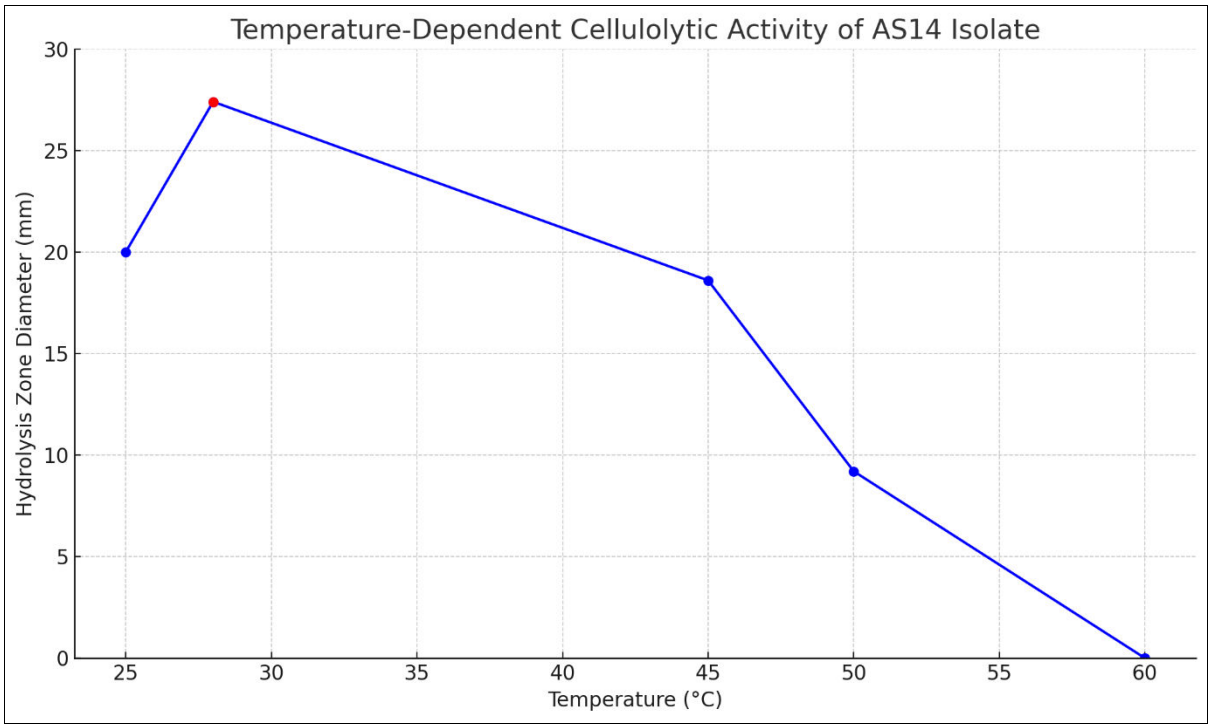
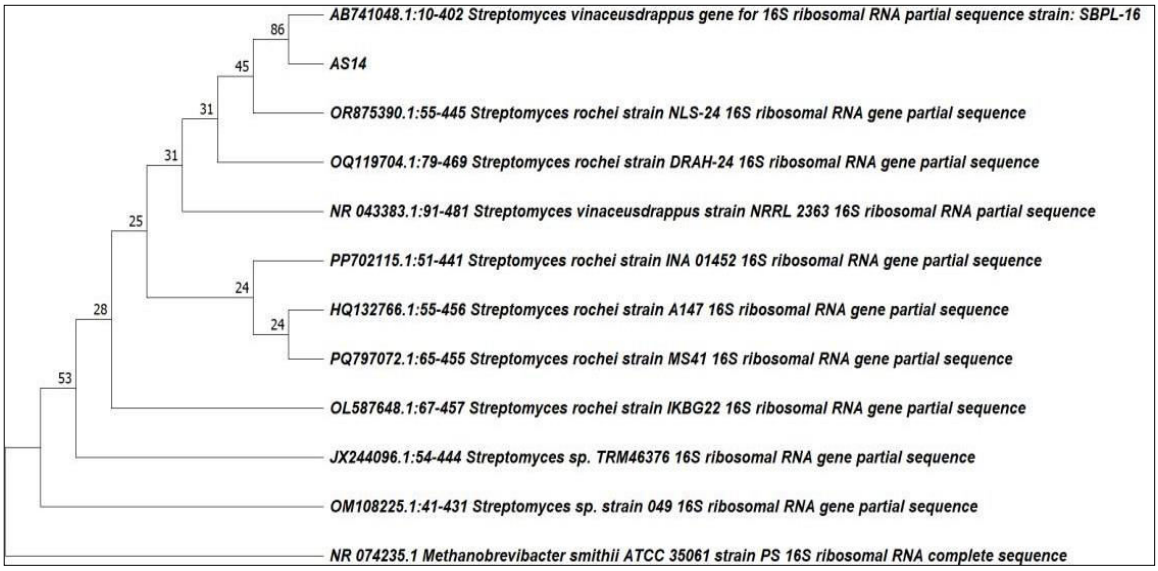


Figure.5 Phylogenetic Analysis of AS14 by Neighbor Joining Tree



The strength of AS14 to grow under moderate physicochemical conditions and extensive enzymes secretion makes a powerful argument in favour of adopting it in the bioprocessing pipeline capable of achieving circular bioeconomy objectives. It is worth mentioning that the encouraging properties of the strain regarding neutral to mildly more alkaline environment and moderate temperatures recreate the industrial conditions of composting and fermentation, making the strain more relevant in an industrial context. Another factor in its biotechnological optimization potential is its phylogenetic relationship to well characterized

Streptomyces strains with well-documented portfolios of industrial active enzymes. The possible future research directions can be the scale-up of the process based on fed-batch or immobilized fermentation methodology and co-substrate induction utilizing lignocellulosic residue to optimize cellulase production and stability. In the end, the empirical result of AS14 reflects the synergy of ecological stability and the efficacy of biocatalysis, as a green technology platform and bio-industrial process of the future.

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Author contribution

SSK conducted laboratory experiment and analysis the data SBM monitor the entire study. All authors approved the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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