

Isolation and identification of bacteria with cellulose-degrading potential from soil and optimization of cellulase production

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ABSTRACT

Soil is one of the most promising sources for the presence of a variety of microorganisms which produce different hydrolytic enzymes. Such microorganisms include bacteria, fungi, etc. The ability to produce hydrolytic enzymes makes them potential candidates for hydrolysis of complex polymeric substrates. The present study aims at screening, isolating, and characterizing cellulolytic bacteria isolated from soil samples. Nine different soil samples were collected from different locations near Raigad district, Maharashtra, India. Carboxymethylcellulose (CMC) was used as a sole source of carbon for screening of cellulase-producing isolates. Forty-five different cellulase-producing bacteria were isolated based on their ability to decolorize Congo red and iodine. The morphological and molecular characterization of seven best isolates was carried out for their identification. All seven isolates were identified to be *Bacillus* species using 16S rRNA gene-based sequencing. The optimization of cellulase enzyme production of these seven isolates was carried out by using different parameters such as pH, temperature, and carbon sources. Majority of the cellulase producers identified in the present research work were found to be mesophiles. pH ranging from 6 to 8 was found to be most suitable to produce cellulase enzyme by the isolates. The data suggest that polymeric substances such as starch and CMC act as inducers for cellulase production.

1. INTRODUCTION

Cellulose is one of the most abundantly available wastes from agriculture and industrial origin. It is also obtained in large amounts from industries such as food, paper, and pulp [1]. Due to its availability in large amounts, it has become the most promising renewable energy resource [2]. Cellulose is made up of multiple D-glucose units and is formed due to the condensation of these glucose units through β (1 \rightarrow 4)-glycosidic bonds [3]. There are several studies on the conversion of this lignocellulosic biomass into fermentable sugars. Methods such as acid, alkali, or heat treatments can be used for efficient conversion of cellulosic biomass. These methods have several disadvantages such as use of harsh chemicals, pollution, high cost, high energy consumption, etc. [4,5]. The most effective approach for the hydrolysis of lignocellulosic waste is thought to be the use of microbial

enzymes [6]. The degradation of this biomass using microbial enzymes can help utilize this resource for the production of many economically important products such as biofuels, animal feeds, etc. [7]. The most commonly used enzymes for the hydrolysis of lignocellulosic biomass are cellulases [8]. Cellulase is a set of three enzymes which acts in a synergistic manner to hydrolyze the lignocellulosic biomass. It includes endoglucanases (EC 3.2.1.4), exoglucanases, also known as cellobiohydrolases (EC 3.2.1.91), and β -glucosidases (EC 3.2.1.21) [9]. The endo- β -1, 4-glucanases, and exo- β -1,4 cellobiohydrolases degrade cellulose to get smaller cello-oligosaccharides. The oligosaccharides obtained are then hydrolyzed to glucose by β -glucosidase [10].

Soil, being the richest ecosystem, is the most important source for the presence of hydrolytic enzyme-producing microbes such as bacteria, fungi, actinomycetes, etc. The microorganisms in the soil produce different hydrolytic enzymes such as amylases, proteases, cellulases, lipases, etc. There are several reports available on the production of cellulases from fungi. *Aspergillus* and *Trichoderma* species are the most used fungi for the production of cellulases at commercial scale [11]. Cellulase was also reported to be produced

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from a fungus named *Penicillium funiculosum*. The cellulase enzyme was produced under submerged fermentation using rice husk powder as a carbon source [12]. However, the production of bacterial cellulases at the commercial scale was limited due to its complexity as well as difficulty in the extraction and purification. A wide range of researches has been initiated upon the application of bacterial cellulases [13]. Cellulases from the bacterial sources such as *Pseudomonas* and *Sphingomonas* are commercially used in the textile industry. Cellulase produced from *Bacillus* species has been extensively studied for its use in laundry detergents [14]. Bacteria can be chosen over fungi for the production of cellulases at the industrial scale due to their high growth rate [15]. Moreover, genetic manipulations of bacteria are easier when compared to fungi [16]. The present study was focused on the screening and isolation of the potential cellulase-producing bacteria and the optimization of different parameters to maximize cellulase production. The bacterial isolate with the promising cellulolytic potential could be further explored for the production and purification of cellulase enzyme. The purified enzyme can be utilized in industrial applications as well as for the degradation of lignocellulosic biomass.

2. MATERIALS AND METHODS

2.1. Chemicals

All the chemicals were of analytical grade and purchased from Sisco Research Laboratories Pvt. Ltd. India. Media components and Congo red (microbiology grade) were purchased from HiMedia Private Ltd. India.

2.2. Sample Collection

Nine different soil samples were selected for isolation of cellulase-producing bacteria from different regions of Raigad district. The preferred areas for collection of soil samples were dump yards, undisturbed garden soil, and undisturbed forest soil. The soil samples were collected in sterile polythene bags.

2.3. Screening and Isolation of Cellulase-Producing Bacteria

Serial dilutions of the soil samples were prepared using 0.85% saline and the higher dilutions were plated on the screening medium composed of carboxymethylcellulose (CMC) (0.5%), NaNO_3 (0.1%), K_2HPO_4 (0.1%), KCl (0.1%), MgSO_4 (0.05%), yeast extract (0.05%), and agar agar (2.0%) [17,18]. Plates were incubated at 37°C for 48 hours. Isolated organisms were maintained on fresh agar slants using the same media composition at 4°C. For screening of the cellulase-producing organisms, point inoculation of the isolates was carried out on the same cellulase screening media plates, followed by incubation at 37°C for 48 hours. The plates were flooded with 0.1% Congo red and further washed using 1M sodium chloride to visualize the zone of clearance. Gram's iodine was also used for screening of cellulase-producing isolates [18].

2.4. Identification of Bacteria

The isolated bacteria were characterized by performing Gram's staining to identify their morphology. Genotypic characterization

of bacterial isolates was carried out at the National Collection of Industrial Microorganisms (NCIM), Council of Scientific & Industrial Research- National Chemical Laboratory (CSIR-NCL), Pune, India. The molecular identification of the cellulase-producing bacterial isolates was carried out using 16S rRNA gene sequencing. This was carried out in two steps.

2.4.1. DNA isolation and PCR

Isolated bacteria were subjected to DNA extraction using HiPurA™ bacterial genomic DNA purification kits [19]. Agarose gel electrophoresis was carried out in order to check the integrity of the extracted DNA and the quantification was conducted using NanoDrop Lite Spectrophotometer (Thermo Scientific). Exactly 50 ng of the above-extracted DNA was taken for 16S rRNA gene amplification. The following universal bacterial forward and reverse primers were used: 16F27-(5'AGAGTTTGATCMTGGCTCAG3') and 16R1492-(5'TACGGYTACCTTGTTACGACTT3'). The polymerase chain reaction (PCR) was carried out in 50 µl reaction mixture containing 3.5 pM primers (Eurofins), 5 mM deoxynucleotide triphosphate (dNTPS-Genei), 1U Taq polymerase (Genei), PCR reaction buffer (with 15 mM MgCl_2), DNA template, and PCR water. PCR conditions were 34 cycles of 30 seconds at 95°C, 30 seconds at 55°C, and 1 minute 30 seconds at 72°C and final extension at 72°C for 7 minutes.

2.4.2. DNA sequencing

After the purification of the PCR product partial 16S rRNA gene sequencing was carried out using the ABI 3,500 × 1 genetic analyzer (Invitrogen/Life Technologies) [20]. The sequenced data were further analyzed by Basic Local Alignment Search Tool (BLAST) with closest culture sequence retrieved from the National Centre for Biotechnology Information (NCBI) database [21].

2.5. Production of Cellulase Enzyme

Production of cellulase was carried out for seven efficient cellulase-producing isolates. The organisms were inoculated in a liquid medium containing 1% CMC, 0.1% NaNO_3 , 0.1% K_2HPO_4 , 0.1% KCl, 0.05% MgSO_4 , and 0.05% yeast extract (pH 7.0) and incubated at 37°C for 24 hours. The growing cultures were further transferred into the production medium with the same composition and incubated for 72 hours at constant stirring at 130 rpm at 37°C using incubator shaker (Orbitek[®] LEBT, Scigenics Biotech Pvt Ltd, Chennai, India). The production medium was centrifuged at 10,000 rpm for 10 minutes to get the cell-free supernatant. This cell-free supernatant was used as a crude enzyme extract for cellulase assay.

2.6. Cellulase Assay

The cellulase activity was carried out using the method described by Maravi and Kumar [17]. The assay was carried out by preparing a reaction mixture containing 0.1 ml of crude enzyme extract with 1 ml of 0.5% CMC prepared in 50 mM phosphate buffer (pH 7). 0.9 ml of 50 mM phosphate buffer pH 7.0 was added to the tube in order to make up a volume 2.0 ml. The mixture was further incubated at 50°C for 30 minutes in a water bath. 3.0 ml of dinitrosalicylic acid

(DNSA) reagent was added to reaction tube to stop the reaction. To the control tube, crude enzyme was added after the stop solution, i.e., DNSA. After boiling for 5 minutes, the released sugar units were estimated according to protocol of Miller [22] with certain modifications. The enzyme activity was calculated in terms of the micromoles of glucose units released in 1 minute using the standard graph of glucose.

2.7. Optimization of Cellulase Activity

The optimization of culture conditions was carried out for maximum enzyme production. The parameters analyzed for the optimization were pH, temperature, and carbon source.

2.7.1. pH

The effect of pH on the enzyme production was checked by growing the isolates in a production medium adjusted at different pH, i.e., 2, 4, 6, 7, 8, and 10. The pH of the media were adjusted by using 1N NaOH and 1N HCl.

2.7.2. Temperature

The effect of temperature on enzyme production was checked by growing the isolates in a production medium and incubating it at temperatures 4°C, 10°C, 28°C, 37°C, and 50°C.

2.7.3. Carbon source

The effect of carbon source on the enzyme production was checked by preparing the production medium by replacement of the carbon source CMC, i.e., CMC with glucose, sucrose, starch, and maltose. The isolates were grown at these different production media and incubated at 37°C for 72 hours. The cell-free supernatant in each of the above case was used as crude enzyme sources to check enzyme activity.

2.8. Statistical Analysis

All the experiments in the present study were carried out in triplicates and the data were obtained in the form of mean \pm standard error. The data were subjected to two factor analysis of variance (ANOVA) test with significance levels of $p < 0.05$ using Microsoft excel 2013.

3. RESULTS AND DISCUSSION

3.1. Screening

Forty-five different bacterial cultures were isolated from nine soil samples. The isolates were numbered from S1-1 to S7-6 based on their origin of soil and number of isolates obtained per soil sample. The cellulolytic potential of the isolates was confirmed by flooding the plates using 0.1% Congo red. Gram's iodine was also used for screening of cellulolytic bacterial colonies. Bacterial isolates showed different zones of clearance around the colonies. The zone of clearance around the colony is an indicator of cellulolytic potential of the isolates [23]. Isolate S1-4 did not show the zone of clearance with 0.1% Congo red. However, it was significantly visible with Gram's iodine.

3.2. Identification of Bacteria

The isolates showing greatest cellulolytic potential on screening plates were further selected for morphological characterization and molecular identification. All the seven isolates were found to be Gram-positive in nature. The morphology of all the isolates was found to be rod-shaped.

All the seven bacterial isolates were identified by 16S rRNA gene-based sequencing which was carried out at NCIM, CSIR-NCL, Pune. The molecular characterization revealed that all the isolates belonged to genus *Bacillus*. NCBI BLAST analysis suggested that although all the isolates were identified to be *Bacillus*, their species were different namely *Bacillus subtilis*, *Bacillus flexus*, *Bacillus licheniformis*, and *Bacillus paralicheniformis*. Table 1 presents the details of identification of all the isolates with their accession numbers received for the genes from NCBI Genbank. The data suggest that the *Bacillus* species has the great potential to produce cellulolytic enzymes. Maravi and Kumar [17] isolated and identified two potential cellulolytic strains, one of which was found to be *B. licheniformis*. Sadhu *et al.* [24] isolated *Bacillus* strain from a cow dung sample. The isolated strain had a potential to produce thermotolerant endoglucanase enzyme (CMCase). Shanmugapriya *et al.* [8] also reported similar findings with purification of cellulase enzyme from *Bacillus* species. The isolation of the bacteria was carried out from the cow dung sample. Saha *et al.* [25] isolated eight potential cellulolytic bacteria

Table 1. Molecular identification of the isolates.

S. No	Isolate	Accession number received from NCBI Genbank	Organism found with identity	Percentage identity
1.	S1-1	MZ342583	<i>B. subtilis</i> with accession number CP053102.1	100%
2.	S1-4	MZ342581	<i>B. flexus</i> with accession number NR_113800.1	100%
3.	S3-3	MZ342584	<i>B. subtilis</i> with accession number CP053102.1	100%
4.	S4-1	MZ342585	<i>B. licheniformis</i> with accession number CP034569.1	99.86%
5.	S4-2	MZ342586	<i>B. licheniformis</i> with accession number CP034569.1	100%
6.	S7-2	MZ342582	<i>B. paralicheniformis</i> with accession number KY694465.1	99%
7.	S7-6	MZ342587	<i>B. licheniformis</i> with accession number CP034569.1	100%

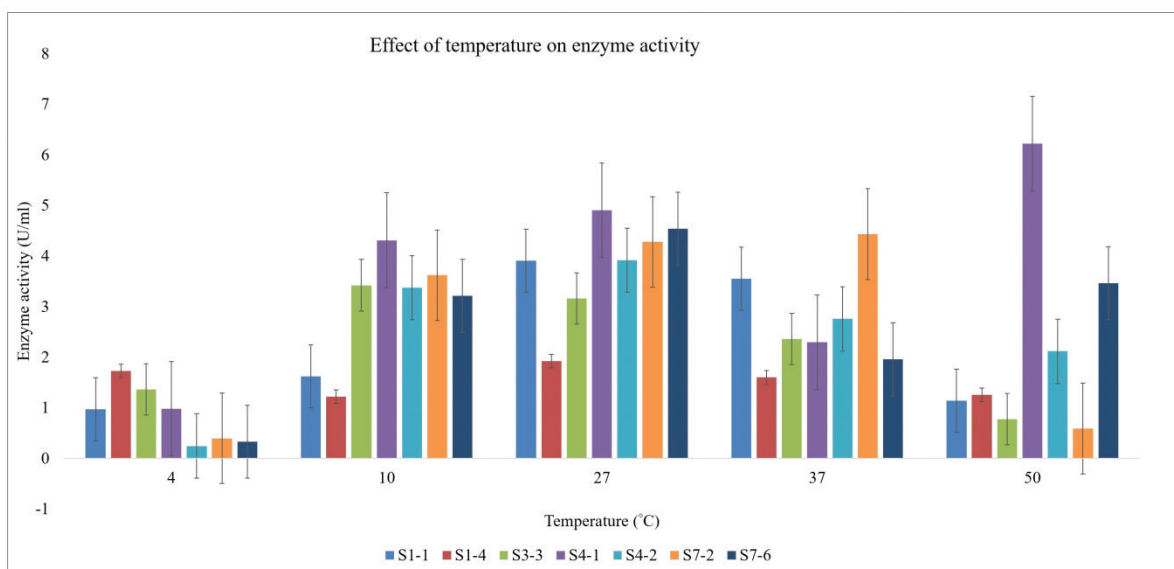


Figure 1. Effect of temperature on cellulase activity of different isolates.

from soil samples. Three out of these eight bacterial species were identified to be *Bacillus* sp., *Bacillus cereus*, and *Bacillus megaterium*. Along with *Bacillus* species, other bacterial genera also have the capability to produce cellulolytic enzymes. Lednická *et al.* [26] isolated and identified different cellulolytic strains from Belgian and Czech soils. The enrichment of these samples was carried out on flax or sisal fibers as sole sources of carbon. The bacteria isolated in the study were found to be strains of *Cellulomonas*, *Flavobacterium*, *Cellvibrio*, *Achromobacter*, and *Pseudomonas*. There are many reports available on the isolation of cellulolytic fungi. A fungus named *Myceliophthora thermophila* SH1 was explored for its cellulolytic and xylanolytic potential. The isolate was originally obtained from a source of hot spring and was able to show efficient cellulase and xylanase production under solid state fermentation [27]. Ja'afaru [28] isolated 110 different cellulase and xylanase producing fungi from saw dust, soil, and decaying wood samples. The isolate showing maximum cellulolytic potential was found to be *Trichoderma* species. The literature suggests that both bacteria and fungi have the ability to produce cellulase enzymes which can be further explored for their cellulolytic potential.

3.3. Optimization of Cellulase Production

The above-mentioned seven best isolates were optimized for their cellulolytic potential at different conditions. The effect of pH, temperature, and different carbon sources was checked on these selected isolates with respect to their cellulase activity. The optimum temperature for the enzyme activity of six out of seven isolates was found to be 27°C. The isolates named S1-1, S1-4, S3-3, S4-2, S7-2, and S7-6 were found to show maximum cellulase activity at 27°C. The highest activity at this temperature was shown by isolate S7-6 and it was found to be 4.544 U/ml (Fig. 1). These results were found to be comparable to the findings of Premalatha *et al.* [4] wherein *Enhydrobacter* species were isolated from leaf litter compost sample for the production of cellulase enzyme and the activity was found to be highest at 30°C. The isolates named S1-1, S1-4, and S7-2 showed almost comparable activities at

37°C. The enzyme production of all above-mentioned isolates was found to be reduced at 50°C. The data suggest that the cellulase enzyme can be produced at 27°C–37°C by these isolates. Similar results were reported by Islam *et al.* [29], in a known *Bacillus* strain where the highest cellulase production was observed at 35°C and the enzyme production was found to be reduced with an increase in the fermentation temperature above 40°C. Rasul *et al.* [30] also reported that the enzyme activity increases gradually up to 40°C and is found to be decreased at higher temperatures. The isolate named S4-1 was found to show highest activity, i.e., 6.226 U/ml at 50°C, which suggests that this *Bacillus* strain may be a type of thermotolerant bacterium. The results were comparable to Sadhu *et al.* [24]. The *Bacillus* strain isolated in this study was found to have maximum cellulase activity at 50°C. Gautam *et al.* [31] also reported the novel cellulolytic fungi from municipal solid wastes which were found to produce the cellulolytic enzyme at the temperature ranging from 40°C to 50°C.

The effect of pH on cellulase production was checked and it was found that all the isolates needed a different pH for maximum enzyme production (Fig. 2). Isolates S1-1, S7-2, and S7-6 showed maximum enzyme production at pH 6. The highest enzyme activity was reported by isolate S1-1 at pH 6 and it was found to be 3.082 U/ml. pH 7 was found to be optimum for enzyme production by isolate S1-4. Isolates S3-3 and S4-2 were found to show maximum enzyme activity at pH 8. The above-mentioned data suggest that slightly acidic to slightly alkaline pH, i.e., 6–8, was found to be the most suitable pH range for cellulase production. Shanmugapriya *et al.* [8] reported that slightly acidic to neutral pH supports maximum production of cellulase enzyme. The isolate named S4-1 showed the maximum enzyme production at pH 4. However, the enzyme activity shown by this isolate was found to be poor when compared to the other isolates under the study. The data are comparable to the findings reported by Maryam *et al.* [32] on *Bacillus cellulosilyticus* which showed optimum cellulase production at pH 5 using alkali pretreated substrate as a source of carbon. Nandimath *et al.* [33] isolated *Bacillus* and

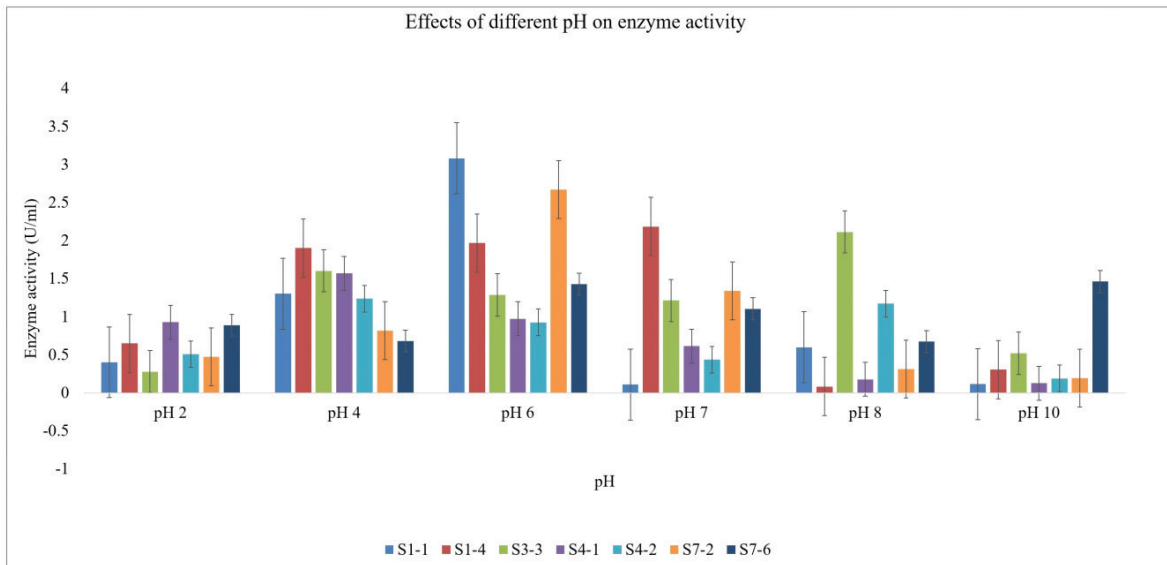


Figure 2. Effect of pH on cellulase activity of different isolates.

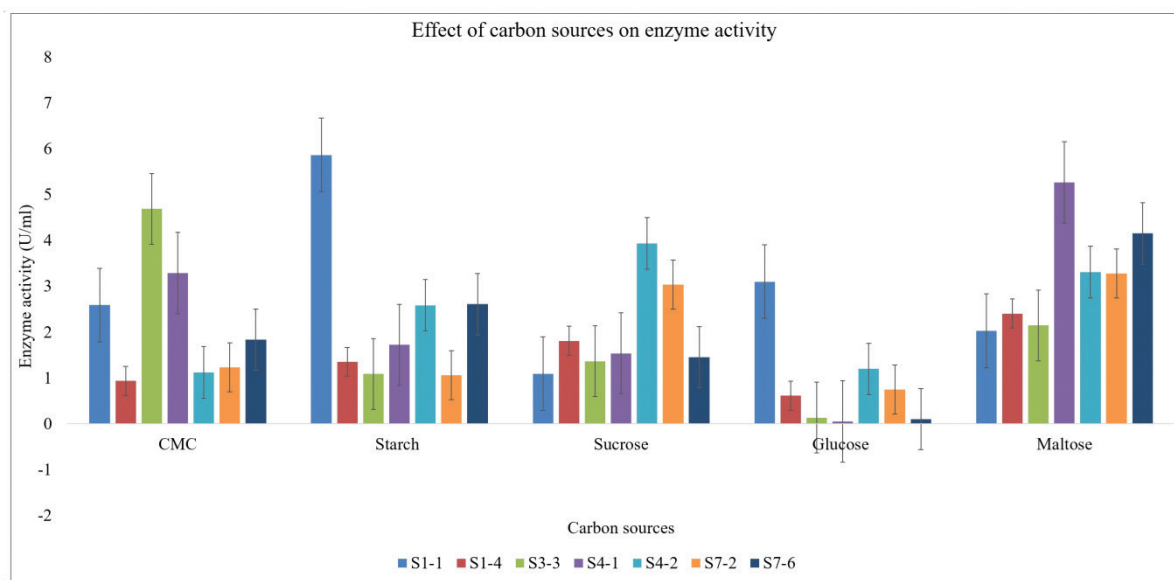


Figure 3. Effect of carbon sources on cellulase activity of different isolates.

Pseudomonas species having cellulolytic potential. The optimum pH for cellulase production by these two isolates was found to be 5. The data indicate that *Bacillus* species have the capability to produce cellulase enzyme in a wide pH range.

The impact of carbon source on enzyme production was checked by using different carbon sources in the production medium. The data show that the polysaccharides such as starch and CMC support the production of cellulase enzyme (Fig. 3). The results were comparable to Sadhu *et al.* [24]. The *Bacillus* strain under the investigation was found to produce maximum cellulase enzyme in the presence of CMC as a carbon source. The highest enzyme activity was shown by S1-1, i.e., 5.871 U/ml, in presence of starch as a sole source of carbon. The enzyme activities were found to be

almost comparable in the presence of sucrose and maltose as a sole source of carbon. The enzyme activity was found to be negligible when glucose was used as a carbon source in the production medium. The above-mentioned data suggest that glucose does not support the cellulase production for the above-mentioned isolates. In contrast to this, Sethi *et al.* [15] found that the glucose was the best carbon source to produce cellulase enzyme by the bacterial isolates. The above-mentioned data suggest that CMC and starch were the most suitable carbon sources for cellulase production. However, disaccharides such as sucrose and maltose can also support the enzyme production. Shajahan *et al.* [34] reported that CMC is a crucial factor to produce cellulase enzyme. The data suggest that polysaccharides such as CMC and starch are needed for the production of cellulase enzyme.

Table 2. Cellulase activity of isolates at different pH.

Strain number	pH 2	pH 4	pH 6	pH 7	pH 8	pH 10
S1-1	0.403 ± 0.13	1.302 ± 0.09	3.082 ± 0.58	0.109 ± 0.02	0.599 ± 0.06	0.116 ± 0.01
S1-4	0.649 ± 0.06	1.902 ± 0.24	1.967 ± 0.26	2.184 ± 0.16	0.084 ± 0.02	0.304 ± 0.11
S3-3	0.28 ± 0.14	1.603 ± 0.72	1.286 ± 0.06	1.214 ± 0.09	2.115 ± 0.14	0.521 ± 0.04
S4-1	0.928 ± 0.05	1.569 ± 0.07	0.974 ± 0.06	0.613 ± 0.08	0.179 ± 0.01	0.127 ± 0.02
S4-2	0.509 ± 0.07	1.237 ± 0.24	0.927 ± 0.12	0.435 ± 0.03	1.171 ± 0.12	0.19 ± 0.05
S7-2	0.476 ± 0.08	0.819 ± 0.17	2.672 ± 0.33	1.343 ± 0.43	0.315 ± 0.05	0.196 ± 0.03
S7-6	0.89 ± 0.13	0.68 ± 0.1	1.428 ± 0.24	1.105 ± 0.07	0.677 ± 0.06	1.463 ± 0.2
		CD	<i>p</i> -value			
	Strain	0.682111	0.000159			
	pH	0.631512	2.47E-22			
	Strain × pH	0.556941	2.25E-17			

Table 3. Cellulase activity of isolates at different temperature.

Strain number	4°C	10°C	27°C	37°C	50°C
S1-1	0.973 ± 0.06	1.622 ± 0.51	3.913 ± 0.18	3.553 ± 0.36	1.141 ± 0.28
S1-4	1.727 ± 0.34	1.221 ± 0.15	1.923 ± 0.56	1.602 ± 0.15	1.255 ± 0.24
S3-3	1.364 ± 0.11	3.425 ± 0.5	3.165 ± 0.64	2.359 ± 0.48	0.779 ± 0.21
S4-1	0.983 ± 0.09	4.316 ± 0.58	4.909 ± 0.95	2.3 ± 0.06	6.226 ± 0.73
S4-2	0.245 ± 0.06	3.376 ± 0.3	3.92 ± 0.37	2.759 ± 0.13	2.117 ± 0.37
S7-2	0.397 ± 0.11	3.622 ± 0.83	4.282 ± 0.48	4.437 ± 0.23	0.587 ± 0.2
S7-6	0.331 ± 0.07	3.219 ± 0.32	4.544 ± 0.07	1.96 ± 0.28	3.466 ± 0.55
		CD	<i>p</i> -value		
	Strain	1.169461	1.87E-10		
	temperature	0.83533	2.4E-20		
	Strain × temperature	0.649701	1.08E-12		

Table 4. Cellulase activity of isolates with different carbon sources.

Strain number	CMC	Starch	Sucrose	Glucose	Maltose
S1-1	2.593 ± 0.3	5.871 ± 0.63	1.098 ± 0.03	3.106 ± 0.6	2.033 ± 0.17
S1-4	0.939 ± 0.28	1.356 ± 0.05	1.814 ± 0.83	0.616 ± 0.09	2.407 ± 0.61
S3-3	4.695 ± 0.79	1.093 ± 0.11	1.368 ± 0.14	0.135 ± 0.03	2.153 ± 0.09
S4-1	3.295 ± 0.76	1.725 ± 0.35	1.542 ± 0.43	0.055 ± 0.02	5.272 ± 0.75
S4-2	1.125 ± 0.06	2.589 ± 0.29	3.94 ± 0.68	1.201 ± 0.28	3.315 ± 0.71
S7-2	1.238 ± 0.26	1.06 ± 0.14	3.043 ± 0.46	0.749 ± 0.23	3.287 ± 0.54
S7-6	1.843 ± 0.45	2.614 ± 0.76	1.46 ± 0.16	0.106 ± 0.06	4.158 ± 0.94
			CD	<i>p</i> -value	
		Strain	1.756032	8.78E-05	
		Carbon sources	1.484118	1.94E-12	
		Strain × carbon sources	0.755676	1.36E-12	

Recent reports on the isolation of cellulolytic bacteria suggest that *Bacillus* is one of the potential genera of bacteria which produces cellulase enzyme [8,23,24,29,34,35]. The cellulases produced by different microbial sources could be further explored for their potential to degrade different lignocellulosic waste materials [4,12,23]. Sibiyi *et al.* [36] tested the efficacy of cellulase enzyme for degradation of waste newspapers. The *Bacillus* strains identified under the present study could be further explored for

their ability to produce cellulase enzyme for degradation of several natural substrates that are rich in lignocellulose.

3.4. Statistical Analysis

The results of optimization were analyzed using a two-factor ANOVA test with significance level of $p < 0.05$ using Microsoft excel 2013 and the results are tabulated for enzyme activities of

the isolates for different parameters (Tables 2–4). The data reveal that the enzyme activities shown by different isolates were found to be significantly different. Although all the isolates belonged to *Bacillus* species, their cellulolytic potential was unique with respect to each strain. Also, there was a significant difference in the enzyme activities of the isolates at different pH, temperatures, and carbon sources.

4. CONCLUSION

The main aim of the present work was to screen and isolate the best cellulase-producing bacteria from soil sample. Seven best cellulase-producing isolates were found during the work. All the isolates were found to be Gram-positive in nature and were identified to be *Bacillus* species using 16S rRNA gene-based sequencing. *Bacillus* is the most abundant bacterium present in soil with a great cellulolytic potential. The results of optimization indicate that the most optimum temperature range for cellulase production by the above-mentioned isolates was 27°C–37°C. It may be concluded that *Bacillus* strains isolated in the present study can produce cellulase enzyme at a pH range of 6–8. The data suggest that the enzymes produced from these isolates could be used for the hydrolysis of mild acid or alkali treated substrates. The presence of polysaccharides and disaccharides in the medium may have an inductive effect on cellulase production. The future study aims at partial purification and characterization of the cellulase enzymes produced above-mentioned isolates. The purified enzyme could be used further for hydrolysis and saccharification of lignocellulosic biomass such as rice straw, rice husk, sugarcane bagasse, and other lignocellulosic substrates, which is a major step during bioethanol production.

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6. AUTHORS' CONTRIBUTION

Both the authors contributed equally to the design of the research experiments. Both have been actively involved in the finalization and revision of the manuscript. Analysis and interpretation of the data are also carried out by both the authors.

7. FUNDING

There is no funding to report.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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