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Research Article
Sugarcane Baggase Agro-waste Material Used for Renewable Cellulase Production from *Streptococcus* and *Bacillus* sp.

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Abstract

Background and Objective: The growing disquiets about the dearth of remnant fuels, the release of green house gasses and air pollution by incomplete incineration of fossil fuel have also resulted in an increasing focus on the use of cellulases to perform enzymatic hydrolysis of the lignocellulosic materials for the generation of bioethanol. The aim of this study was to isolate a potential thermotolerant cellulase producing bacterium from natural resources for the bioethanol production. **Materials and Methods:** The soil samples were collected aseptically from different site of university campus to isolate cellulase producing bacteria by serial dilution method on CMC agar plates (pH 7.0) at 55°C. Fifty bacterial strains isolated from the soil samples were screened for cellulase production. Among them, two cultures were adjudged as the best cellulase producer and were identified as *Streptococcus* and *Bacillus* sp. on the basis of biochemical characterization. After growth, incubated plates were overlaid with congo-red solution (0.1%) for 10 min and then washed with 1 N NaOH solution for de-staining and effective cellulolytic bacterial culture were screened out on the basis of clear zone around the colony for further study. The selected bacterial isolates were identified from MTCC Chandigarh. Data were analyzed by one way ANOVA. **Results:** The *Bacillus* sp. showed maximum cellulase production (170 U mL^{-1}) in the presence of sugarcane baggase, ammonium sulphate and Mn^{2+} ions at 55°C, pH 7.0 within 72 h of incubation while *Streptococcus* sp. showed maximum cellulase production (130 U mL^{-1}) in the presence of wheat bran, ammonium sulphate and K^{2+} ions at 50°C, pH 6.5 within 96 h. The enzyme showed maximum activity in the presence of Triton-X-100. Tween-40, Tween-60 and Tween-80 (100 mM), was also found to stimulatory effect, respectively. **Conclusion:** These isolates (*Bacillus* and *Streptococcus* sp.) may be useful in several industrial applications owing to its thermotolerant, heavy metal resistant and surfactant resistance characteristics.

Key words: Bioethanol, surfactants, cellulase, thermotolerant, *Bacillus* sp., *Streptococcus* sp., sugarcane baggase

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Energy use has increased gradually over the last century as the world population has raised and more countries have become developed. Crude oil has been the major resource to meet the increased energy demand. Cellulose is one of the most ample natural carbon reservoirs on this planet and its annual biosynthesis by plants occurs at a rate of 0.85×10^8 M t/annum. The treatment of this cellulosic carbon by cellulase degradation is very important for universal carbon sources demands[1]. The conversion of lignocellulose is a renewable source of energy on earth, to glucose and other important soluble sugars[2]. The extensive research has demonstrated that the enzyme mediated lignocellulose conversion to valuable soluble sugars. The sustained research has yielded cellulases which shown their biotechnological potential in various industrial sectors viz, food, brewery and wine, animal feed, textile and laundry, pulp and paper, agriculture as well as in research and development[3]. The demand of these cellulases is increasing more frequently than ever before and this need has become the motivating force for research on cellulases. Recently, exploration of the biodiversity of soil cellulolytic systems has increased on the conversion of cellulosic biomass into fuel and other products[4,5]. The current research shows the consumption of lignocellulosic materials as an excellent substrate for ethanol production[6].

Cellulases have a group of three enzymes namely endo-1,4-β-glucanase (Endoglucanase), exo-1,4-β-glucanase (Exoglucanases) and β-glucosidase that synergistically hydrolyzed cellulose into soluble sugars and glucose[7.] Cellulases are inducible enzymes which are produced by microorganisms during their growth on cellulosic substrates. Numerous microorganisms like fungi, bacteria and actinomycetes can synthesize cellulases. Currently, the majority of the commercial and laboratory cellulases are attained by fungi due to their maximum enzyme activity, but several reasons suggest that bacteria may have admirable potential[8]. Bacteria frequently have a higher growth rate than fungi allowing for higher rate of enzyme production. Most significantly, they show affinity to be more heat stable and are easier for genetic purpose. Various bacterial genera reported for cellulolytic activities include *Bacillus*, *Clostridium*, *Cellulomonas*, *Rumminococcus*, *Alteromonas*, *Acetivibrio* etc. Among bacteria, *Bacillus* sp. including *B. coagulans*, *B. pumilus*, *B. aquimaris* and *Bacillus subtilis* SV1, are well recognized cellulase production under submerged condition[9-12]. The present study was the isolation and screening of potential thermophilic cellulase producing bacteria from natural ecosystem. After that a comparative study of different process parameters were optimized for enhanced cellulase enzyme production using different agro-waste material as a carbon source.

MATERIALS AND METHODS

Isolation, screening and identification of thermotolerant cellulase producing bacteria: The soil samples were collected aseptically from different sites of university campus to isolate cellulase producing bacteria during December, 2016. One gram soil was suspended in 9.0 mL sterile distilled water, agitated for a min and 0.1mL suspension was spread over CMC agar plates (pH 7.0) containing, 2.0%, CMC: 0.5%, ammonium sulphate and 2%, agar. The inoculated plates were incubated at 55°C, till sufficient growth appeared. After sufficient growth incubated plates were overlaid with congo-red solution (0.1%) for 10 min and then washed with 1 N NaOH solution for de-staining. If a strain was cellulolytic then it started hydrolyzing the cellulose present in the surrounding and in the zone degradation there was no red color formation. Selection was done as per colonies with and without clear and transparent zone as cellulase producing and cellulase non-producing strain, respectively. Bacterial colonies showing clear zones were selected, streaked twice on CMC agar plates for purification and maintained as pure culture over CMC agar slants (pH 7.0, 4°C). The isolate having maximum clearance zone was selected for further studies. The selected bacterial isolates was identified by morphological and biochemical characterization as per the Bergey's Manual of Systematic Bacteriology[13].

Inoculum preparation: Mother culture was prepared by inoculating one full loop of 24 h grown culture on CMC agar plate in 50 mL CMC broth and incubated at 55°C for 24 h to achieve active exponential phase containing of 2.8×10^8 CFU mL^{-1}. Suitable amount (0.5%, v/v) of cell suspension were used to inoculate the test flasks.

Enzyme assay: Cellulase was assayed by measuring the reducing sugar released by reaction on CMC. Cellulase assay was done through Nelson[14] and Smogyi[15] methods by using a reaction mixture consisting 500 µL of substrate solution (1.0% soluble CMC in 1.0 M phosphate buffer pH 7.0.), 100 µL of the enzyme solution and 1 mL volume make up by adding 400 µL distilled water. The reaction mixture was incubated for 10 min at 65°C. Reaction was stopped by adding 1 mL of alkaline copper tartrate solution and incubated in boiling water bath for 10 min and cooled, then added arsenomolybdate solution for color stabilization. Optical

density of each sample with reaction mixture was taken at 620 nm in a spectrophotometer (Shimadzu, Japan). One unit of enzyme activity was defined as the amount of enzyme that liberates 1.0 µg of glucose min mL^{-1}.

Biomass determination: Bacterial cells in broth were harvested by centrifugation (Remi, Mumbai: Sigma, USA) (1000 rpm for 10 min at 4°C), washed with distilled water and dried in an oven at 80°C until getting a constant weight. The biomass was reported in the form of dry cell mass (g L^{-1}).

Optimization of different process parameters for cellulase production: The various process parameters influencing cellulase production were optimized individually and independently of the others. Therefore, the optimized conditions were subsequently used in all the experiments in sequential order. For optimization, the CMC medium was inoculated and incubated at different temperature viz., 35-70°C under the standard assay conditions. The samples were withdrawn at every 24 h interval up to 120 h to study the effect of incubation period. The influence of pH on the enzyme production was determined by varying the pH of the broth is adjusted to 4.5-9.0 in different flasks using 1N HCl and 1N NaOH. For measuring the enzyme activity at varying pH values ranging from 4.5-9.0 at 55°C using the appropriate buffers at concentration of 100 mM citrate buffer (pH 5.0-6.0, 1M), phosphate buffer (6.0-7.5) and Tris-HCl buffer (pH 8.0-10.0), respectively under standard assay conditions. The growth medium was supplemented with different carbon sources viz., CMC, starch, wheat bran, baggase, xylan, fructose, glucose, (at the level of 1%, w/v). Different organic and inorganic nitrogen sources (beef extract, malt extract, peptone and yeast extract) and inorganic nitrogen sources (sodium nitrate and ammonium sulphate) (at the level of 0.5 %, w/v) were also used for enzyme production. Thereafter, optimization of different heavy metals ($MnCl_2$, $MnSO_4$, KCl, $CaCl_2$, $FeSO_4$, $MgSO_4$, $CuSO_4$, $NiCl_2$, NaCl and $HgCl_2$ at the level of 0.1 %, w/v) and different surfactant (Tween-20, Tween-40, Tween-60, Tween-80, Triton-X-100 and Polyethylene glycol at the level of 100 mM) were used for enhanced enzyme production.

Statistical analysis: All experiments were carried out in triplicates and the results are presented as the mean of three independent observations. Standard deviation for each experimental result was calculated using Microsoft Excel 2003 (independent one way analysis of variance (ANOVA) from Minitab 15 with 95% of confidence interval)[16].

RESULTS

Isolation, screening and identification of thermotolerant cellulase producing bacterial cultures: Fifty bacterial isolates producing variable cellulolytic zones on CMC agar plates which stained with congo-red solution followed with the NaOH solution were isolated from the soil samples. The zones of clearance by isolates reflect their extent of cellulolytic activity. Those having clearance zone greater than >1.0 cm were considered as significant. Among 50 bacterial isolates, 29 exhibited good cellulase activity which was reassessed by loading their culture broth in the wells on CMC agar plates which stained congo-red solution followed with the NaOH solution (pH 7.0). The culture broth of good cellulase producers cleared more than >1.0 cm zone within 4-5 h of incubation at 55°C, thereby indicating an extra-cellular nature of the cellulase. The isolate P-15 and P-35 showing maximum clearance zone diameter were selected for further studies.

The efficient strain P-15 was rod-shaped, Gram-positive, aerobic and facultative, motile, with positive TSI, Voges-Prausker, citrate utilization and catalase test. It grew over a wide range of pH (4.0-11), temperature (10-85°C), NaCl concentration (0.0-8%) and was able to hydrolyze gelatin, casein, starch, Tween-20, Tween-40 and Tween-80 and produce acid from glucose, sucrose, lactose, maltose and mannitol while strain P-35 was cocci-shaped, Gram-positive and aerobic and facultative, with positive, catalase test. It grew over a wide range of pH (4.0-10.5), temperature (25-80°C), NaCl concentration (0.0-7%) and was able to hydrolyze gelatin, casein, starch, Tween-20 and produce acid from lactose and fructose. The strain was halotolerant as it grew in the presence of 0.0-7% NaCl. On account of morphological and biochemical characteristics, the efficient strain P-15 and P-35 were identified as *Bacillus* and *Streptococcus* sp. (Table 1).

Effect of incubation periods on cellulase production: The effect of incubation periods on the cellulase enzyme production by bacterial strains *Bacillus* and *Streptococcus* sp. were examined at different incubation periods range from 24-120 h (Fig. 1). The *Bacillus* and *Streptococcus* sp. showed a wide range of incubation periods (48-96 h) for cellulase enzyme production, but maximum enzyme production was achieved within 72h. The *Bacillus* sp., showed maximum 89.2 U mL^{-1} enzyme production with 0.65 mg mL^{-1} biomass production within 72 h while *Streptococcus* sp. showed 63 U mL^{-1} with 0.6 mg mL^{-1} biomass production within 96 h of incubation. Above and below this incubation periods, the enzyme production was lower.

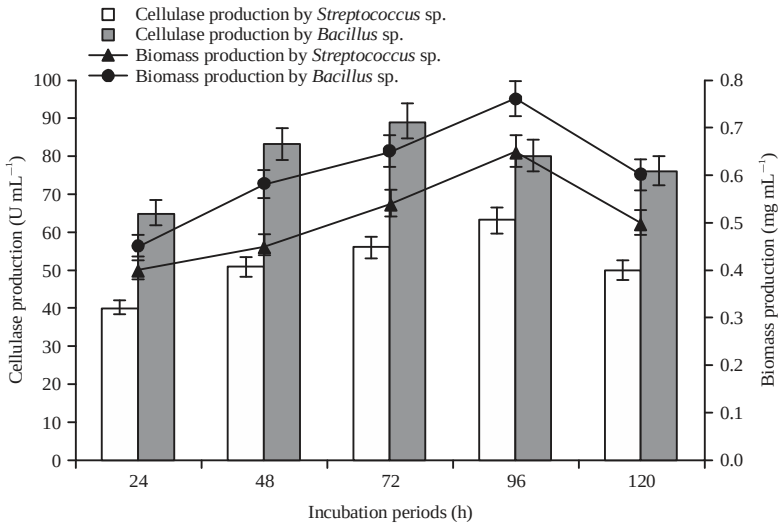

Fig. 1: Influence of Incubation periods on cellulase production and biomass production by *Streptococcus* and *Bacillus* sp.
The flasks were inoculated with culture were incubated at different incubation periods (24-120 h). Error bars presented mean ± standard deviation of triplicates of three independent experiments

Table 1: Identification of cellulase producing bacteria

Biochemical tests	P-15	P-35
Gram's nature	+	+
Shape	Rod	Coccus
Motility	Motile	Non-Motile
Temperature (°C)	10-85	25-80
pH	4-11	4-10.5
NaCl (%)	8.0	7.0
Glucose fermentation	A	-
Sucrose fermentation	A	-
Lactose fermentation	A	A
Maltose fermentation	A	-
Fructose fermentation	-	A
Mannitol fermentation	A	-
TSI	A/A, H_2S	-
Indol production	-	-
Methyl red	-	-
Voges-Prausker	+	-
Citrate utilization	+	-
Catalase	+	+
Oxidase	-	-
Isolate identification	*Bacillus subtilis*	*Streptococcus* sp.

TSI: Triple sugar iron, A: Acid, AG: Acid gas, H_2S: Hydrogen sulphite, -: Negative, +: Positive

Effect of temperature on cellulase activity: The bacterial strains *Bacillus* and *Streptococcus* sp. were examined for cellulase enzyme production along with biomass at different temperatures from 35-70°C. In this experiment bacterial strains showed better enzyme production from 45-60°C, but 55°C was found to be the most effective temperature for optimum enzyme production by *Bacillus* sp. Maximum 94 U mL^{-1} enzyme production with 0.8 mg mL^{-1} biomass production was achieved by *Bacillus* sp. at 55°C while *Streptococcus* sp. showed 68 U mL^{-1} and 0.6 mg mL^{-1} at 50°C. Above and below this temperature, the enzyme production was less (Fig. 2).

Effect of pH on cellulase production: The effect of pH on the crude cellulase enzyme production by bacterial strains *Bacillus* and *Streptococcus* sp. were examined at different pH values from pH 4.5-9.0 (Fig. 3). The *Bacillus* and *Streptococcus* sp. showed a wide range of pH tolerance (pH 5.5-7.5) capacity for cellulase enzyme production, but maximum enzyme production was achieved at pH 7.0. The *Bacillus* sp., showed maximum 105 U mL^{-1} enzyme production with 0.95 mg mL^{-1} biomass production at pH 7.0 while *Streptococcus* sp., showed 88 U mL^{-1} with 0.8 mg mL^{-1} biomass production at pH 6.5. Above and below this pH, the enzyme production was lower.

Effect of carbon sources on cellulase activity: Addition of different carbon sources had both stimulating and inhibitory effects on cellulase production (Fig. 4). *Bacillus* and *Streptococcus* sp. reported maximum cellulase production with sugarcane baggase and wheat bran (120 and 100 U mL^{-1}) with 1.1 and 0.97 mg mL^{-1} biomass production followed by CMC. Starch, fructose, glucose had no significant effects on cellulase production (Fig. 4). From the result, it was confirmed that sugarcane baggase and wheat bran could be effective for production of cellulase by the organisms.

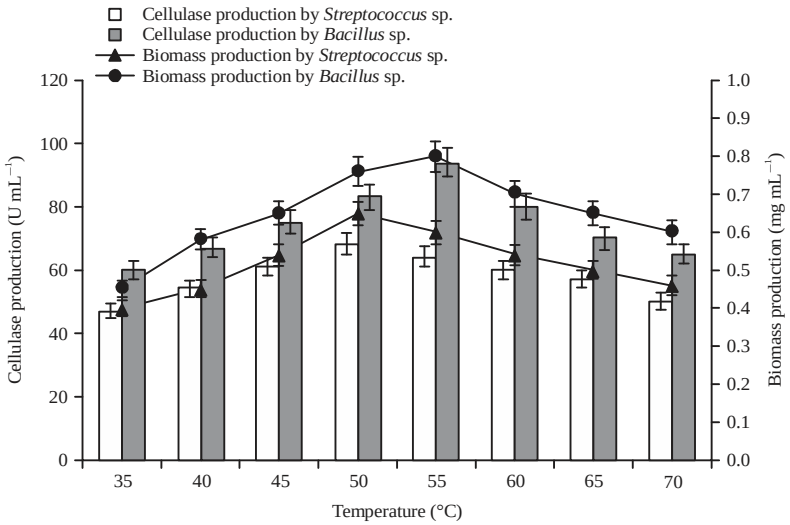

Fig. 2: Effect of temperature on cellulase production and biomass production by *Streptococcus* and *Bacillus* sp. at optimum incubation period

The flasks were inoculated with culture in the medium were incubated at different temperature (35-70°C) for 72 h at pH 7.0. Error bars presented mean±standard deviation of triplicates of three independent experiments

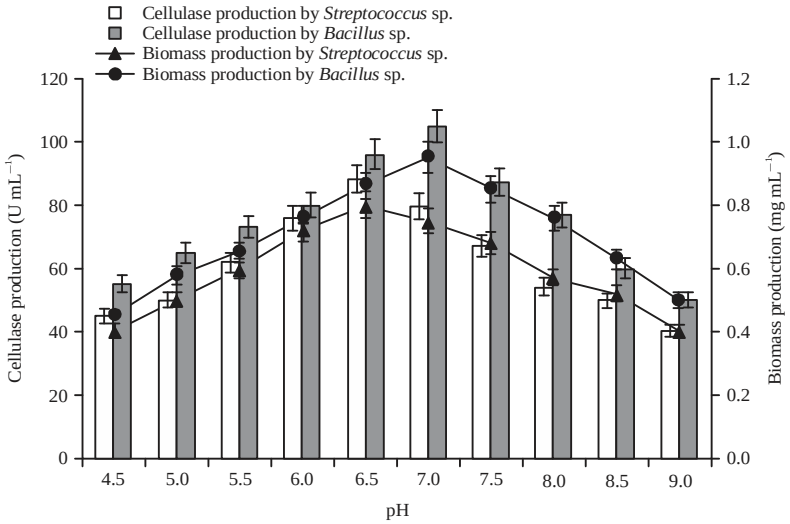

Fig. 3: Impact of pH on cellulase production and biomass production by *Streptococcus* and *Bacillus* sp. at optimum incubation period and temperature

The flasks were inoculated with culture were incubated at different pH (4.5-9.0). Error bars presented mean±standard deviation of triplicates of three independent experiments

Effect of organic and inorganic nitrogen sources on cellulase production: The influence of different organic and inorganic nitrogen source was also optimized for better cellulase production. It was observed from Fig. 5 that all the organic nitrogen sources showed decreased cellulase production when compared with inorganic nitrogen sources. Cellulase productions by *Bacillus* and *Streptococcus* sp. were showed maximum cellulase production (140 and 105 U mL^{-1}) with 1.2 and 1.0 mg mL^{-1} biomass production in the presence of ammonium sulphate at 0.5% concentration, other nitrogen sources showed inhibitory effects on cellulase production as indicated by Fig. 5.

Effect of heavy metal ions on cellulose production: In the present study, the effect of different metal ions on cellulase

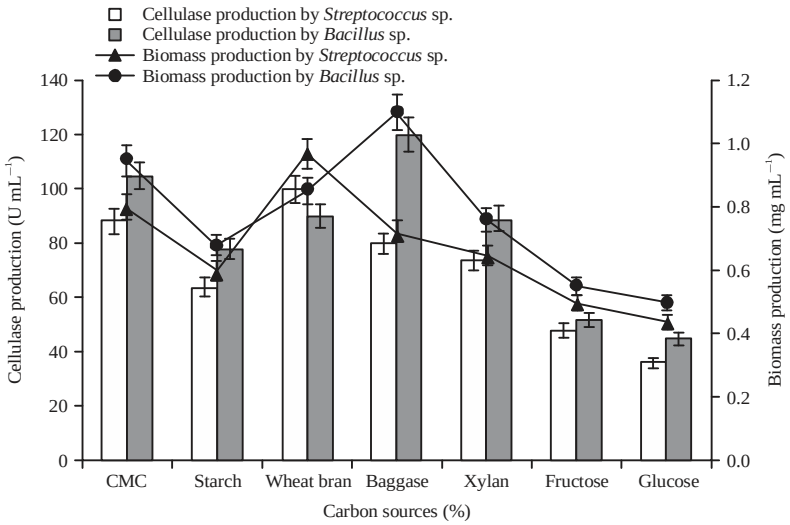

Fig. 4: Influence of different carbon sources on cellulase production and biomass production by *Streptococcus* and *Bacillus* sp. at optimum incubation period, temperature and pH

Test flasks contained different carbon sources in the medium at a level of 1% (w/v). Error bars presented are mean values±standard deviation of triplicates of three independent experiments

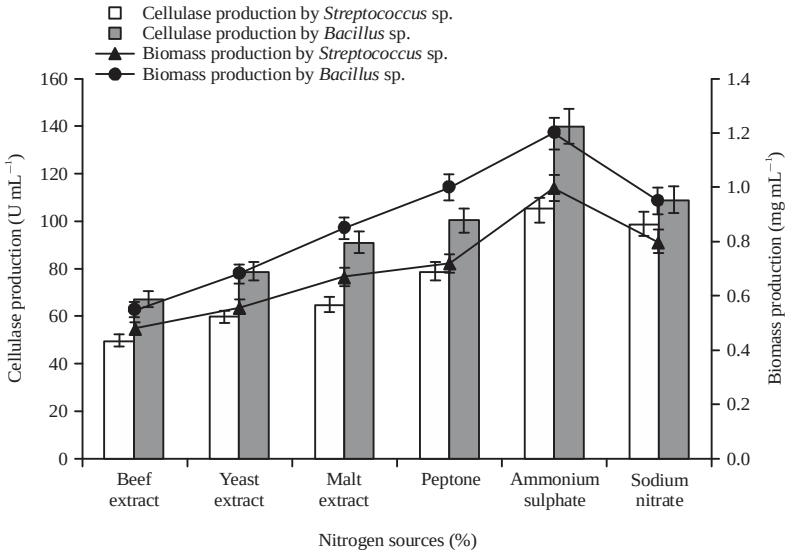

Fig. 5: Impact of different nitrogen sources on cellulase production and biomass production by *Streptococcus* and *Bacillus* sp. at optimal condition

Test flasks contained different nitrogen sources in the medium at a level of 0.5% (w/v). Error bars presented are mean±standard deviation of triplicates of three independent experiments

production by *Bacillus* and *Streptococcus* sp. were investigated. The results indicated that cellulase production by *Bacillus* sp. was maximum (160 U mL^{-1}) with 1.3 mg mL^{-1} biomass production in the presence of magnesium sulphate, whereas *Streptococcus* sp. showed maximum enzyme production (120 U mL^{-1}) with 1.1 mg mL^{-1} biomass production in the presence of potassium chloride (Fig. 6).

Effect of surfactant on cellulose production: In the present study, the effect of additional surfactants on enzyme yield was tested using production medium with addition of Tween-20, Tween-40, Tween-60, Tween-80, Triton-X-100, SDS and Polyethylene glycol. The result depicted that, Triton-X-100 showed maximum (170 and 130 U mL^{-1}) cellulase production by *Bacillus* and *Streptococcus* sp. with maximum (1.3 and 0.86 mg mL^{-1}) biomass production (Fig. 7).

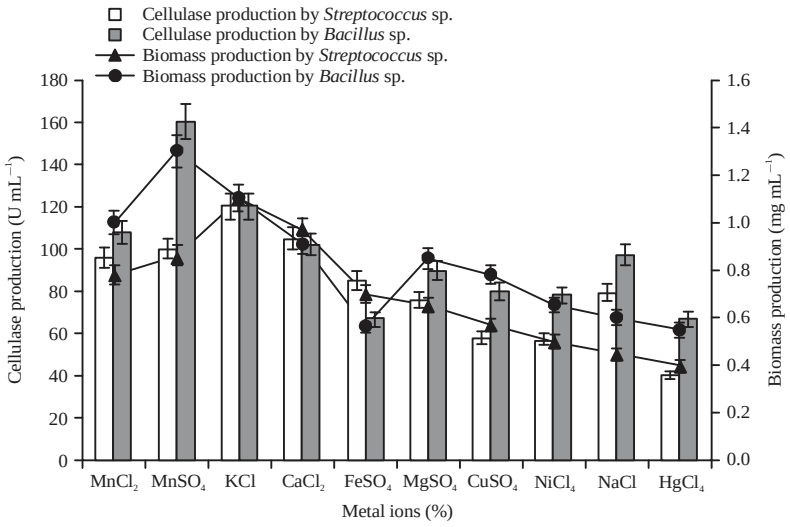

Fig. 6: Effect of different metal ions on cellulase production and biomass prodcution by *Streptococcus* and *Bacillus* sp. at optimal conditional parameters

The control flask does not contain any metal ions. Test flasks contained different metal ions in the medium at a level of 0.5%. Error bars presented mean±standard deviation of triplicates of three independent experiments

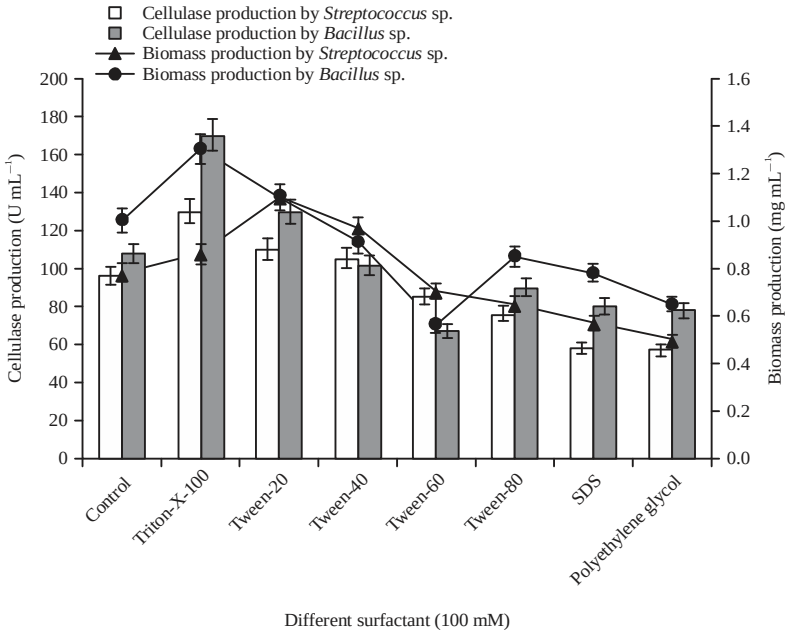

Fig. 7: Effect of different surfactants on cellulase production and biomass production by *Streptococcus* and *Bacillus* sp.

The control flask does not contain any surfactant. Test flasks contained different surfactant in the medium at a level of 100 mM. Error bars presented mean±standard deviation of triplicates of three independent experiments

DISCUSSION

Cellulase production by *Bacillus* and *Streptococcus* sp. was examined at different incubation periods (24-120 h) depicted in Fig. 1. The *Bacillus* and *Streptococcus* sp. were showed a broad range of incubation periods (48-96 h) for cellulase production, but maximum enzyme production was attained within 72 h. The *Bacillus* sp. showed maximum 89.2 U mL^{-1} enzyme production with 0.65 mg mL^{-1} biomass production within 72 h while *Streptococcus* sp., showed 63 U mL^{-1} with 0.6 mg mL^{-1} biomass production within 96 h of incubation. Above and below this incubation periods, the

enzyme production was lower. Similarly, Selvankumar *et al*.[17] have been reported that maximum cellulose production was reported by *Bacillus amyloliquefaciens* within 72 h. Ibrahim *et al*.[18] have also been reported that *Bacillus amyloliquefaciens* showed maximum cellulose production within 96 h. The decline in cellulose production above 96 h by isolates is due to their late stationary phase. Production of enzymes is usually initiated during the log phase of the growth and reaches maximum levels during the initial stationary phase[19]. Even though extracellular enzymes are produced from log phase to initial stationary phase, within the phases the production may vary[20].

The bacterial strains *Bacillus* and *Streptococcus* sp. were studied for cellulase production along with biomass at different temperatures (35-70°C). In this experiment, bacterial strains showed better enzyme production from 45-60°C, but 55°C was found to be the most efficient temperature for maximum (94 U mL^{-1}) enzyme production with 0.8 mg mL^{-1} biomass production by *Bacillus* sp. while *Streptococcus* sp., showed maximum enzyme production (68 U mL^{-1}) with 0.6 mg mL^{-1} biomass production at 50°C. Above and below this temperature, the enzyme production was less (Fig. 2). Similarly, Ibrahim *et al*.[18] have been accounted that *Bacillus amyloliquefaciens* showed maximum cellulose production at 55°C within 96 h. From results, it was concluded that the *Bacillus* sp., could be able to tolerate wide range of temperatures for higher cellulase production. The reported alkaline cellulases from *Bacillus* sp., present an optimum activity from 40-70°C[21-24]. This result shows that the endoglucanase produced by *Bacillus* sp., is a thermotolerant endoglucanase with promising potentials for exploitation by industries or processes that operate at this temperature.

Cellulase enzyme production by *Bacillus* and *Streptococcus* sp. was examined at different pH (4.5-9.0) depicted in Fig. 3. The *Bacillus* and *Streptococcus* sp. showed a wide range of pH tolerance (pH 5.5-7.5) capacity for cellulase production, but maximum enzyme production was reported at pH 7.0. The *Bacillus* sp. showed maximum (105 U mL^{-1}) enzyme production with 0.95 mg mL^{-1} biomass production at pH 7.0 while *Streptococcus* sp., showed maximum enzyme production (88 U mL^{-1}) with 0.8 mg mL^{-1} biomass production at pH 6.5. Above and below this pH, the enzyme production was lower. Acharya and Chaudhary[25] have also been reported that maximum cellulose production was reported at 6.5 and 7.0 by *Bacillus licheniformis* MVS1 and *Bacillus* sp. MVS3. Immanuel *et al*.[26] reported that the cellulolytic enzyme, endoglucanase from *Cellulomonas*, *Bacillus* and *Micrococcus* sp., isolated from the estuarine coir netting effluents hydrolyzes substrate in the pH range of 4.0-9.0, with maximum activity at pH 7.0.

Addition of different carbon sources had both stimulating and inhibitory effects on cellulase production. *Bacillus* and *Streptococcus* sp., were showed maximum cellulase production with sugarcane baggase and wheat bran (120 and 100 U mL^{-1}) with 1.1 and 0.97 mg mL^{-1} biomass production followed by CMC. Other carbon sources like starch, fructose and glucose had no significant effects on cellulase production (Fig. 4). From the result, it was confirmed that sugarcane baggase and wheat bran could be efficient for cellulase production by the organisms. Some other investigators also reported that agro-industrial residues such as rice bran, rice straw, sugarcane bagasse and wheat bran used as carbon sources for cellulase production[27-28]. For example, *B. subtilis* CBTK 106, *B. subtilis* BC 62 and *B. pumillus* exhibited their maximum cellulase production when wheat bran, banana fruit stalk and soybean were supplemented to the production media. Das *et al*.[29] have also been reported that *Bacillus* sp., utilized CMC as carbon source for maximum cellulase production. A higher production of cellulase when CMC served as substrate may be a result of induction of the enzyme, since cellulose is known to be a universal inducer of cellulase synthesis.

The effect of different organic and inorganic nitrogen sources were also optimized for better cellulase production. It was observed from Fig. 5 that all the organic nitrogen sources inhibited cellulase production while an inorganic nitrogen source enhances the cellulase production. *Bacillus* and *Streptococcus* sp., were showed maximum cellulase production (140 and 105 U mL^{-1}) with 1.2 and 1.0 mg mL^{-1} biomass production in the presence of ammonium sulphate at 0.5% concentration, other nitrogen sources showed inhibitory effects on cellulase production as indicated by Fig. 5. Similarly, Sreeja *et al*.[28] reported that *B. licheniformis* exhibited maximum cellulose production in the presence of ammonium sulphate substituted medium than the other organic and inorganic nitrogen sources. Many other researchers have also been found that ammonium sulphate give maximum cellulase production by *B. pumilus*, *Ruminococcus albus*, *Bacillus* sp. B21, *Streptomyces* sp., BRC2, respectively[30-33,27]. Utilization of different inorganic nitrogen sources in this experiment revealed that these isolates could obtain nitrogen from naturally available nitrogen sources in soil and from fertilizers.

The effect of different metal ions on cellulase production by *Bacillus* and *Streptococcus* sp., were depicted in Fig. 6. The results indicated that cellulase production by *Bacillus* sp., was maximum (160 U mL^{-1}) with 1.3 mg mL^{-1} biomass production in the presence of magnesium sulphate, whereas, *Streptococcus* sp., showed maximum enzyme production (120 U mL^{-1}) with 1.1 mg mL^{-1} biomass production in the

presence of potassium chloride (Fig. 6). Lee *et al.*[34] have also been reported that K^+ and Mn^+ activated cellulase production by *Bacillus thuringiensis*. Metal ions such as Ca, Mg, Fe, Co and Zn were necessary for cellulase synthesis by *Trichoderma viride* QM6a[35]. The major action of these metal ions is to work as cofactor of the enzyme. The present study revealed that enzyme is inactivated by Hg^{2+} and Fe^{2+}. Similar findings reported by Irfan *et al.*[36] for *Cellulomonas* sp. This is possibly due to binding of Hg^{2+} with thiol groups and interaction with carboxyl or imidazol groups of amino acids[37].

In the present study, the effect of surfactants on enzyme production was tested using production medium with addition of Tween-20, Tween-40, Tween-60, Tween-80, Triton-X-100, SDS and Polyethylene glycol. The result depicted that, Triton-X-100 showed maximum cellulase (170 and 130 U mL^{-1}) and biomass (1.3 and 0.86 mg mL^{-1}) production by *Bacillus* and *Streptococcus* sp. (Fig. 7). Generally, surfactants modify the cell membranes of microbes to facilitate enzyme release[38]. Surfactants also improve the cellulase stability and prevent the denaturation of enzymes during hydrolysis by desorbing it from cellulose substrate. Similarly, Sreeja *et al.*[28] reported that Triton-X-100 supplemented medium showed maximum cellulase production by *B. altitudinis* and *B. licheniformis*. Bhardwaj *et al.*[39] have also been reported that Tween-80 and Triton-X-100 enhanced the production of amylase enzyme. Sun and Liu[40] reported that surfactants increased the hydrolysis of lignocellullosic substances.

CONCLUSION

A thermo tolerant, heavy metal and surfactant stable cellulase is produced by *Bacillus* and *Streptococcus* sp. The organisms appear to have greater potential for enhanced enzyme production through optimization of nutritional and physical process parameters. Tolerance against surfactant and metal ions facilitates its use for various processes under stressed conditions. Owing to its thermotolerant nature, its cellulases may have potential uses in industries such as detergent, food, pharmaceutical, leather, agriculture, kraft pulp prebleaching process as well as molecular biology techniques.

SIGNIFICANCE STATEMENTS

This study discovers a thermo-tolerant, heavy metal and surfactant stable cellulase from *Bacillus* and *Streptococcus* sp., isolated from soil and using lignocellulosic material as a carbon source. The organisms appear to have greater potential for enhanced enzyme production. Both isolate were showed tolerance against surfactant and metal ions which facilitates its use in various industries under stressed conditions like at high temperature, pH, salts, detergents etc. This study will help researchers to uncover the critical area of energy demand and potential uses in industries such as detergent, food, pharmaceutical, leather, agriculture, kraft pulp pre bleaching process as well as molecular biology techniques. Such findings will explore a new awareness of research in the area of maximum cellulase production from prominent bacterial culture using waste lignicellulosic material as a carbon source which further used for bioethanol production.

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