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Sustainability Studies of Bacteria Derived Cellulase and Its Evaluation for Wash Performance Analysis

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Abstract: Wild marine strain *Bacillus licheniformis* KY962963, producing thermostable alkaline carboxymethyl cellulase (exoglucanase, E.C.3.2.1.91) was acquired from the Experimental Salt Farm of CSIR-CSMCRI of Bhavnagar district. Main prospective of cellulase derived from *B. licheniformis* is in the laundry industry. It is a first report pertaining to the wash performance ability of the extracted cellulase both in the presence and absence of commercial detergent. The reflectance data showed that cellulase concentration of 1690 U leads to better strain removal efficiency. The purified CMCase was found to be of 38 kDa. The enzyme activity was enhanced by Mn^{2+} (metal ion), EDTA (chelator) and 1% Triton-X (surfactant) whereas β-Me (chelator) resulted in significant reduction of enzyme activity. Activity of cellulase in the presence of disparate agronomical wastes such as wheat bran and sugarcane bagasse are quite higher when compared to chemical substrate such as CMC. Maximum cellulase production was obtained in the presence of wheat bran i.e., 447.79 U/mg which indicated that the chemical substrate could successfully be replaced with agronomical substrate to make the process more economic as well as ecofriendly. Thermostability, alkalinity and stability in the presence of salt (NaCl), metal ions, inhibitors, surfactants, commercial detergents and organic solvent delineates the application of cellulase as the potential laundry additive.

Keywords: Alkali-halotolerant Cellulase, Solvent Stability, Wash Performance Analysis

1. Introduction

Extracellular enzymes like cellulase are seeking attention globally now a day as it has the broader physiological and catalytic convention range to overcome the environmental issues as cellulose is the most abundant carbohydrate in nature which fulfills high energy demand of the world [1-2]. Cellulase is one of the most imminent commercial enzyme (system) having varied miscellaneous applications in paper industry, textile industry, oil extraction and in the processing of animal feed [3-4]. The role of cellulase as a supplement in commercial laundry have recently gained the attention of researchers. However, the chemical characteristics of surfactants presents in the detergents considerably manipulate the enzyme's stability. Therefore, essential criteria to use cellulolytic enzymes in detergent preparation is its functional activity and stability towards alkaline pH when mixed with different surfactants and commercial detergents. In this regard, considerable research has been made to screen cellulase producing microbes having robust enzymatic activity under extreme conditions including high temperature, salt concentration and pH [5]. Therefore, thermophilic cellulolytic bacteria have been isolated from the sites where environmental conditions are quite harsh such as soil [6],

compost systems [7], wastewaters [8] and marine habitat [9]. The main purpose of using extremophilic microbes is to make overall process environmentally friendly and economically feasible (cost effective). Usage of such microbes can make process environment friendly when compared to chemical source for enzyme production. To fulfill this aspect, certain *Bacillus* as well as *Geobacillus* spp. [10], some Actinobacteria such as *Nocardiodes* sp. [11], and *Marinobacter* sp. [12] are used as the potential source of cellulase. This study focuses on the isolation of such microbes capable of producing extracellular cellulase and its characterization for promising applications in laundry industries. The main aim of the present investigation is to gain highly thermoactive and thermostable cellulase form the wild strain of *Bacillus licheniformis* KY962963 isolated from experimental salt farm of CSMCRI Bhavnagar with distinct functional properties. The main goal of our study is to explore functionality of cellulase under different ranges of physical and chemical parameters along with their collaborative effect for its possible application as an additive in detergent or laundry base matrices.

2. Experimental

2.1. Materials

All the chemicals used in the study were of analytical grade. Other media elements and chemical reagents used in the present study were bought from Sigma-Aldrich (USA) and Hi Media laboratories (India).

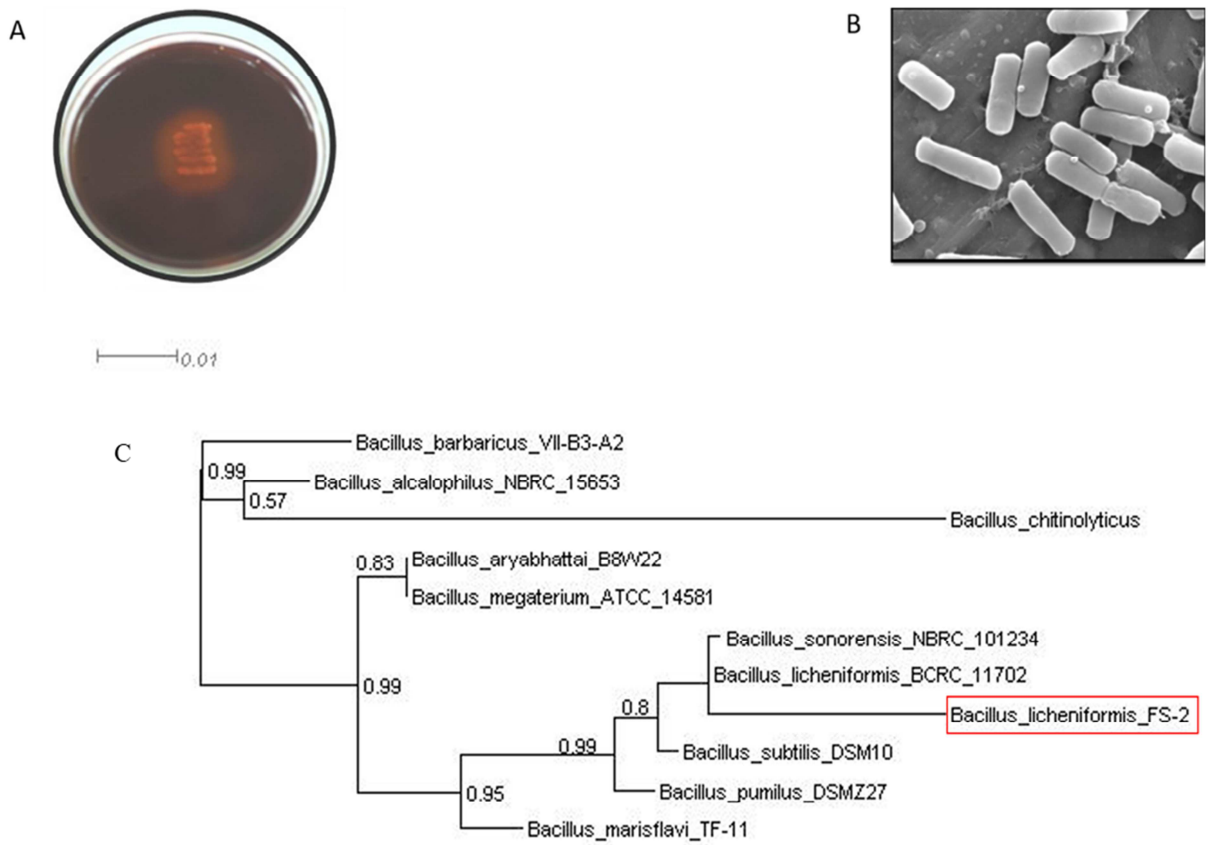

Figure 1. *(A) Screening for cellulase producer by CMC agar plate assay; (B) SEM image of Bacillus licheniformis KY962963; (C) Phylogenetic analysis of Bacillus licheniformis KY962963.*

2.2. Isolation and Screening of Cellulolytic Microorganisms

The bacterial strain involved in the experiment was isolated from experimental salt farm of CSIR-CSMCRI (N21°47.735", E072°07.626"). During isolation we got more than 93 isolates. Further they were screened for extracellular cellulase production by spotting the bacterial culture on carboxymethylcellulose (CMC) agar medium plates containing (g/l): CMC 10.0; yeast extract 1.0; sodium dihydrogen phosphate 1.0; magnesium sulphate 0.1; calcium chloride 0.05; sodium chloride 35.0; agar 20.0. Plates were incubated at 30°C for 72 hrs after inoculation. A clear zone of cellulose hydrolysis was observed after flooding the plates with Gram's iodine solution which confirmed the production of extracellular cellulase (Figure 1A) [13]. After screening one promising bacterial strain was selected on the bases of zone of clearance on the CMC agar plate.

2.3. Production Media for Enzyme and Growth Condition

The production of cellulase enzyme was carried out in the

primary medium containing (g/l) peptic digest of animal tissue 5.0, sodium chloride 35.0, beef extract 1.5, yeast extract 1.5, agar 20.0, CMC 10.0. The pH of the medium was adjusted to 7.4±0.2. The sterilization of the basal media was done using autoclave at 121°C for 15 minutes. 1% of 12-hour old bacterial culture was inoculated into the sterilized media and incubated it at 37°C on rotary shaker at 120 rpm for 3 days more precisely 72 hrs. After incubation, the production media was centrifuged at 12,000×g for 20 min at 4°C and the collected supernatant was assayed for cellulase activity.

2.4. Biochemical Analysis and Molecular Identification of the Bacterial Strain Through 16S rRNA Sequencing Along with the Growth Curve

Bergey's Manual of Systematic Bacteriology was referred for biochemical analysis [14]. Scanning electron microscopy (SEM) was done using LEO 1430VP (UK) system for the analysis of bacterial surface formation (Figure 1B).

Genomic DNA of bacterial strain was extracted using a CTAB method [15-16]. The PCR amplification was carried out in 50µl reaction mixture containing 10x PCR buffer containing 200mM $MgCl_2$, 200µM of dNTPs, 1.25U Taq Polymerase, 0.5µM of each 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') and 50ng of template DNA. The PCR reaction was run at 95°C for 2 min, followed by 30 cycles of 95°C: 30 s, 55°C 30s, and 72°C 45s, followed by 72°C for 10 minutes in a PCR machine. PCR amplified products were then purified, and sequenced bi-directionally. The sequences were cleared of vector and low-quality regions and assembled to form a consensus sequence. The consensus sequences were identified in EzBioCloud server (Figure 1C).

The bacterial culture was withdrawn from growth medium after every 2h of interval. Absorbance was measured at 600 nm uptil 28h. The activity of enzyme was also checked simultaneously with the growth. The halotolerant nature of the selected strain was examined with growth at different concentrations of NaCl (0%, 1%, 3%, 5% 7%, 9%, 11%, 13%, 15%, 17%, 19%, 21%, 23%, 25%) (Figure 2).

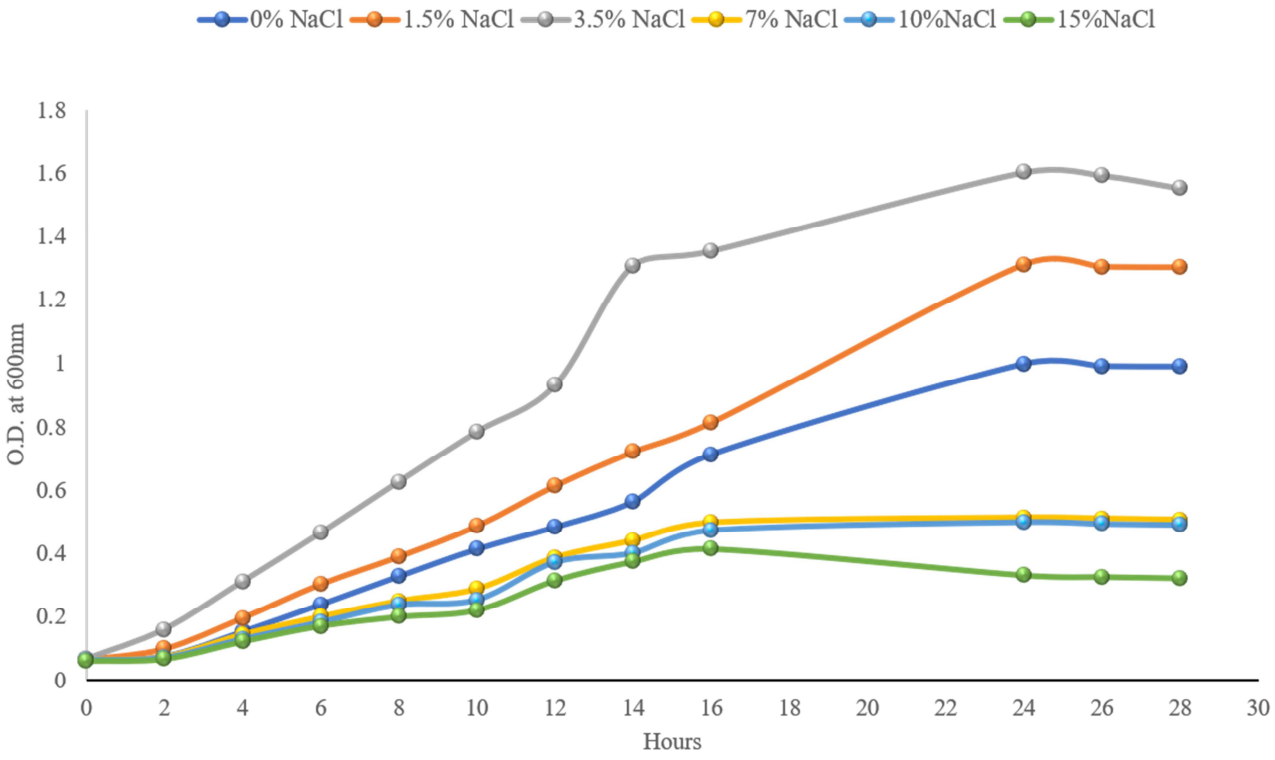

Figure 2. *Bacterial growth curve at different NaCl concentration.*

2.5. Assay for Cellulase Activity

Quantification of the cellulase activity was done spectrophotometrically using 3,5-dinitrosalicylic acid (DNS) [17]. The whole procedure for assaying cellulase activity was done according to Jain et al., 2012. Bradford method was followed to measure concentration of protein content in an enzyme and standard was prepared using bovine serum albumin (BSA) [18].

2.6. Three Step Purifications, Molecular Weight Detection and Enzyme Kinetics of Cellulase

Bacterial cells removal from culture broth was done by centrifugation at 12,000×g for 20 min at 4°C. Further, supernatant was precipitated with 80% $(NH_4)_2SO_4$ (ammonium sulphate) at 4°C. After precipitation, the material was centrifuged. Supernatant was discarded and

remaining pellet was recovered. The collected precipitate was dissolved in 50 mM glycine-NaOH buffer of pH 9 and dialyzed for 48 hrs. The removal of extra ammonium sulphate was observed by using Nesseler's reagent. Partially purified cellulase was then applied to DEAE Sepharose column equilibrated with 50 mM glycine-NaOH buffer of pH 9 pertaining to ion exchange chromatography. The fractions were eluted with a linear or one-dimensional gradient of 0.1-1 M NaCl using same buffer. The fractions showing highest enzyme activity were collected and further proceed to next step of purification that is gel filtration using DEAE Sephadex A-50 column equilibrated with 50mM glycine-NaOH buffer (pH 9). Fractions showing maximum enzyme were collected and further scrutinized for purity and other characterization experiments.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out for purity check of the final product and molecular weight determination on 8% gel [19].

Km and Vmax values of the enzyme were determined using *Lineweaver-Burke* plot. This can be done by measuring the cellulase activity against different concentrations of the substrate CMC (0.25-4 mg/ml).

2.7. Impact of pH and Temperature on Activity and Stability of Cellulase

The pH optima of the cellulase was examined by utilizing a wide range of pH (pH 5-13) buffers in an assay reaction mixture. The stability of the cellulase was assessed after preincubating the enzyme in different buffers ranging from pH 5-6 (citrate buffer), 6-8 (phosphate buffer), 8-10 (glycine-NaOH buffer), 10-11 ($NaHCO_3$/ NaOH buffer), and 11-13 (KCl/ NaOH buffer) for 30 min. Standard assay conditions were followed to determine the residual enzyme activity.

By measuring the cellulase activity at different temperature range (10-130°C), we can get an idea of temperature optima for maximum cellulase production. Glycine NaOH buffer of pH 9 is used as a substrate in this study and it will remain constant for the experiment. The stability of cellulase at higher temperature was evaluated by calculating the residual activity after 30 min of pre-incubation in 50mM glycine NaOH buffer of pH 9 at temperature span of 10-130°C.

2.8. Impact of Agronomical Wastes on the Cellulase Production

The effect of various agronomical cellulosic waste materials on the production of cellulase was examined by replacing the chemical substrate CMC in the production medium with rice straw, wheat straw, wheat bran, sugarcane bagasse and corn stover at a concentration similar to that of a chemical substrate i.e., of 1% (w/v) each. The media for the production of cellulase was inoculated with 12 hrs old culture (*B. licheniformis* KY962963) and kept for incubation at 37°C on a rotary shaker at a speed of 120 rpm for 72hrs. The wastes investigated in the study are the prospective substrates for various industrially important bioconversion processes. The purpose of the analyses was to evaluate the effect of these different cellulosic waste materials on enzyme production, which can lead to the cost reduction if replaceable with costly substrates in enzyme production. The other benefit would be the waste management if we can replace chemical substrate with these types of agronomical substrates. This leads to the environment friendly approach.

2.9. Significance of Organic Solvents, Chelators / Inhibitors, Bleaching Agents, Surfactants and Detergents on Cellulase Stability

To check the stability of cellulase towards various organic solvents, the enzyme was mixed with such solvents like acetone, benzene, butanol, DMSO, cyclohexane, toluene, methanol and chloroform in the ratio of 50% v/v. The solvent enzyme mixture was incubated at 37°C on a rotary shaker at a speed of 120 rpm for 5 days. Residual cellulase activity was measured in accordance optimized assay conditions with respect to control.

Effect of various additives on the activity of cellulase was estimated by executing the reaction of enzyme and substrate in the presence of miscellaneous metal ions such as Ca^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+} and inhibitors such as EDTA, Phenylmethane Sulfonyl Flouride (PMSF) and β-mercaptoethanol (β-Me) at 5mM concentration. Activity of cellulase was evaluated as relative activity (%) in comparison with control (without additives). The result of control can be considered as 100% of relative activity.

The appropriateness of the enzyme as a laundry additive was examined by determining its stability in the presence of different surfactants like Sodium dodecyl sulphate (SDS), Triton-X, Tween 80, and bleaching agent like Sodium hypochlorite (NaClO). The enzyme was preincubated with all these supplementary additives for 30 min at 70°C. Residual enzyme activity was calculated under the optimal assay conditions. This experiment further investigated the affinity of cellulase with commercial solid detergents. The other commercial detergents such as Ariel, Wheel, Rin and Surf Excel were used by diluting it in a tap water to a final concentration of 7mg/ml. To inactivate the internal enzymes of the detergents, the diluted detergent was heated at 100°C for 30 min. The mixture containing enzyme and diluted detergent was then incubated for 30 min and the residual cellulase activity was measured under optimum condition.

2.10. Wash Performance Assay

Tomato sauce and turmeric paste was used to stain the clean pieces of cotton cloth of the size 4cm×6cm. The cotton pieces were then dried. The stained clothes were washed according to the method described by Jain et al., 2012. The resulting reflectance (%) of the cloth was measured after drying the washed cloth in the range of wavelength of 300-

800 nm in reflectance mode on UV/Vis spectrophotometer (Shimadzu, Japan). Visual examination of the washed cotton cloth pieces with enzyme, detergent, both enzyme and detergent were evaluated.

3. Results

3.1. Identification of Bacterial Strain

The bacterial strain employed in this study is capable of producing cellulase formed irregular, undulate, white pigmented and opaque colonies with rough surface on the Nutrient agar plate. The strain was Gram positive with short rods and aerobic. It was found to be motile in nature. The biochemical characterization of the selected strain is summarized in Table 1.

Table 1. *Biochemical characterization of Bacillus licheniformis KY962963.*

Biochemical Test	Result	Biochemical test	Results
Arginine dehydrolase	+	Sorbitol	+
Esculin hydrolysis	+	Trehalose	+
β-galactocidase	+	Lactose	+
Phenylalanine deamination	+	D- cellobiose	+
Lysin decarboxylase	-	D- fructose	+
Ornithine decarboxylase	-	D-mannose	+
Tryptophan deaminase	-	Galactose	+
Urea hydrolysis	-	Salicin	+
Sugar hydolization Test		Starch	+
Glucose	+	D&L fucose	-
Sucrose	+	D-arabinose	-
Maltose	+	Adonitol	-
Ribose	+	Xylitol	-

The strain was identified as *Bacillus licheniformis* on the basis of Bergey's manual of systamatic bacteriology 8th edition and 16s rRNA sequencing and submitted in NCBI with accession no. KY962963 (Figure 1C). Strain *Bacillus licheniformis* KY962963 has been shown cellulose hydrolyzing activity on CMC agar plate by adding Gram's iodine after 72 hrs of incubation which was collected from the Experimental salt farm of CSMCRI.

The *Bacillus licheniformis* KY962963 showed optimum growth at 3.5% salt (NaCl) concentration with an acceleration phase near to 14 hrs. On the other hand, at a high NaCl concentration (7 and 9%) the acceleration phases got compressed but, with extended stationary phase (Figure 2). Further, the concurrent assessment of growth along with cellulolytic activity revealed that 72hrs of incubation period is optimum for the extracellular cellulase yield.

3.2. Three Step Purifications of Cellulase

The cell free supernatant derived from the production media can be used for the purification. There are three steps for the purification of cellulase which includes ammonium sulphate precipitation, ion exchange and size exclusion chromatography. The purified enzyme demonstrated a 42.04-fold increase in the activity and it shows the final yield of 30.80% (Table 2).

Table 2. *Summary of three-step purification of cellulose.*

No.	Purification step	Protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification fold	Yield (%)
1	Crude enzyme	1040	660	1.0	1	100
2	Ammonium sulphate precipitation	90	338	4.48	7.29	52.07
3	DEAE Sepharose	30	295	12.29	20.48	45.44
4	DEAE Sephadex A50	10	200	25	42.04	30.80

The purity of the enzyme was established by SDS-PAGE with a single band of protein having a molecular weight 38 kDa (Figure 3).

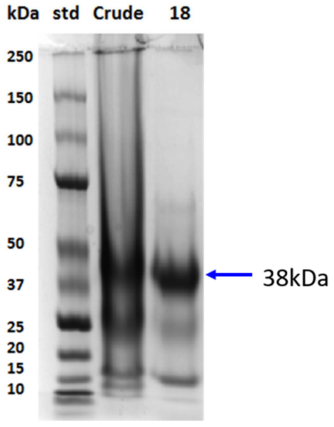

Figure 3. *SDS-PAGE analysis of cellulase produced by Bacillus licheniformis KY962963.*

3.3. Impact of pH and Temperature on Stability and Activity of Cellulase

The cellulase from *Bacillus licheniformis* KY962963 *is* active over a broader pH range (pH 7.0- 12.0). It shows the highest enzyme activity at pH 9 i.e., 371.49 U/mg. The pH stability of an enzyme was evaluated after incubation of the reaction mixture for 30 min at 37°C, and the enzyme was found to be stable at pH 9.0 (Figure 4). Like pH the enzyme has also an extensively large range of temperature tolerance between 10 and 130°C. After the experiment performed under the optimum assay condition it is found that the optimum temperature for maximum cellulase production was observed to be 70°C as shown in Figure 4. The stability studies of enzyme were carried out at diverse range of temperature for 30 min and detected that the cellulase gives the highest stability at 70°C (Figure 4).

Carboxymethyl cellulase optimal temperature activity was at 70°C for *Bacillus licheniformis* KY962963.

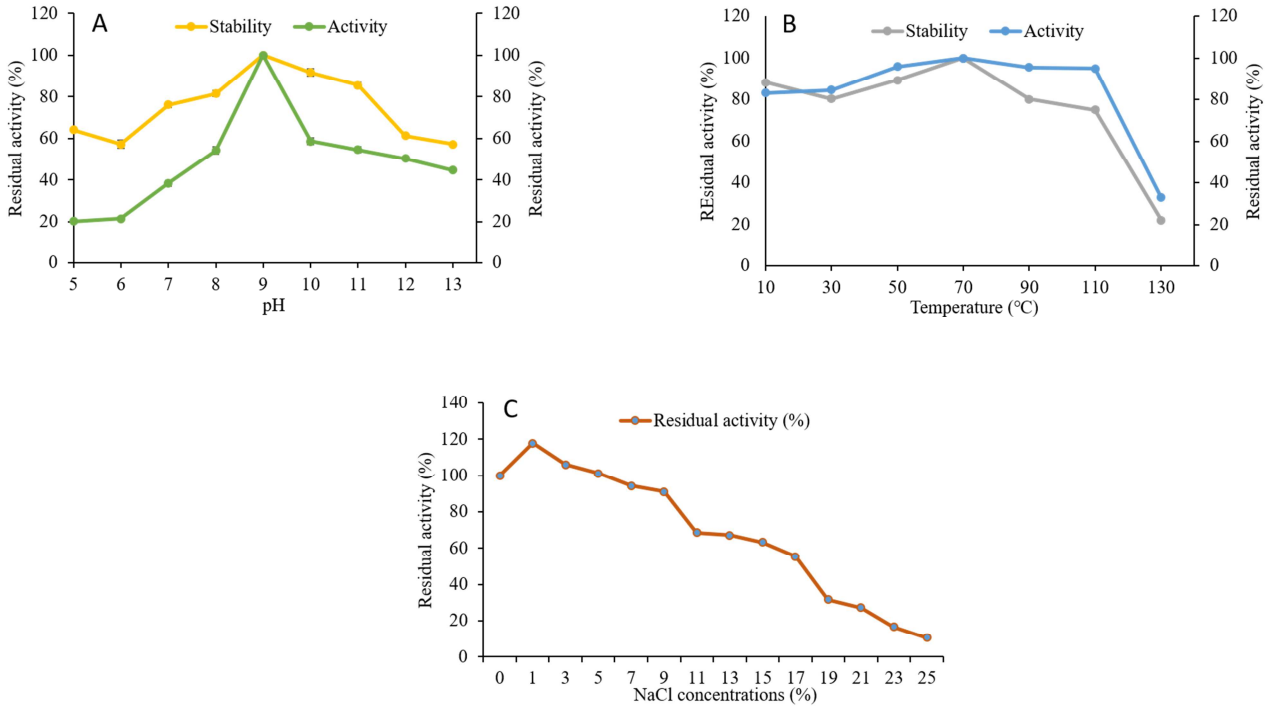

Figure 4. *Impact of temperature, pH and salinity on enzyme activity and stability.*

3.4. Effect of Salt Concentrations on Cellulase Activity

The enzyme has a wide range of salinity tolerance between 1-17% of NaCl concentration. The optimum salt concentration was observed to be 3% (Figure 2). As shown in figure enzyme retain its 40% activity even at a high salt concentration at 10% which proves its origin from marine source.

3.5. Effect of Cellulosic Wastes

The selected strain of bacteria showed significant growth in the presence of all the cellulosic wastes as a carbon source used in this study. It is found that the production of cellulolytic enzyme was highest in the presence of wheat bran (447.79 U/mg). Residual activity (%) of cellulase had been demonstrated in the Figure 5.

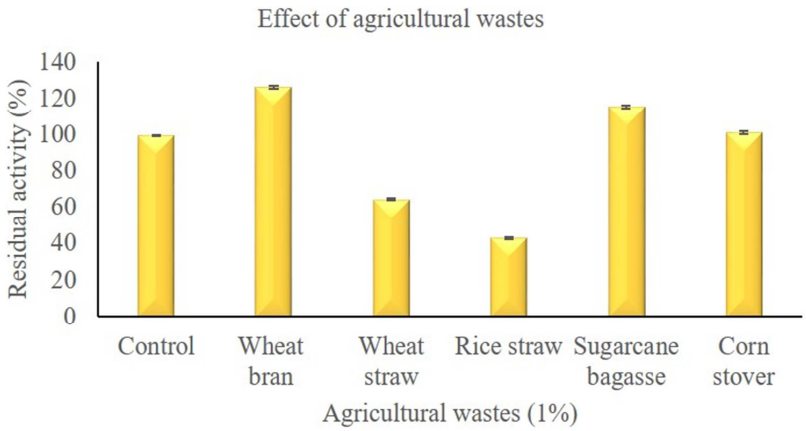

Figure 5. *Effect of agricultural waste materials on enzyme activity.*

3.6. Effect of Chelators / Inhibitors

The impact of different metal ions and inhibitors on the activity of cellulase was examined at 5mM of constant concentration. This study shows that cellulase activity increased by Mn^{2+}, Zn^{2+} and Ca^{2+} to 240, 155 and 100% respectively (Figure 6). The inducing effect of divalent metals such as Mn^{2+} and tolerance to the high salt concentration (NaCl 10%) revealed the novel feature of the cellulase from *Bacillus licheniformis* KY962963. On the other hand, the activity of cellulase is significantly enhanced by the EDTA, which is a unique characteristic of cellulase and this feature is could be interested to investigate at the molecular level.

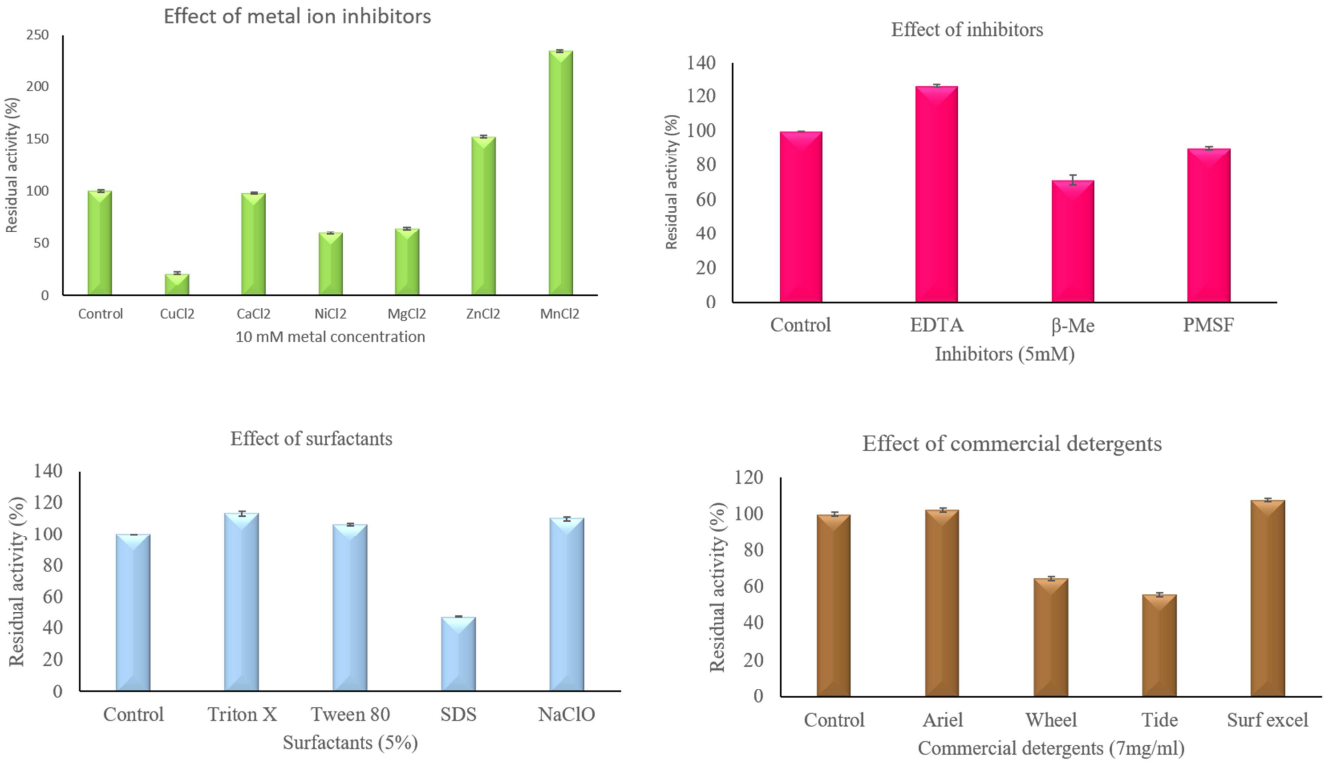

Figure 6. *Effect of different additives on enzyme activity.*

3.7. Effect of Surfactants and Commercial Detergents

The detergents induced the cellulase hydrolyzing activity as residual enzyme activity increased to 112% with Triton-X 100 and 108.79% with NaOCl which is known as nonionic detergent, but SDS which an ionic detergent reduced the activity drastically by 46% (Figure 6). Cellulase showed greater stability in the presence of Surf Excel and Ariel, when compared to control by i.e., 108% and 101% respectively after incubation at 50°C for 30 min (Figure 6).

3.8. Impact of Organic Solvents on the Cellulase Stability

In the case of organic solvents, the residual enzyme activity increased by 192%, 169% and 122% when methanol, DMSO, and acetone were present respectively. No adverse effect has been seen in the case of butanol and toluene [20]. No sharp decline in the residual activity of cellulase has been seen even after incubation of 5 days in case of acetone, methanol, and chloroform. Whereas the residual enzyme activity was found to be decreased in presence of cyclohexane and benzene by 60% and 57% respectively (Figure 7) [20].

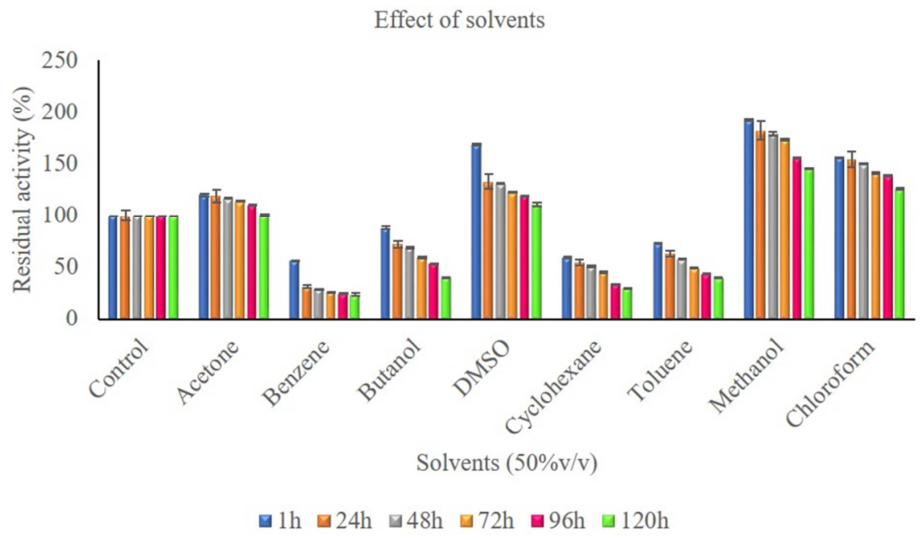

Figure 7. *Impact of different organic solvents on enzyme activity.*

3.9. Kinetic Determination

The kinetic study of cellulase derived from the *Bacillus licheniformis* KY962963 towards a particular range of CMC divulged value of Km and Vmax as 10mg/ml and 1000µmol/min/mg (Figure 8).

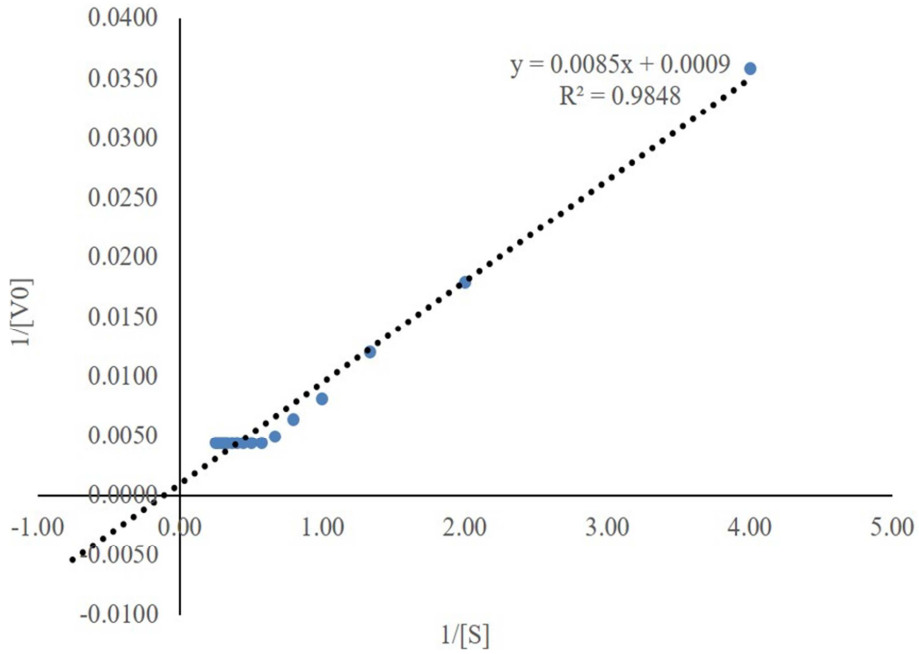

Figure 8. *Lineweaver-Burke plot for enzyme kinetics.*

3.10. Wash Performance Assay

We are using stained cotton cloths for experimental evaluation of this particular analysis. Tomato sauce and turmeric paste were used to stain the clean cloth to evaluate the stain removal capability. Commercially available detergent powder which was diluted with tap water supplemented with crude cellulase showed a quite good stain removal treatment than employing detergent alone. The stained cloth washed with only detergent showed lower reflectance than the cloth washed with the mixture of crude cellulase and detergent (Table 3). The reflectance was marked at wavelength 433 nm and 430 nm for tomato sauce and turmeric paste respectively. Best result by means of maximum reflectance was found to be at of 89.36% at 50°C for tomato sauce stained cloth and 49.25% at 60°C for turmeric paste stained cloth (Table 3).

Table 3. *Wash performance analysis of cellulase at different temperatures.*

	Reflectance (%) at temperatures		
	25°C	**50°C**	**60°C**
Tomato sauce stain			
Tap water	45.51±1.20	51.25±1.86	60.21±1.21
Detergent (7mg/ml)	54.12±1.35	60.28±1.31	80.25±1.75
Cellulase+ detergent	54.55±0.49	89.36±1.32	78.64±0.35
Turmeric stain			
Tap water	9.23±0.51	10.01±0.79	10.92±0.46
Detergent (7mg/ml)	15.92±0.12	12.52±0.12	16.95±0.14
Cellulase+ detergent	18.95±0.15	28.21±0.18	49.25±1.39

Maximum reflectance for all the staining agents was found at 1690U concentration of cellulase (Table 4).

Table 4. *Effect of cellulase concentration and incubation period on wash performance analysis.*

	Tomato sauce Reflectance in %	**Turmeric paste**
Enzyme concentration (U)		
854	70.12±1.39	61.23±1.31
1690	80.92±1.68	63.19±1.15
Incubation time (min)		
15	77.23±1.35	58.23±1.22
30	75.68±1.38	60.11±1.12

At incubation period of 15 min maximum reflectance was observed for tomato sauce stained cloth and 30 min of incubation period swas found to be optimum for turmeric stained cloth.

4. Discussion

Strain *Bacillus licheniformis* KY962963 has been shown cellulose hydrolyzing activity on CMC agar plate by adding Gram's iodine after 72 hrs of incubation which was collected from the Experimental salt farm of CSMCRI. Besides we identified few other bacteria that also can hydrolyze carboxymethyl cellulose, but the activity was less. Among the screened bacteria, *Bacillus licheniformis* KY962963 showed strong signs of growth on carboxymethyl cellulose agar and exhibited more positive results in the staining test (Gram's iodine). Carboxymethyl cellulose was the sole carbon source in the medium; therefore, cellulase might produce in order to effectively digest cellulosic materials.

Morphological observation of selected strain concludes to be the Gram-positive rods belong to the genus *Bacillus*. Other biochemical tests also confirmed that the selected strain is *Bacillus licheniformis*. The purified enzyme showed 200U of total activity and 25U/mg specific activity (Table 1) which is closely similar to *Bacillus* sp., recorded previously for the production of cellulase [20].

The *Bacillus licheniformis* KY962963 carboxymethyl cellulase kinetic analysis showed the Km value of 10 mg/ml and Vmax 1000 mol/min/mg which has the similarity with *Paenibacillus campinasensis* cellulase had a Km of 11.25 mg/ml and a Vmax of 1250 mol/min/mg with carboxylmethyl cellulose [21]. On the other hand, *Bacillus circulans* Km value on CMcellulase 7.2 mg/ml, Vmax value 210 mol/min/mg [22]. Carboxymethyl cellulase optimal temperature activity was at 70°C for *Bacillus licheniformis* KY962963. Similar findings have also been reported in marine microorganisms like *Bacillus sp.* KSMN 252 [23], *Rhodothermus marinus* ATCC 43812 [24], and *Geobacillus* sp. [25]. For the industrial process, this thermostable property of cellulase is considered to be the most important [26]. This particular study revealed that the optimum temperature for maximum cellulase production was found to be at 70°C. Optimum cellulolytic activity was found at pH 9.0, this observation shows the similarity with *Bacillus* sp. KSM635 [27], and *Bacillus subtilis* AS3 [28]. Optimum cellulolytic activity was found at pH 9.0, this observation shows the similarity with *Bacillus* sp. KSM635 [27], and *Bacillus subtilis* AS3 [28].

The enzyme shows salinity tolerance upto 10% which proves its origin from marine source. Most of the *Bacillus* sp. which produced alkaline cellulase exhibited a different type of inhibition as well as activation with the addition of different metal ions or organic solvents.

The cellulase showed higher activity in the presence of agronomical waste residue, which confirms the digestion ability of cellulase. The inducing effect of wheat bran on cellulase production shows similarity with that of *Trichoderma reesei* RUT C-30 when performed under solid state fermentation [29]. The purified cellulase was found to be active in the presence of agronomical waste residues, thus confirming the digestion ability in the agricultural waste materials.

The inducing effect of metal ions such as Mn^{2+}, Zn^{2+} and Ca^{2+} shows the similarity with the report of Sanchez-Torres et al., 1996 [30] where Cel5A activity was found to be induced when low concentrations of manganese is present. This finding has a similarity with the cellulase from *Bacillus* [31] which was reported earlier. While cellulase activity was strongly inhibited by divalent ions of Cu^{2+}, Ni^{2+}, and Mg^{2+}. These findings have the similarity to those results acquired by *Geobacillus* sp.70PC531 [25]. When EDTA is added to the reaction mixture, it induces the enzyme activity. These findings are similar to those obtained by *Geobacillus* sp.70PC531 [25]. Howbeit the EDTA is known as chelator, it increases the enzymatic activity it proves that the enzyme is not a metalloenzyme. The inducing effect of divalent metals such as Mn^{2+} and tolerance towards high salt concentration (NaCl 10%) revealed the unique characteristic of the cellulase from *Bacillus licheniformis* KY962963.

The potentiality of the cellulase evaluated for its affinity towards detergent components after assessing its stability with different surfactants. It shows greater stability in presence of commercial detergents. The reason behind this could be that nonionic surfactant can modify the enzyme surface property and minimize the cellulase irreversible inactivation [32]. These findings are better than the results found by Sadhu et al., 2013 [33].

In the presence of organic solvents cellulase enzyme does not show any drastic decline in its activity which proves its characteristic to solvent stability. There are several reports showing that different carbon sources have different influences on extracellular cellulase production by different strains. The inducing effect of wheat bran on cellulase production is similar to that observed for *Trichoderma reesei* RUT C-30 under solid state fermentation [29].

The potential of the cellulase enzyme evaluated for its compatibility with detergent formulations after assessing its stability with different non-ionic, anionic surfactants and oxidizing agents and it shows greater stability in presence of commercial detergents as well as non-ionic and anionic surfactants, the reason behind this could be that nonionic surfactant can modify the enzyme surface property and minimize the cellulase irreversible inactivation [32]. These findings are better than the results found by Sadhu et al., 2013 [33]. Wash performance analysis is the main experiment which proves that cellulase can be used as laundry additive. A similar type of study has been done before for the enzyme protease, but according to our knowledge, this type of analysis for cellulase enzyme has been done for the first time.

In conclusion, we can say that a wild strain of *Bacillus licheniformis* KY962963 is haloalkaline, thermoactive, solvent stable cellulase having functional activity at a wider pH range (7-12), temperature (30-110°C) and salinity up to 14%. Enzyme is also found to be capable of hydrolyzing cellulosic waste materials by showing higher activity. In addition to these features, the cellulase stability towards surfactants, bleach, and inhibitors along with assay for wash performance explicated the valuable aspects of cellulase for laundry-based matrices. Further, statistical optimization of the growth conditions for the scale up of downstream and upstream processing is required for its commercial feasibility.

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Compliance with Ethical Standards

Human or animal rights are not applicable in this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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