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Enhancement of Production of Cellulases from UV (Ultraviolet light) treated *Bacillus subtilis*

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Abstract: ***Cellulases are the enzymes that hydrolyze cellulosic biomass and are produced by the microorganisms that grown over cellulosic matters. Bacterial cellulases possess more advantages when compared to the cellulases from other sources. In present study 18 Cellulase producing bacteria were isolated from wood furnishing area, soil under dry leaves, cow dung, forest area. All the isolates were later purified and screened for their cellulase activity. Result indicate that bacteria MJTP 2015 09 shows best result. Cellulase production was enhanced by mutation with U.V. radiation and Ethidium Bromide. The selected bacteria when treated with U.V. radiation for 15 minute (MJTP 2015 09 15') shows highest enzyme activity. The organism was then identified using morphological, cultural and biochemical analysis and was found as Bacillus subtilis. Cellulase was produced and the culture conditions like Peptone and Beef extract (1.5%) as nitrogen source, $MgSO_4$ (0.02g) as metal ion, CMC (1.0%) as substrate, temperature 37°C and pH 7 were optimized. The enzyme was further purified using ammonium sulphate precipitation and dialysis. Cellulase was then characterized for the stability and better activity under the effect of different pH, temperature, activators and inhibitors. The application of Cellulase in Biostoning, detergent compatibility and biodegradation was also analyzed.***

Keywords: cellulose, cellulase, cellulosic biomass, ***Bacillus subtilis***

1. Introduction

Today large amount of agricultural and industrial cellulosic wastes have been accumulating in the environment. Cellulose is the primary product of photosynthesis in terrestrial environments, and the most abundant renewable Bioresource produced in the biosphere (100 billion dry tons/year) (**Zhang and Lynd, 2004**).

Cellulases are the enzymes that hydrolyze β -1,4 linkages in cellulose chain. They are produced by large variety of microrganisms like fungi, bacteria, actinomycetes. These microorganisms can be aerobic, anaerobic, mesophilic or thermophilic. Among them, the genera of *Clostridium*, *Cellulomonas*, *Thermomonospora*, *Trichoderma*, and *Aspergillus* are the most extensively studied cellulase producer.

Cellulases are mainly classified into 5 types on the basis of types of reaction catalyzed-

Table 1: Types and action of cellulase

Type	*Reaction*
Endocellulase	Randomly cleaves internal bonds at amorphous sites that create new chain ends.
Exocellulase	Cleaves two to four units from the ends of the exposed chains produced by endocellulase
Cellobiase	Hydrolyses the exocellulase product into individual monosaccharides.
Oxidative cellulases	Depolymerize cellulose by radical reactions
Cellulose phosphorylase	Depolymerize cellulose using phosphates instead of water.

Cellulases were initially investigated for bioconversion of biomass, which gave way to research in the industrial application of enzyme. The major industrial applications of cellulases are in textile industry for "bio-polishing" of fabrics and producing stonewashed look of denims, as well as in household laundry detergents for improving fabric softness and brightness. Besides, they are used in animal feeds for improving the nutritional quality and digestibility, in processing of fruit juices, in baking etc. Utilisation in de-inking of paper is yet another emerging application **(Tolan and Foody, 1999).** The cellulases that are used so far for the above-mentioned industrial applications are those from fungal sources **(Tolan and Foody, 1999)**. With the shortage of fossil fuels and the arising need to find alternative renewable source of energy and fuels, there is a renewal of interest in the bioconversion of lignocellulosic biomass using cellulose and other enzymes.

The aim of this study is to enhance production of cellulases from bacteria isolated from various sources by using biological techniques. To attain this aim project was started with following objectives:

- Sample collection
- Isolation of bacteria from sample
- Purification of bacteria
- Screening of cellulolytic bacteria
- Strain improvement
- Identification of cellulolytic bacteria
- Fermentation
- Purification of enzyme
- Characterization of purified enzymes
- Application of purified enzyme

2. Material and Method

Collection of soil sample

Soil samples were collected from four areas in Lucknow like wood furnishing area(S1) R.K. Timber, Gomtinagar; soil under dry leaves (S2) Vibhutikhand, Gomtinagar; cow dung(S3) Stable behind Gomtinagar railway statio; forest area(S4) Arjunganj, Gomtinagar.

Isolation of Bacteria from Soil

Bacterial colonies were isolated from soil by serial dilution agar plating method. Eighteen different bacterial isolates

were selected for further studies based on their colony morphology studies. They were tentatively named as MJTP 01 to MJTP 18. All the eighteen were sub cultured on sterile NA plates by discontinuous quadrant streaking.

Screening of cellulolytic bacteria

The screening was done by streaking the isolated colonies on screening medium i.e. CMC agar media. After 72 hours incubation the plates were flooded with 0.1% Congo red solution and left undisturbed for 15 minutes. To visualize clear zones formed by cellulase positive strains the plates were destained using 1M NaCl solution. Positive and better zone producing strain was chosen and continued for further studies.

Strain Improvement

Out of the 18 isolates evaluated, MJTP 09 was found to be the most potent for cellulase production; therefore, this strain was used for strain improvement by mutation. Two methods of mutation were employed for strain improvement: physical method U.V.radiation for different time intervals (3, 6, 9, 12 and 15 min) and chemical method Ethidium Bromide treatment at different concentrations (1, 2, 3, 4 and 5 ug/ml). After mutation culture from each plate was inoculated in fermentation media. After 72 hr the crude extract was isolated and DNS assay was performed.

Identification of isolate having maximum cellulase producing potential

Isolate MJTP 09 mutated by U.V. treatment for 15 minutes showing good cellulose producing potential was selected for further studies and identified by performing various staining and biochemical activities based on Bergey's manual.

Study of growth parameters

The study of physical parameters such as Growth kinetics, effect of temperature and effect of pH for growth of isolate MJTP 09 15' was done. For studying the growth curve of the culture 100 ml of NB was inoculated and incubated in shaker120 rpm for 24 hrs. After that O.D. was read at 600 nm everyday till decline phase was not reached; for the effect of pH four flasks with 20 ml of NB were maintained at pH 5, 7, 9 and 11 and was inoculated and incubated at 37°C for 24 hrs at 120 rpm. After 24 hrs of incubation OD was read at 600 nm. For the effect of temperature culture was streaked on sterile NA plates, the plates were incubated at different temperature i.e.4°C, 32°C, 37°C and 50°C. After 24 hrs of incubation, growth was observed.

Optimization of production media for fermentation

Optimization of physiochemical factors such as pH, nitrogen source, different concentration of substrate, metal ions of isolate MJTP 09 15 min was done for cellulase producing potential. For this production media was modified for each factor and after incubation of 72 hours enzyme assay of each modified media(MM) was performed and O.D. was measured at 540 nm.

Table 2: different modified media for optimization

Modified Media(MM)	*Factors*	
PM		Peptone (0.5%) +Yeast extract(0.5%)+ $MgSO_4$(0.2g/l)+ CMC(1.0%)+ pH 7
MM1	N_2 Source	Peptone (1%)
MM2		Yeast extract (1%)
MM3		Beef extract (1%)
MM4		NH_4CL (1%)
MM5		Urea (1%)
MM6	CMC conc.	0.5%
MM7		0.75%
MM8		1.0%
MM9		1.25%
MM10	Combination of different N_2 sources	Peptone(0.5%)+ Beef extract(0.5%)
MM11		Peptone(0.5%) + NH_4Cl(0.5%)
MM12		Peptone(0.5%) + Urea(0.5%)
MM13		Beef extract (0.5%) + Yeast extract(0.5%)
MM14		Peptone(0.25%)+ Beef extract(0.75%)
MM15		Peptone(0.75%)+ Beef extract(0.25%)
MM16		Peptone(0.1%)+ Beef extract(0.9%)
MM17		Peptone(0.9%)+ Beef extract(0.1%)
MM18		Peptone(0.3%)+ Beef extract(0.7%)
MM19		Peptone(0.7%)+ Beef extract(0.3%)
MM20		Peptone(0.6%)+ Beef extract(0.4%)
MM21		Peptone(0.4%)+ Beef extract(0.6%)
MM22		Peptone+ Beef extract(0.75%)
MM23		Peptone+ Beef extract(1.25%)
MM24		Peptone+ Beef extract(1.5%)
MM25		Peptone+ Beef extract(1.75%)
MM26	Metal ions	Ca(0.2g/l)
MM27		Pb(0.2g/l)
MM28		Zn(0.2g/l)
MM29		Cu(0.2g/l)
MM30		Fe(0.2g/l)
MM31	Combination of different metal ions	Fe(0.1g/l)+Mg(0.1g/l)
MM32		Fe(0.5g/l)+Mg(0.15g/l)
MM33		Fe(0.15g/l)+Mg(0.5g/l)
MM34	pH	pH5
MM35		pH7
MM36		pH9
MM37		pH11

Fermentation

MJTP 09 15 min culture was inoculated in 100 ml of Optimized production media containing Peptone and Beef extract (1.5%), $MgSO_4$ (0.02g), CMC (1.0%), KH_2PO_4 (0.1g), NaCl (0.1g), pH7 and incubated at 37°C, 120 rpm for 7 days.

Downstream processing of enzyme

Downstream processing involves recovery and purification of product of interest from fermented broth. First cell free extract was prepared by centrifugation of fermented broth at 5000 rpm for 5 min at 4°C . Supernatant separated referred as crude enzyme. Then enzyme was precipitated by adding solid ammonium sulphate to the culture filtrate to 70% saturation. After 24 h the resulting precipitate was collected by centrifugation at 10,000 rpm for 10 min and dissolved in 100 Mm Tris buffer. The salt precipitated protein was then dialysed overnight against three changes of the same buffer. After dialysis enzyme collected was purified cellulase. DNS assay of crude and purified enzyme was done to determine

the amount of cellulase produced and specific activity of the enzyme. Protein estimation was done by Lowery's method.

Characterization of purified enzyme

The purified enzyme was characterized for the stability and better activity at different pH, temperature and the effect of activators and inhibitor were also studied. The optimum pH for the purified enzyme was determined by incubating enzyme with substrate (1% CMC) prepared in appropriate buffer at different pH (5,7,9 and 11). Crude enzyme mixture in those buffers was incubated for 15 min at 37˚C.

The effect of temperature on activity of cellulose was determined by incubating enzyme with 1% CMC in 100mM tris buffer (pH 7) at temperatures between 10 to 50°C.

Various metal ions activators including Ca, Cu, Pb, Mg and inhibitors EDTA , SDS was applied to check the optimum activity of enzyme. Each metal ions and inhibitors were used at concentration of1mg/ml. Cellulase activity was assayed by DNS method.

3. Application of cellulase

Biostoning of Denim fabric : Denim fabric was taken and prewashed with detergent for 10 minutes at 60ºC and was cut into two 2×2 size. The cellulase treatment was done in two conical flasks each containing 20ml of 100Mm tris buffer and the prewashed denim fabric. One was kept as a test in which 5 ml of purified enzyme was added. Another flask was assigned as a control in which 5 ml of distilled water was added. The conical flasks were kept 30 minutes (50ºC). The fabrics were then soaked for 10 min in 100 ml of 10 mM NaOH and rinsed with 10 mM NaOH for 2 min followed by tap water. The fabrics were dried for one hour at 50ºC and air dried overnight at room temperature. The colour change occurred on both side of the fabrics were observed.

Detergent Compatibility of Cellulase : Four locally available detergent brands (Surf excel, Ariel, Wheel and tide) were used for studying com- patibility of purified cellulase under nor- mal conditions. 1% Detergent solutions were prepared . Carboxy-methyl cellulose solution (1%) was used as substrate and prepared in tris buffer of pH 7. A reaction mixture comprising 3 mL of substrate solution, 1.1 mL, detergent solution and 0.9 mL, purified cellulase was incubated at 55°C for 10 - 15 minutes followed by normal enzyme assay as described earlier. A control sample was also incubated in parallel to reaction mixture solution.

Biodegradation of cellulosic materials: Most efficient isolate was selected and used for

Filter paper and cotton degradation. For this a sterile 50 ml of 100 mM tris buffered solution with pH 7 was individually supplemented with filter paper strips and cotton as a sole source of carbon and the medium was supplemented with two drops of 10 mM glucose to possibly induce cellulose production(Maki *et al*., 2011). Then, the log phase culture of selected most efficient isolate was separately inoculated into this medium. The culture was incubated for maximum 6 days at 37°C in shaking condition at 120rpm and observed daily for visual evidence of degradation.

4. Result

Isolation of Bacteria from Soil

Bacteria from soil were isolated by serial dilution method and spread plate method, mixed colonies obtained were differentiated on the basis of morphological characteristics.. These cultures were named as MJTP 01, MJTP 02, MJTP 03, MJTP 04, MJTP 05, MJTP 06, MJTP 07, MJTP 08, MJTP 09, MJTP 10, MJTP 11, MJTP 12, MJTP 13, MJTP 14, MJTP 15, MJTP 16, MJTP 17 and MJTP 18. All the colonies were purified by quadrant streaking

Screening of cellulolytic bacteria

The primary screening was done by streaking on CMC agar media, then secondary screening was done with 0.1% Congo red solution and destained using 1M NaCl solution. Plates were checked for clear zone of hydrolysis. Table 4 shows the remark for screening. It was concluded that MJTP 02 and MJTP 09 showed best results.

Table 3: Results of screening

S.no.	Culture no.	Primary screening	Secondary screening
1	MJTP-01	+	_
2	MJTP-02	++	+++
3	MJTP-03	++	++
4	MJTP-04	_	_
5	MJTP-05	+++	+
6	MJTP-06	++	+
7	MJTP-07	++	+
8	MJTP-08	+	+
9	MJTP-09	++	+++
10	MJTP-10	+++	_
11	MJTP-11	+++	_
12	MJTP-12	_	_
13	MJTP-131	+++	_
14	MJTP-14	++	+
15	MJTP-15	++	++
16	MJTP-16	+	_
17	MJTP-17	+	_
18	MJTP-18	+	+

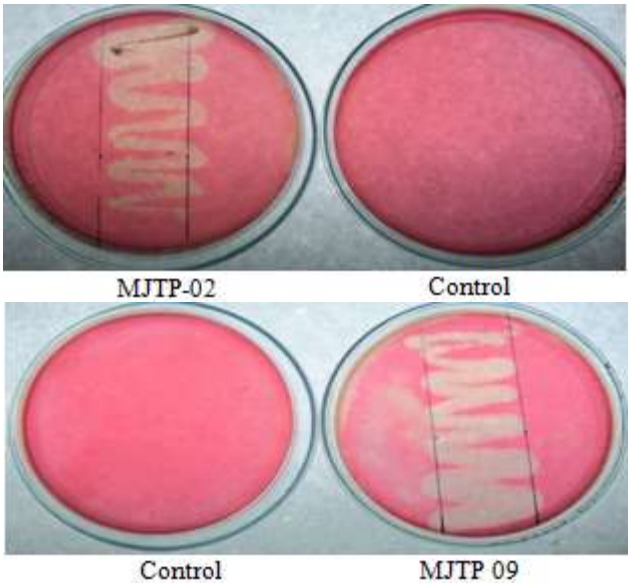

Figure 1: Zone of hydrolysis on CMC Agar plate

Tertiary screening: MJTP 02 and MJTP 09 showed approx same result in primary and secondary screening therefore they were further screened for production of cellulase .The activity of Cellulase was assayed using DNS method. After which it was concluded that MJTP 09 showed maximum cellulose activity and therefore use for further studies.

Table 4: Tertiary screening

S.no.	Culture no.	O.D. at 540 nm	Enzyme activity (U/ml/min)
1	MJTP-02	0.14	0.00216
2	MJTP-09	0.21	0.003

Strain improvement

After stain improvement by U.V.radiation and Ethidium Bromide treatment followed by DNS assay MJTP 09 treated with U.V. radiation for 15 min named as **MJTP 09 15'**showed highest cellulose activity, thus it was used for further studies.

Table 5: Enzyme Assay after UV Treated colonies

S.no.	Mutant bacteria	O. D. at 540 nm	Enzyme activity (U/ml/min)
1	Control	0.26	0.00396
2	3 min	0.29	0.00444
3	6 min	0.31	0.00468
4	9 min	0.25	0.00385
5	12 min	0.24	0.0036
6	**15 min**	0.34	0.00516

Table 6: Enzyme Assay after EtBr Treatment

S.no.	Concentration of EtBr(µg/ml)	O. D. at 540 nm	Enzyme activity (U/ml/min)
1	Control	0.21	0. 00031
2	1	0.20	0.00300
3	2	0.29	0.00432
4	3	0.22	0.00324
5	4	0.29	0.00432
6	5	0.26	0.00396

Identification of isolate showing maximum cellulose activity

The selected culture MJTP 09 15' was identified by Bergey's Manual. Various Biochemical and staining test were performed, the result of which are given in **table 11**

Table 7: Staining & Biochemical Tests of MJTP 09 15min

S.no.	*Test*	*Result*
1	Gram staining	Positive (*Bacillus*)
2	Endospore staining	Positive
3	Catalse test	Positive
4	Mannitol test	Positive
5	V.P. test	Positive

Thus from Bergey's Manual it was identified that the isolate MJTP 09 15' was *Bacillus subtilis.*

Study of growth parameters

Growth kinetics of Bacterial strain MJTP 09 15' was studied by taking the absorbance reading of the culture broth at 600nm after every 24 hrs. The stationary phase was observed on 6-7 days. The maximum growth was observed at 37ºC at pH 7.0

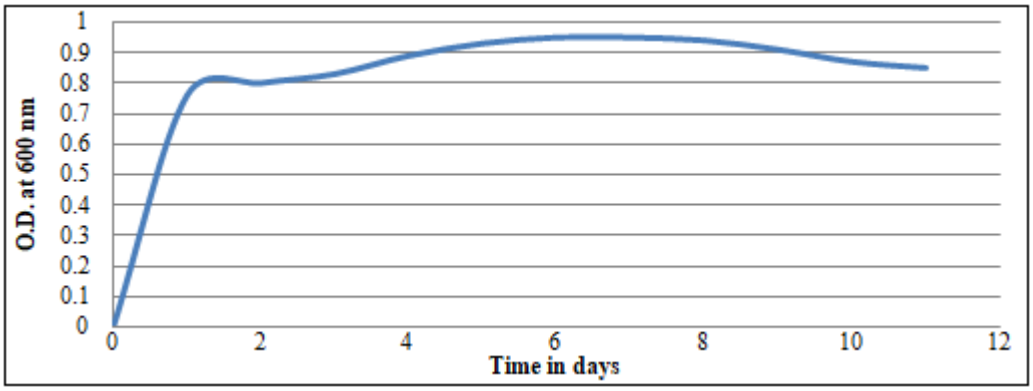

Figure 2: Graph showing growth curve of the culture MJTP 2015 09 15'

Optimization of production media for fermentation

It was discovered that the enzyme activity by bacteria MJTP 09 was best when production media supporting the growth has nitrogen source as Peptone and Beef extract (1.5%), metal ion as Mg^{2+}, CMC 1.0%, pH7.

Table 8: Different Modified Media for optimization

Modified Media (MM)	Factors		O.D. at 540nm	Enzyme activity (U/ml/min)
PM		Peptone (0.5%) +Yeast extract (0.5%) + $MgSO_4$ (0.2g/l) + CMC (1.0%) + pH 7	0.23	0.00360
MM1		Peptone (1%)	0.15	0.00240
MM2	N_2 Source	Yeast extract (1%)	0.18	0.00276
MM3		Beef extract (1%)	0.17	0.00096
MM4		NH_4CL (1%)	0.06	0.00204
MM5		Urea (1%)	0.13	0.00360
MM6	CMC conc.	0.5%	0.14	0.00216
MM7		0.75%	0.15	0.00240
MM8		1.0%	0.19	0.00288
MM9		1.25%	0.18	0.00276
MM10		Peptone(0.5%) + Beef extract(0.5%)	0.27	0.00408
MM11		Peptone(0.5%) + NH_4Cl(0.5%)	0.18	0.00276
MM12		Peptone(0.5%) + Urea(0.5%)	0.19	0.00288
MM13		Beef extract(0.5%) +	0.24	0.00276

	Combination of different N_2 sources	Yeast extract(0.5%)		
MM14		Peptone(0.25%)+ Beef extract(0.75%)	0.21	0.0030
MM15		Peptone(0.75%)+ Beef extract(0.25%)	0.17	0.00264
MM16		Peptone(0.1%)+ Beef extract(0.9%)	0.22	0.00336
MM17		Peptone(0.9%)+ Beef extract(0.1%)	0.20	0.0030
MM18		Peptone(0.3%)+ Beef extract(0.7%)	0.22	0.00336
MM19		Peptone(0.7%)+ Beef extract(0.3%)	0.21	0.00312
MM20		Peptone(0.6%)+ Beef extract(0.4%)	0.20	0.00300
MM21		Peptone(0.4%)+ Beef extract(0.6%)	0.20	0.0030
MM22		Peptone+ Beef extract(0.75%)	0.36	0.0054
MM23		Peptone+ Beef extract(1.25%)	0.37	0.00552
MM24		Peptone+ Beef extract(1.5%)	0.42	0.00636
MM25		Peptone+ Beef extract(1.75%)	0.41	0.00624
MM26	Metal ions	Ca(0.2g/l)	0.25	0.00384
MM27		Pb(0.2g/l)	0.25	0.00384
MM28		Zn(0.2g/l)	0.20	0.00300
MM29		Cu(0.2g/l)	0.28	0.00420
MM30		Fe(0.2g/l)	0.32	0.00480
MM31	Combination of different metal ions	Fe(0.1g/l)+Mg(0.1g/l)	0.25	0.00384
MM32		Fe(0.5g/l)+Mg(0.15g/l)	0.21	0.00300
MM33		Fe(0.15g/l)+Mg(0.5g/l)	0.26	0.00396
MM34	pH	pH5	0.24	0.00360
MM35		pH7	0.40	0.00600
MM36		pH9	0.26	0.00396
MM37		pH11	0.30	0.00456

Enzyme assay and protein estimation of crude and purified enzyme

Protein concentration of crude enzyme collected after fermentation and purified enzyme after dialysis was determined by Lowry's method, and enzyme activity by DNS assay. result of which are given bellow:

Table 9: Protein Concentration Estimated by Lowry's method

S.no.	Enzyme used	O.D. at 680nm	Protein conc. (mg/ml)
1	crude enzyme	2.0	0.48
2	purified enzyme	0.17	0.04

Table 10: Enzyme Activity estimated by DNS assay

S.no.	Enzyme used	O.D. at 540nm	Enzyme activity (U/ml/min)
1	crude enzyme	0.18	0. 0.0027626
2	purified enzyme	0.10	0.00156

Characterization of purified enzyme

Effect of pH on enzyme activity

Enzyme shows approx similar activity at all pH, but slightly higher at pH 9.

Table 11: Enzyme Activity at different pH

S.no.	pH	O.D. at 540nm	Enzyme activity (U/ml/min)
1	5	0.14	0.00216
2	7	0.14	0.00216
3	9	0.15	0.00240
4	11	0.14	0.00216

Effect of temperature on enzyme activity

Enzyme shows approx similar activity at all temperatures, slightly higher at 22°C and 50°C.

Table 12: Enzyme Activity at different temperature

S.no.	Temperature	O.D. at 540nm	Enzyme activity (U/ml/min)
1	22°C	0.15	0.00240
2	28°C	0.14	0.00216
3	37°C	0.14	0.00216
4	50°C	0.15	0.00240

Effect of activators on enzyme activity

Enzyme shows maximum activity with calcium followed by Pb.

Table 13: enzyme activity with different activators

S.no.	Activator	O.D. at 540nm	Enzyme activity (U/ml/min)
1	Without activator	0.14	0.00216
2	Ca	0.20	0.00300
3	Cu	0.15	0.00240
4	Mg	0.14	0.00216
5	Pb	0.17	0.00264

Effect of inhibitors on enzyme activity

Enzyme activity is inhibited more by SDS then EDTA.

Table 14: enzyme activity in presence of different inhibitors

S.no.	Inhibitor	O.D. at 540nm	Enzyme activity (U/ml/min)
1	Without inhibitor	0.14	0.002160.00
2	SDS	0.07	0.00108
3	EDTA	0.11	0.00168

5. Application of cellulase

Biostoning of Denim fabric

The observation revealed that the purified enzyme was effective in removing the stain from the fabric. But stone washing after enzyme treatment is needed for best result.

Before Treatment After Treatment

Figure 3: Biostoning of Denim fabric

Detergent Compatibility of Cellulase

The enzyme incubated with deter-gent solution revealed maximum compatibility with Ariel and followed by Surf Excel. Therefore, their suitable controls were also run and their activities were found low as compared to those supplemented with cellulase. This revealed that the cellulase is compatible with local detergents and suggesting its potential as suitable additive to detergents.

Table 15: Detergent Compatibility of Cellulase

S. No	Detergent		O.D. at 540nm	Enzyme activity (U/ml/min)
1	Wheel	Control	0.10	0.00156
2		Test	0.10	0.00156
3	Tide	Control	0.10	0.00156
4		Test	0.11	0.00168
5	Ariel	Control	0.10	0.00156
6		Test	0.12	0.00180
7	Surf	Control	0.12	0.00180
8		Test	0.13	0.00204

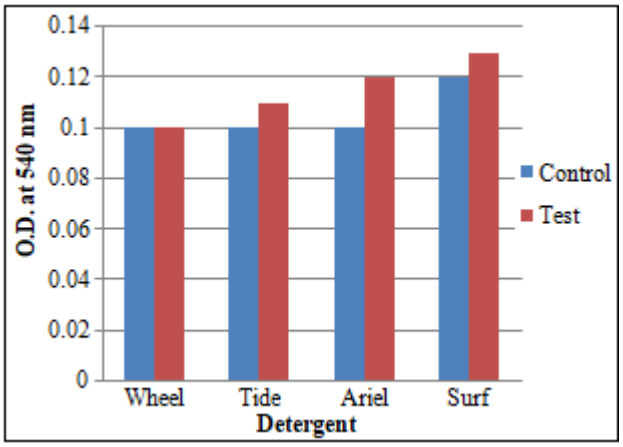

Figure 4: Detergent Compatibility of Cellulase

Biodegradation of cellulosic materials

The purified enzyme shows degradation of filter paper, but do not degrade cotton.

Before incubation After incubation

Figure 5: Showing degradation of cotton

Before incubation After incubation

Figure 6: Showing degradation of filter paper

6. Discussion

Microorganisms were isolated from soil by serial dilution agar plate method as previously done by **Cordeiro, *et al.*, 2002, Ibrahim, and EI- diwany, 2007** and 18 bacterial isolates were purified which were named as **MJTP 2015 01 to MJTP 2015 18**.

The culture were then grown on minimal agar medium supplement with 1%CMC and then plates were screened for cellulolytic microorganisms, as done earlier by **Alam, *et al.*,**

2004, Kotchoni, *et al*., 2006, Ibrahim, and EI- diwany, 2007. By flooding with congo red solution colony **(MJTP 2015 02 and MJTP 2015 09)** that showed largest zone was picked, as previously done by **Cordeiro, *et al*., 2002, Lo, *et al*.,2009.** Then MJTP 09 was further screened for potential cellulose activity by DNS assay of **Mandels, *et al*., 1969.**

To increase the cellulose producing potential the strain MJTP 2015 09 was mutated with U.V. radiation and Ethidium Bromide as done by **Chand, *et al*., 2004.** U.V. radiation for 15 minutes yielded maximum cellulase-producing mutants named as **MJTP 2015 09 15'** which was used for further studies.

Morphological properties and taxonomic characteristics of isolate **MJTP 2015 09 15'**was studied according to the methods in Bergey's Manual of Systematic Bacteriology, as prior done by **Ponpium, *et al*., 2000** and was identified as *Bacillus subtilis.*

Production media used for cellulose producing microorganisms composed of soluble CMC, peptone, yeast extract, KH2PO4, MgSO4, NaCl, as used before by **Lo, *et al*.,2009, Li, 2009, Govender, *et al*.,2009** and was optimized for different nitrogen source, metal ions, substrate concentration done earlier by **Jayant, *et al*., 2011.** The optimized culture contained nitrogen source as Peptone and Beef extract (1.5%), metal ion as Mg^{2+}, CMC 1.0%, pH7.

Partial purification was subjected to fractional ammonium sulphate precipitation (upto 70% saturation). Ammonium sulphate crystals were added to the supernatant to bring the saturation in ice bath and the suspension was dialyzed against 100mM Tris buffer of pH7, as done by **Makky, 2009, Odeniyi, *et al*., 2009.**

Total protein and activity of purified cellulase were determined before and after dialysis of ammonium sulfate precipitation. Protein concentration was measured with Lowry's method as done earlier by **Zambare, *et al*., 2011.** Enzyme activity was assayed by DNS method as done by **Yin, *et al*., 2011, Samira, *et al*., 2011.**

Characterization of purified cellulase was done by studying the effect of different pH values (5-11), incubation temperatures (22°C to 50°C), various compounds and metal ions (SDS, EDTA, Ca^{2+}, Cu^{2+}, Mg^{2+}, Pb^{2+}) as activators and inhibitors on purified cellulase as done previously by **Iqbal, *et al*.,2011.**

Applications of cellulase in various industries were also studied like biostoning of jeans as done earlier by **Mukesh kumar, *et al., 2011***, enhancing detergents activity as done by **Iqbal, *et al*., 2011** and biodegradation of cellulosic materials previously done by **Shaikh, *et al*., 2013.**

7. Conclusion

The present study was carried out for isolation of potential cellulase producing bacterial strain from different environmental waste which were rich in cellulosic biomass. Two isolates MJTP 02 and MJTP 09 were selected after screening on CMC agar media to show maximum cellulose producing potential and the source for the isolates were soil from wood furnishing area and cow dung respectively. Then MJTP 09 was further screened for potential cellulose activity by DNS assay.

The strain MJTP 09 was then mutated with U.V. radiation and Ethidium Bromide to increase the cellulose producing potential of the strain. It was found that strain improvement by U.V. radiation for 15 minute enhance the production of cellulose. MJTP 09 15' culture was characterized from their morphological, cultural and biochemical analysis and identified as *Bacillus subtilis.* Growth parameters of the isolate MJTP 09 15' was also studied at different temperature (optimum temperature 37°C), pH (optimum pH 7) and growth kinetics (stationary phase was seen from 6^{th} day to 8^{th} day).

Then optimization of different physiochemical factor like pH, different nitrogen source, metal ions, substrate concentration was checked for maximum cellulase production. It was discovered that the enzyme activity by bacteria MJTP 09 was best when production media supporting the growth has nitrogen source as Peptone and Beef extract (1.5%), metal ion as Mg^{2+}, CMC 1.0%, pH7.

Purification of cellulase was done and the enzyme activity and specific activity was determined. The optimum parameters required for the stability and better activity of were also studied. The activity of the enzyme was found to be stable at wide range of temperature, from 22°C to 50°C, and pH range of 5to11. Enzyme shows maximum activity with Ca^{2+} followed by Pb^{2+} thus they can be used as an activator. It was also determined that SDS and EDTA inhibit the enzyme activity, SDS inhibit more than EDTA.

It is also found that cellulases are not only served as the cellulosic enzyme but also have applications in various industries including biostoning of jeans, enhancing detergents activity and biodegradation of cellulosic materials.

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