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Studies on the effects of ethidium bromide and DMSO on cellulase producing isolated bacterial strain

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Abstract

Cellulase enzymes hydrolyse cellulose and are discharged by the microorganisms that grown over cellulose rich matter. Production of cellulase at marketing level is most actively grown research area now a day. Screening of potential strain from new source and there by optimizing production condition for industrial cellulase. Cellulase is one of the several profit making enzymes which have been serviced in different industries like paper and pulp, textile, production of bio-fuel, detergents, feed and food industry and brewing Bacterial cellulose possess more advantages when compared to the cellulose from other sources. In present study 14 Cellulase generating bacteria were deserted from wood degrading area, soil under dead leaves, dung of cow, and forest area. Purification and screening for cellulase production of isolates were done. Results indicate that bacteria SPCP1910 showed best result. Isolated strain was made better by treatment with DMSO and Ethidium Bromide.

Keywords: Cellulose, cellulase, radiation, diet, strain improvement.

Introduction

In this era the large quantity of industrial and agricultural cellulosic wastes has been accumulating in the environment[1]. Cellulose is the basic results of photosynthesis in earthly environments, and the most a plentiful revival Bio resource produced in the biosphere (100 billion dry tons/year). Cellulose is the largest plentiful organic mixture on earth, in the higher plant of the cell wall the maximum structural compound. Cellulose is maximum found in cotton (90%), flax (80%), jute (60–70%), and wood (40–50%). Cellulose paste can be also acquired from many forming products such as sorghum bagasse, sugarcane corn and straws of rye, wheat, oats, and rice[3]. Cellulose is a straight polysaccharide consisting of β-1,4-glycosidic attached d-glucose units (that's why called an hydro-glucose unit)[2]. Cellulose is the bio molecule that concentric most of the plant's cell wall. In earth probably the most abounded organic compound[2]. Away from being basic constructed dwelling material for plants, cellulose has many more other uses. Film, paper, explosive, and plastic can be used and it can be also used in industries. In our diet plan cellulose play a considerable source of fiber in our requirements[4].

Methodology

Sample selection: There were different cellulose containing samples were selected on counting cellulosic richness. Paying attention on bacterial diversity different location for sample selection was picked out.

Bacteria isolation: Cellulase producing bacteria were isolated by serial dilution from selected sample, picked out from different location. For serial dilution 0.85 % normal saline were used and samples were diluted serially[5].

Pure culture: After serial dilution mix culture was isolated that contains many different colonies on same plate. To convert mix culture into pure culture streak plate method was used. Nutrient agar media was used to streak the bacterial colony on plate[5].

Screening: After streaking there were 15 bacterial culture were isolated. To find out celluase producing bacteria screening was done by using 1% CMC with minimal agar media. 1% Congo red was used to see the zone of hydrolysis and for washing 0.1N Naclwas used. Culture SPCP1910 produced best zone of hydrolysis[6].

Strain improvement: Isolated culture SPCP1910 was mutated by using UV rays and EtBr. Culture SPCP1910was spread on 6 nutrient agar media plates and it was treated with UV rays in laminar air flow for different time interval i.e. 4 mint, 6 mint ,8 mint, 9 mint, 12 mint.

Culture SPCP1910 was also treated with different concentration of EtBr i.e.1µg/ml, 2µg/ml, 5µg/ml and 8 µg/ml[7,8,9].

Selection of mutated bacteria: UV mutated bacteria (8mint) and EtBr mutated bacteria (5µg/ml) were streaked on 1%CMC

agar media and to see the maximum zone of hydrolysis and best zone of hydrolysis was shown by UV mutated bacteria.

Media optimization: Before going the production through fermentation media for the bacteria was optimized by one time at a factor. During this different carbon, nitrogen, sources, temperature were checked and best results for bacterial growth were selected.

Bacterial growth curve: To find out stationary phase of bacteria it was inoculated in nutrient broth media and incubated at 37ºC in shaker incubator for one week and optical density was taken through the spectrophotometer[10].

Fermentation and downstream processing: Optimized media components were used to make fermentative media. Bacteria were inoculated in media and shake flask fermentation was used to produce cellulaseenzyme[11]. Further the purification of enzyme is done by salt precipitation (40% ammonium sulphate) and dialysis[12,13].

Enzyme Assay: it is performed by using the DNS test[14,15].

Characterization of enzyme: Effect of different parameters i.e. temperature, pH, metal ions were checked.

Effects of temperature: Purified enzymes were incubated with cellulose at different temperature i.e. 4°C, 25°C, 37°C and 50°C[16,17]. DNS test was performed to check the activity.

Effect of pH: Purified enzyme was incubated with cellulose at different pH i.e. 3,7,9,11[18]. Activity was checked by DNS methods.

Effects of metal ions: Different metals were incubated with enzyme[19,20] and cellulose (substrate) and enzyme activity was checked by DNS methods.

Results and discussion

Sample collection and isolation of bacteria: Soil was collected from the area which was enriched in cellulose. The soil sample was further diluted to decrease the count of bacteria and by spreading the sample on nutrient agar plates the colonies can be easily selected.

Purification and screening of bacteria: The bacteria which were selected on the basis of different morphological parameters were streak in different nutrient agar plates from mixed culture plate to obtain the pure culture. In pure culture was screened for cellulose activity. The culture plate was incubated for 48-72 hr. The minimal salt media was supplemented with 1% CMC. The secondary screening was done with 1% congo red and then it was washed with 1MNaCl. After washing culture SPCP1910 shows zone of hydrolysis among all the bacterial plate.

Table-1: Optimized media.

Factor	Modified Media	Component
Peptone	MM1	5%
	MM2	6%
	MM3	7%
	MM4	8%
Beef extract	MM5	5%
	MM6	4%
	MM7	3%
	MM8	2%
NaCl	MM9	6%
	MM10	7%
	MM11	8%
	MM12	9%
$MgSO_4$	MM13	0.3%
	MM14	0.4%
	MM15	0.5%
	MM16	0.6%
CMC	MM17	0.5%
	MM18	1%
	MM19	1.5%
	MM20	2%
pH	MM21	1
	MM22	5
	MM23	7
	MM24	9
	MM25	11

Table-2: Colony morphology of different bacterial colonies.

Shape	Margin	Elevation	Pigmentation	Surface	Texture	Opacity
Circular	Discrete	Flat	Off white	Rough	Gummy	Opaque
Circular	Entire	Raised	Yellowish	Smooth	Hard	Translucent
Irregular	Lobate	Convex	Off white	Smooth	Soft	Opaque
Circular	Curled	Raised	Off white	Rough	Gummy	Opaque
Irregular	Entire	Flat	Off white	Rough	Soft	Opaque
Circular	Lobate	Convex	Yellowish	Convex	Soft	Opaque
Circular	Lobate	Flat	Off white	Smooth	Gummy	Opaque
Irregular	Lobate	Raised	Off white	Convex	Hard	Opaque
Circular	Curled	Raised	Off white	Convex	Hard	Opaque
Irregular	Entire	Raised	Off white	Smooth	Gummy	Opaque
Circular	Lobate	Convex	Yellowish	Smooth	Soft	Opaque
Circular	Curled	Flat	Off white	Smooth	Soft	Opaque
Circular	Entire	Raised	Off white	Convex	Soft	Opaque
Rhizoidal	Curled	Convex	Off white	Convex	Hard	Opaque

Table-3: Screening for cellulase producing bacteria among the isolated colonies.

Culture no.	Primary screening	Secondary screening
SPCP1901	-	-
SPCP1902	-	-
SPCP1903	+	+
SPCP1904	+	+
SPCP1905	-	-
SPCP1906	++	+
SPCP1907	+	+
SPCP1908	-	-
SPCP1909	-	-
SPCP1910	+++	+++
SPCP1911	+	+
SPCP1912	-	-
SPCP1913	+	-
SPCP1914	+	+

Table-4: Biochemical tests for the identification of the SPCP1910.

Biochemical tests	Results
Gram's staining	Positive, *Bacillus*
Endospore staining	Positive
Catalase test	Negative
Mannitol test	Positive

Strain improvement: It is done by providing the mutation to the culture. The mutation is given in to parameters such as chemical mutation (EtBr and DMSO treatment).

Media optimization: The media optimization is done with the help of EtBr mutated SPCP1910culture.

Fermentation and downstream processing: Fermentation was done by the shake flask method. Purification was done by salt precipitation and dialysis.

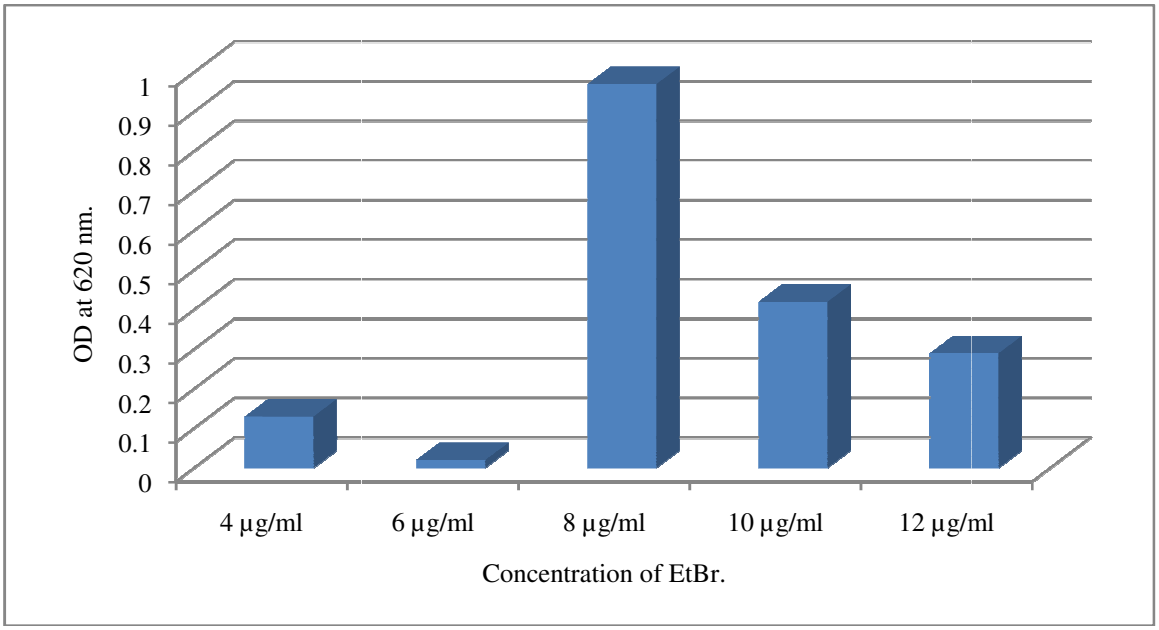

Figure-1: Concentration of EtBr versus OD.

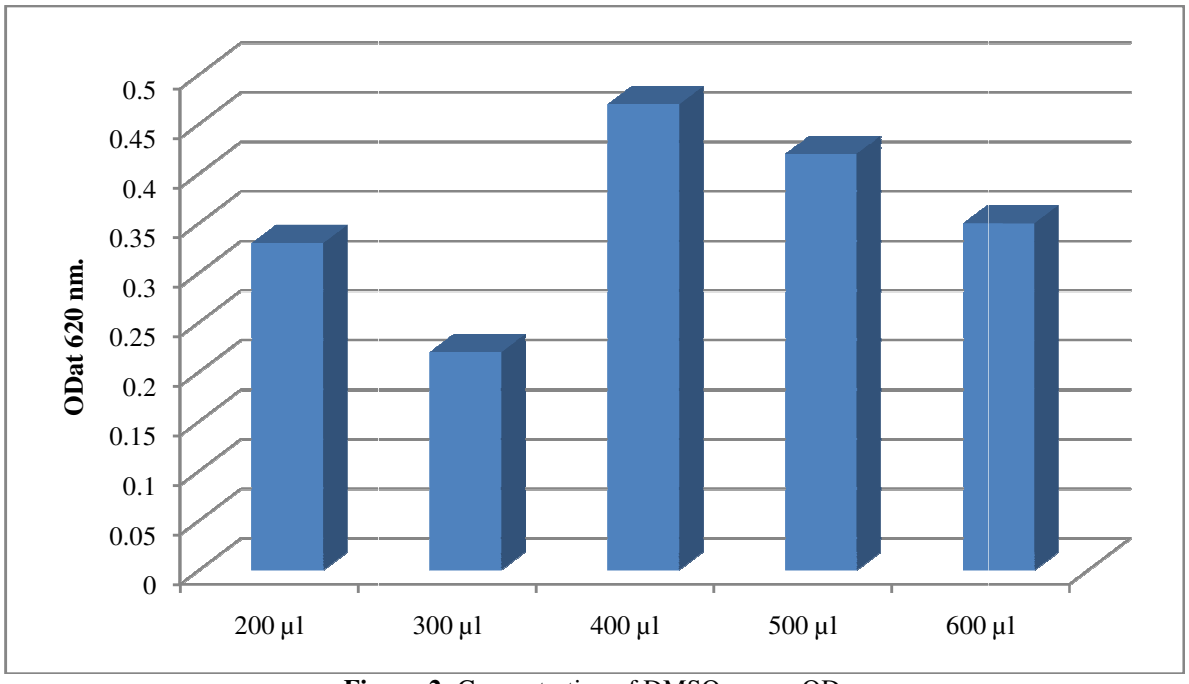

Figure-2: Concentration of DMSO versus OD.

Table-5: Effects of temperature on bacterial growth.

Temperature	Growth
37°C	+++
40°C	+
4°C	-
Room Temperature	+

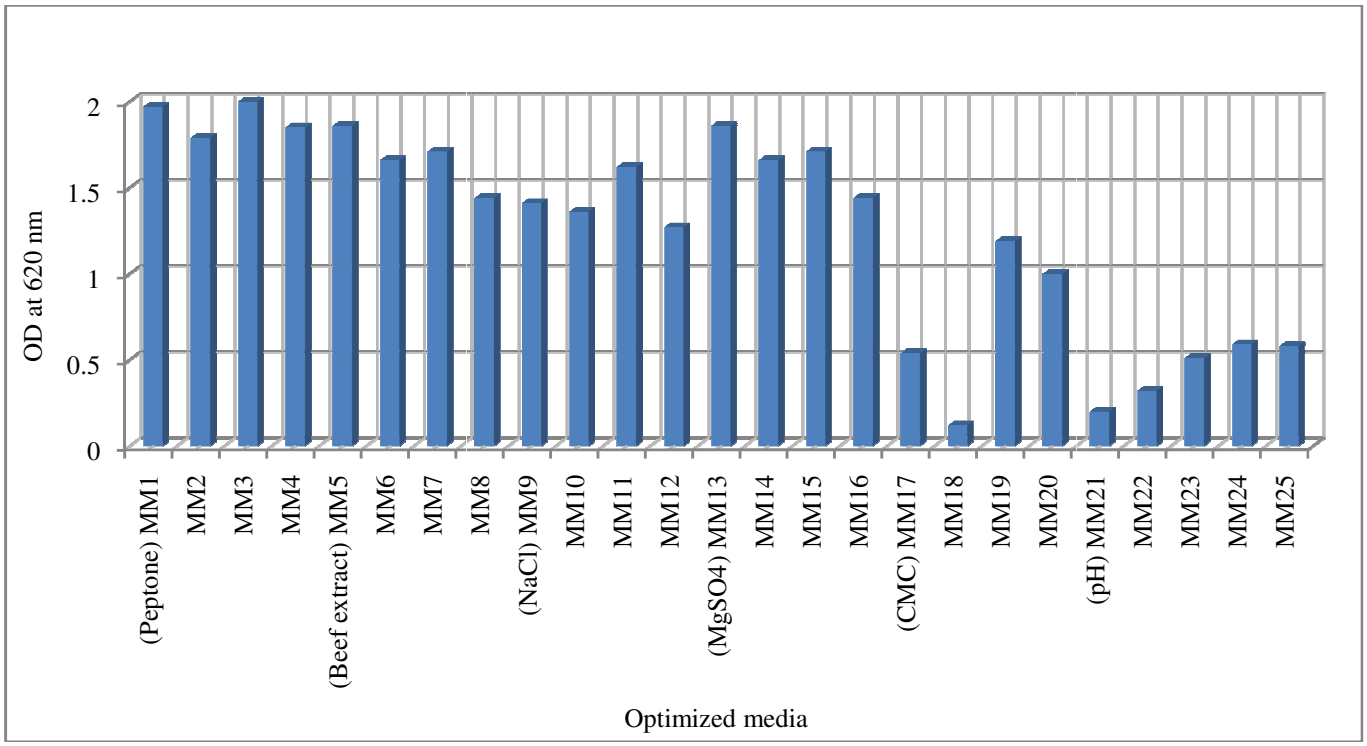

Figure-3: Modified media versus optical density.

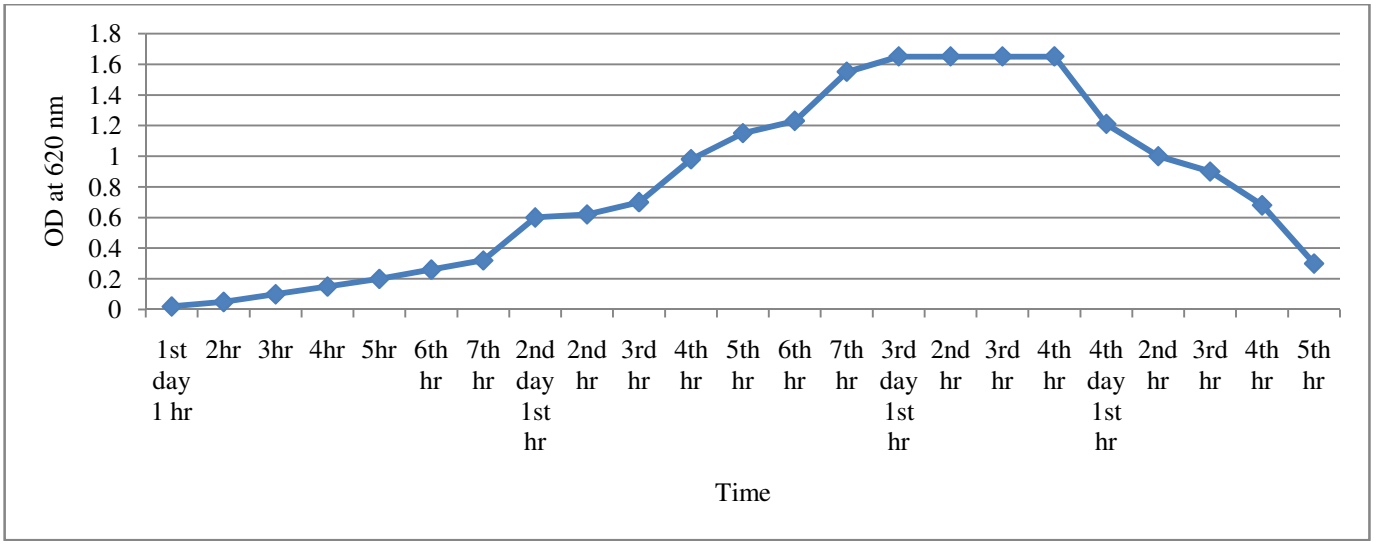

Figure-4: Bacterial growth kinetics.

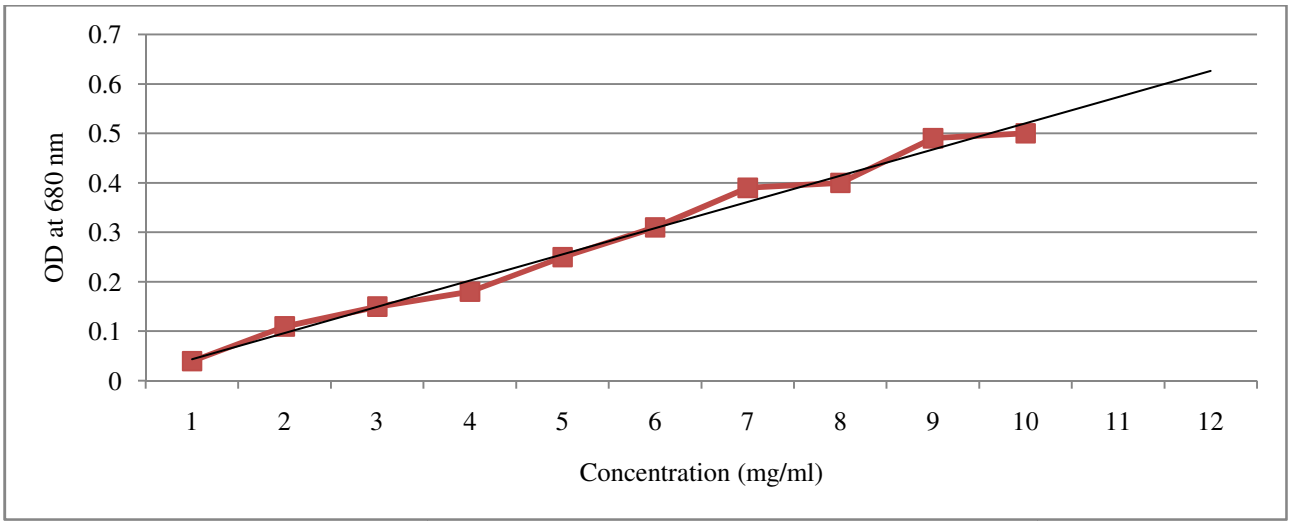

Figure-5: Standard graph of Lowry's method

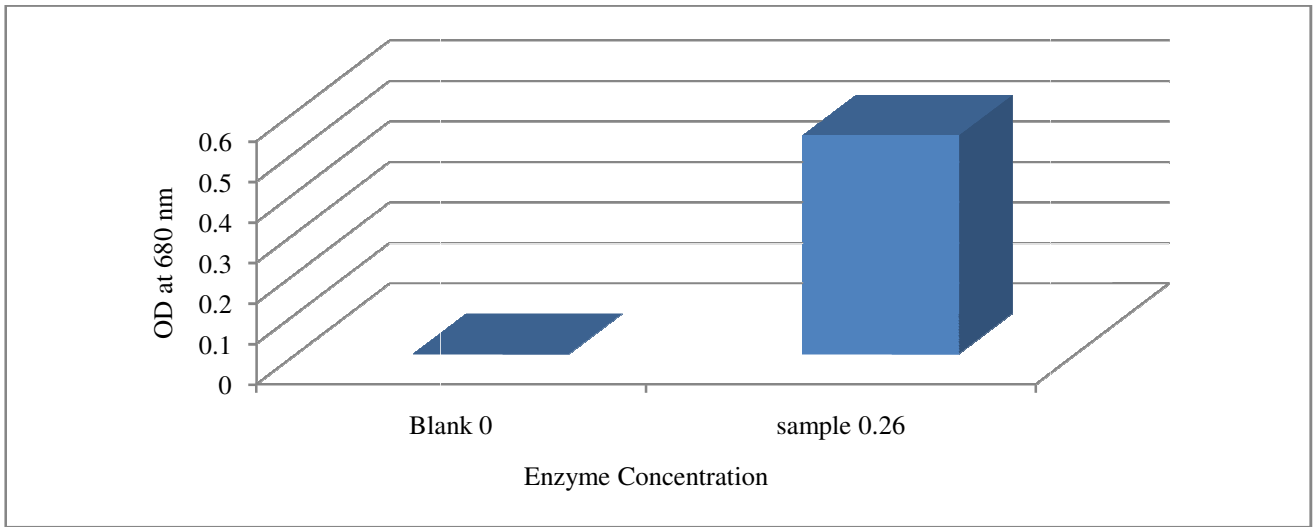

Figure-6: Concentration of enzyme identified by stand graph of Lowry's method.

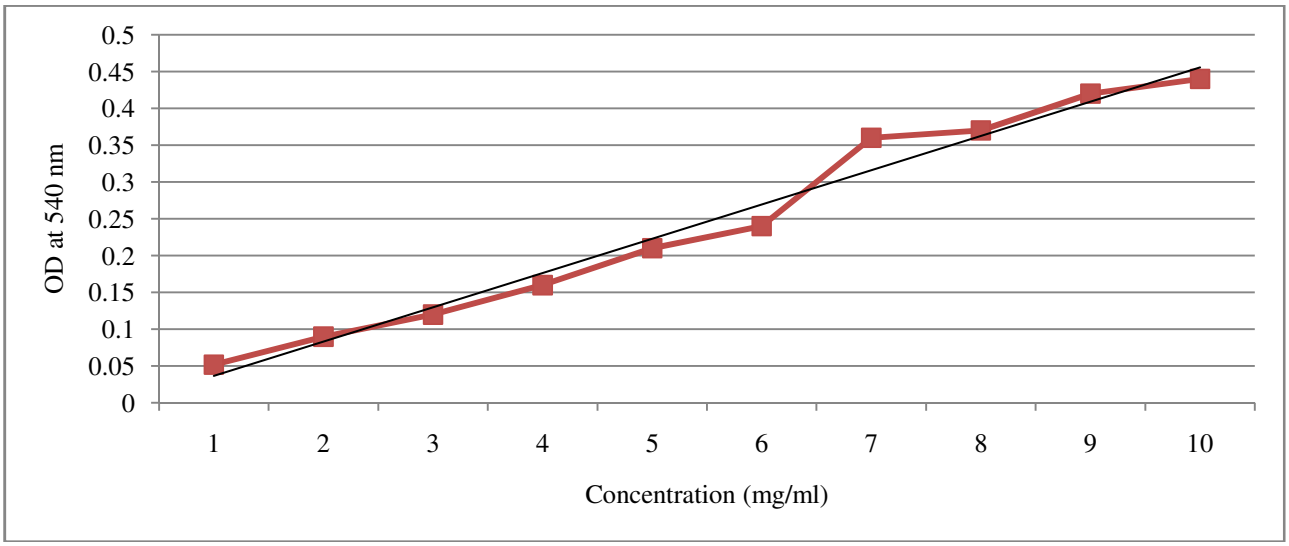

Figure-7: Standard graph of DNS Test.

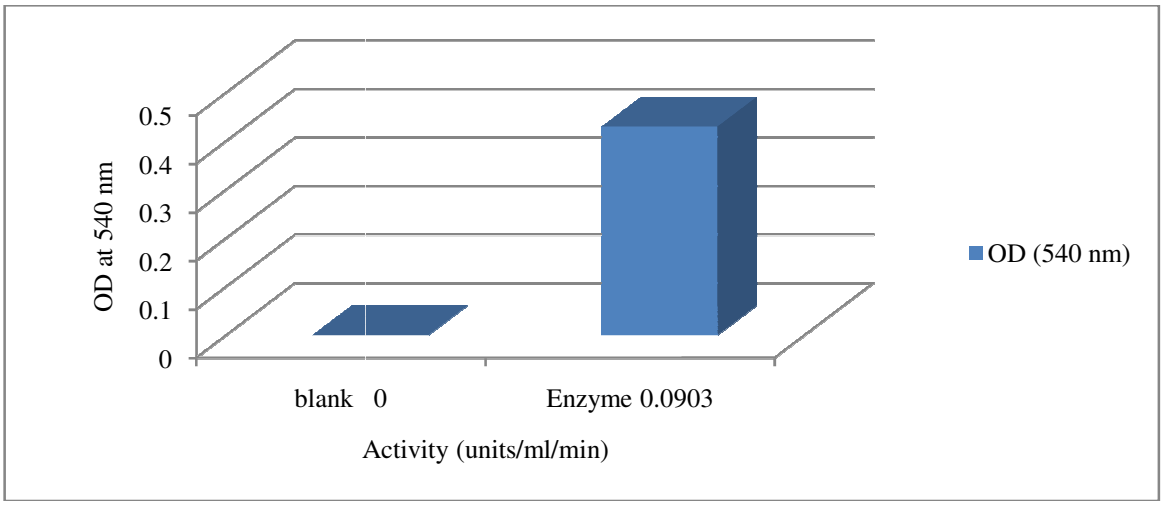

Figure-8: Activity of pure enzyme.

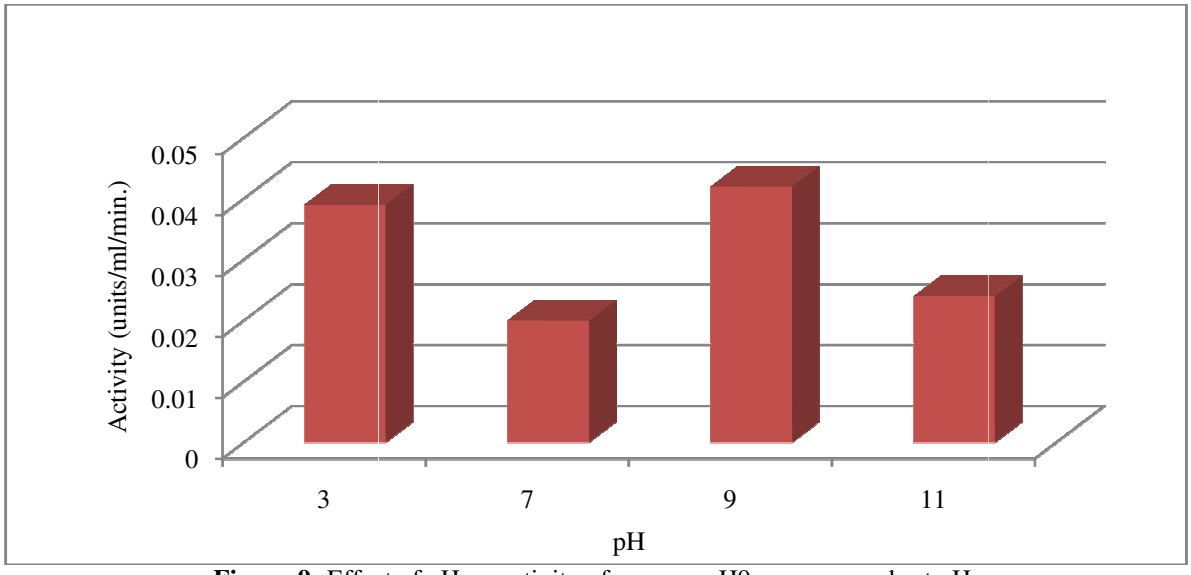

Figure-9: Effect of pH on activity of enzyme pH9 was seen as best pH.

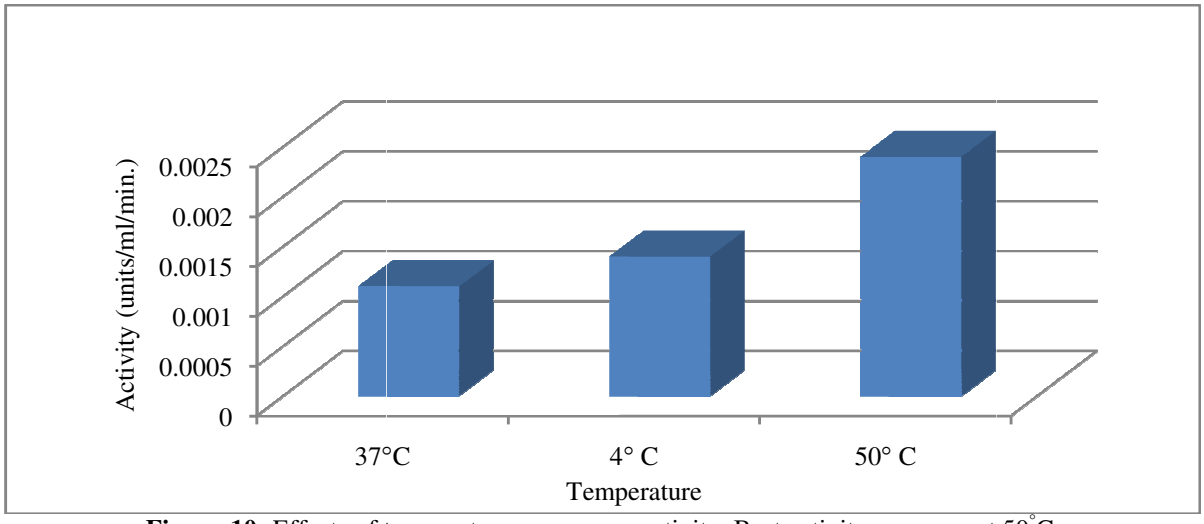

Figure-10: Effects of temperature on enzyme activity. Best activity was seen at 50°C.

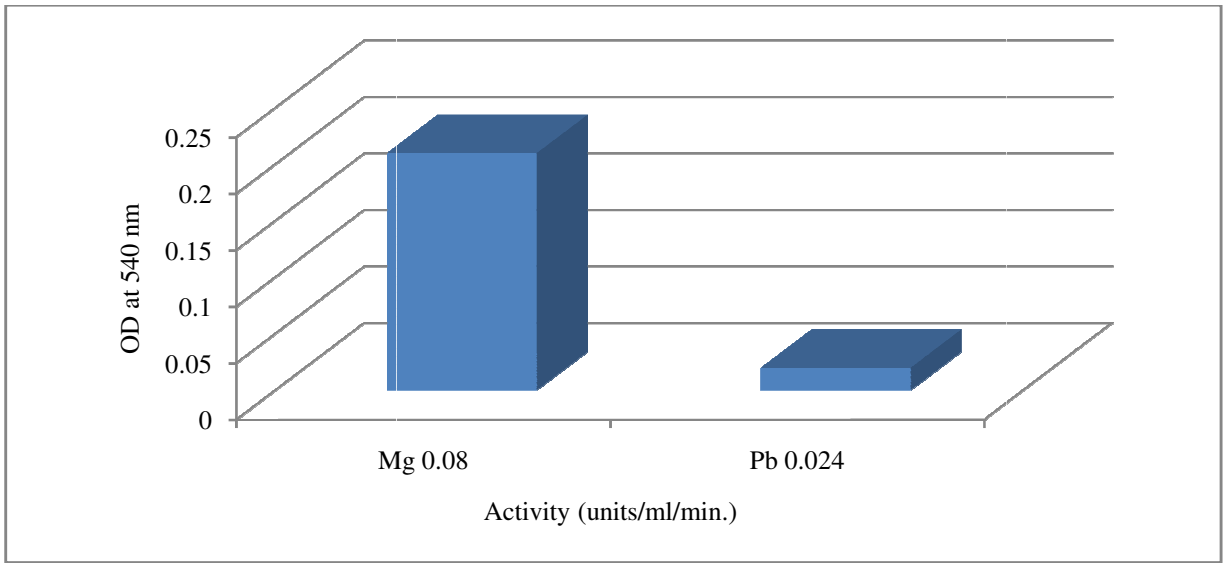

Figure-11: Effects of metal ions as activators on enzyme activity. Best activity was shown by $MgSO_4$.

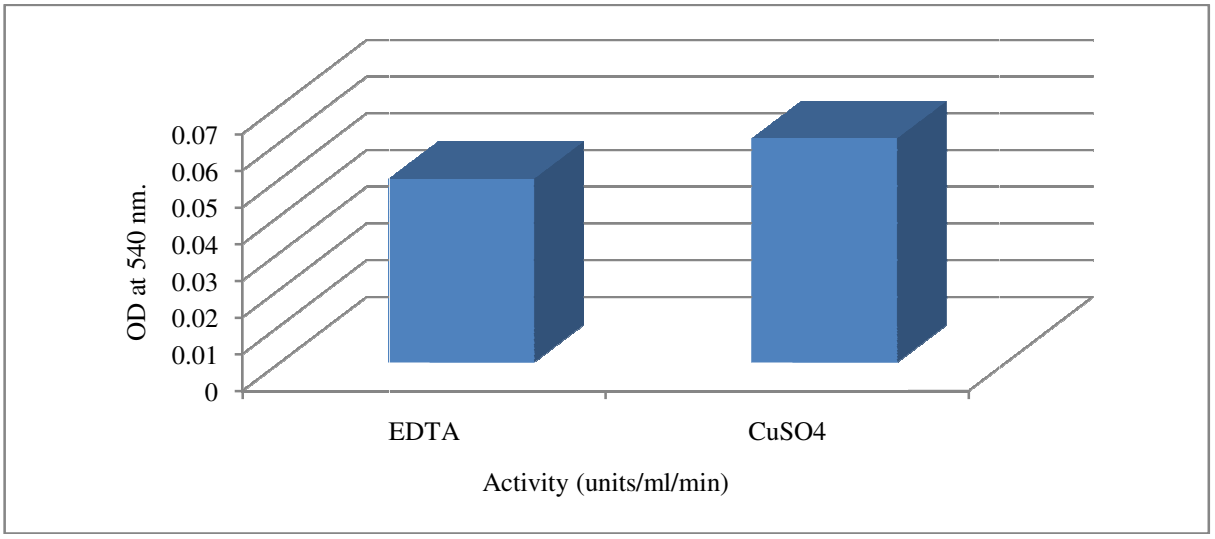

Figure-12: Effects of inhibitors on enzyme activity. Maximum inhibition was shown by $CuSO_4$.

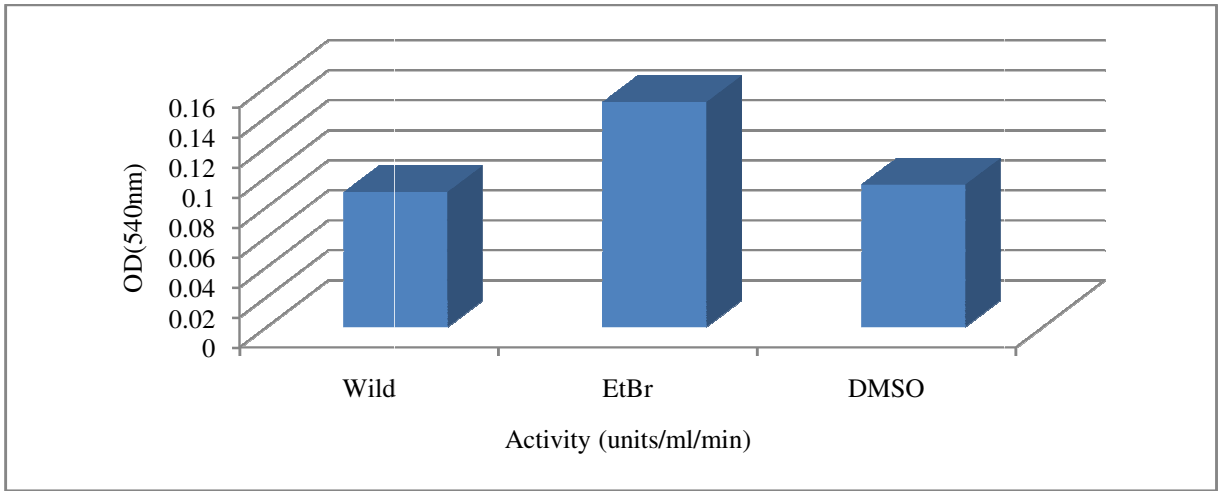

Figure-13: comparison between isolated enzymes from wild and mutated strain.

Discussion: Existing study was accomplished for isolation of potential cellulase discharging bacterial strain from different environmental waste which was rich in cellulosic biomass.

One isolates SPCP1910 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 SPCP1910was further screened for potential cellulose activity by DNS assay.

The strain SPCP1910 was then mutated with Ethidium Bromide and DMSO to increase the cellulose producing potential of the strain. It was found that strain improvement by EtBr enhances the production of cellulase while the treatment with DMSO enhances slightly. SPCP1910 culture was characterized from their structure, cultural and biochemical analysis and scrutinized as *Bacillus subtilis.*

Growth parameters of the isolate SPCP1910 was also studied at different temperature (optimum temperature 37°C), pH (optimum pH9) and growth kinetics (stationary phase was seen from 3rd day).

Then media optimization of different physiochemical factor like pH, different metal ions, substrate concentration was checked for maximum cellulose production. It was identified that the enzyme activity by bacteria SPCP1910 (EtBr mutated) was enhanced when production media was optimized.

Purification of cellulase was done and the enzyme activity was determined. The optimum parameters required for the stability and better activity of enzyme were also studied. The activity of the enzyme was found to be stable at wide range of temperature, from 35°C to 50°C, and pH range of 9.

Conclusion

Throughout the work, the strain SPCP1910 found to be best for the production of cellulase, after the mutation with ethidium bromide to strain, the rate of production of cellulase enhances. SPCP1910 culture was scrutinized as *Bacillus subtilis* on the basis of characterization from their structure, cultural and biochemical analysis. The enhancement and stability of activity of enzyme was also maintained by optimizing the production media and by checking the effects of pH, temperature, activators and inhibitors.

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