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Optimization and strain improvement by mutation for enhanced cellulase enzyme production by *Bacillus sp* APCMST10 isolated from coir retting effluent

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Abstract
The goal was to investigate the cellulase enzyme production ability of bacterial strain isolated from coir retting effluent and identified as *Bacillus* sp. on the basis of 16 S rDNA sequence homology. The effects of different nutrient sources like carbon and nitrogen, phosphate, surfactants, metal ions and environmental factors such as temperature and pH on cellulase production by the predominant bacterial strain were examined. Bacillus sp APCMST10 registered maximum cellulose production when cultured in the media supplied with carboxy methyl cellulose (40.27±1.55U/ml), ammonium nitrate (34.81±0.26 U/ml), dipotassium hydrogen phosphate (44.26±0.40U/ml), tritonx100 (45.31±1.44U/ml), zinc sulphate (46.16±1.44U/ml) at 35ºC temperature (49.33±0.63U/ml) and pH6.0(49.33±1.95U/ml), respectively. The efficiency of the test organisms on production of cellulase enzymes after physical and chemical mutation was also analyzed. The mutant strains *Bacillus sp* APCMST10 which were registered higher cellulase production when compared with that of wild strains.

Keywords: *Bacillus* sp., cellulase enzyme, ammonium nitrate, mutation

Introduction

Cellulose, which is the most abundant renewable resource, is a polysaccharide composed of β-D-glucopyranosyl units joined by 1, 4-glycosidic bonds (Gardner and Blackwell, 1974; Kolpak and Blackwell, 1976) [10, 17]. Because cellulose can be utilized to produce ethanol, it is a promising alternative energy source for the production of fossil fuels. Cellulose is degraded by cellulases to reducing sugars and fermented by yeast or bacteria to ethanol (Duff and Murray, 1996). Cellulases are produced by various microorganisms including *Trichoderma* sp, *Chrysosporium* sp, *Fusarium* sp, *Sclerotium* sp, *Phanerochaete* sp, *Aspergillus* sp, *Schizophyllum* sp and *Bacillus* sp. (Selby and Maitland, 1967; Wood and Phillips, 1969; Toyama and Ogawa, 1975; Sternberg, 1976; Fan *et al.*, 1987; Duff and Murray, 1996) [9, 23, 24, 25, 27]. Three major types of cellulases, endoglucanases (EC 3.2.1.4), exo-cellobiohydrolases (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21), have been identified to date (Coughlan, 1985; Coughlan and Ljungdahl, 1988) [7, 8].

Materials and methods

1. Isolation, screening and identification of cellulose production bacteria

The coir retting effluent samples were collected from the retting ground situated in Rajakkamangalam coasts, kanyakumari district. It was brought to the laboratory for isolation of cellulolytic bacteria. In total 12 bacterial strains were isolated from retting effluent and among them only two strains performed maximum cellulase production which was confirmed through the larger zone formation. From the two strain one cellulase positive strain were further identified based on its morphological, physiological and biochemical tests and also by molecular characterization using 16S rRNA sequencing described by Holt *et al*. (1994) [14]. Based on the above results, the potential cellulase producing organisms was identified as *Bacillus species* APCMST.

2. Mutagenesis of cellulase enzyme producing strains

Mutation is a spontaneous event that occurs mainly due to the tautameric changes in nucleic acids. In nature spontaneous mutation occurs at a very low frequency (10^{-9} to 10^{-N}). In the present study, the bacterial strains were mutated by using Physical method (UV irradiation) and chemical method using Ethidium Bromide and Streptomycin.

3. Characterization of enzyme production in mutant strains

Various process parameters that influence cellulase production were characterized individually and independently of the others. For optimization, the CMC medium was inoculated and incubated at different temperature ., 25–55 °C under the standard assay conditions. The effect of pH on enzyme production was determined by varying the pH (ranging from 5.0–8.0) of the broth in different flasks. The enzyme activity at different pH values was measured using the appropriate buffers (citrate buffer) under standard assay conditions. The growth medium was supplemented with different carbon sources *viz*., CMC, sucrose, glucose, fructose, (at the level of 1%, w/v). Different nitrogen sources *viz*, yeast extract, ammonium chloride, ammonium sulfate and sodium nitrate (1%, w/v) were also used for enzyme production. The growth medium was supplemented with different phosphate sources *viz*., calcium phosphate, sodium hydrogen phosphate, dipotassium hydrogen phosphate and potassium dihydrogen phosphate. Cellulase enzyme production using different surfactant was also analyzed by changing the surfactant of the enzyme production medium. The surfactant used was: Tween40, Tween60, Tween80 and Tritonx100. Thereafter, optimization of different metal ions (5 mM) *viz*, magnesium chloride, zinc sulphate, manganesh sulphate and calcium chloride were used for enhanced enzyme production.

Result

1. Identification of the test strain

Based on the morphological, physiological and biochemical characteristic the cellulase positive strain was subjected to 16Sr RNA sequence for molecular identification and was identified as, *Bacillus* sp APCMST10. *Bacillus* sp APCMST10 was established by amplifying the 16Sr RNA region and the sequence of the strain was examined by BLAST analysis. The 16Sr RNA genome sequence of this strain showed 100% similar identity with that of *Bacillus sp.*TN3 and *Bacillus sp.*SI1: *Bacillus* sp APCMST10 (Fig-1).

2. Total viable count of bacterial isolates *Bacillus* sp APCMST10 on mutation with UV irradiation

The TVC of mutated and control plates are shown in Table 3.3.1. For *Bacillus* sp APCMST10, the bacterial count noticed was 123 ± 4.32 CFU/ml in control plate and in 5 mins, 10 mins, 15 mins and 20 mins of UV treated plates, the CFU was ranged from 8.0 ± 0.24 to 72.0 ± 2.72 CFU/ml, respectively.

3. Total viable count of bacterial isolates *Bacillus* sp APCMST10 on mutation with chemical mutagens

Chemical mutation of the test bacterial strains was carried out using selected mutagens such as streptomycin and ethidium bromide at various concentrations. After mutation the colonies were plated on Luria Bertani agar counted and from which the CFU was calculated using the above formula. The number of CFU calculated was tabulated in Table 3.3.2. The results showed that the mutagens decreased the growth rate of the bacterial strains to some extent. For *Bacillus species* APCMST10 the bacterial count recorded was 78 ± 2.51 CFU/ml in control plate and it was 69 ± 2.32, 34 ± 1.61, 12 ± 0.62 and 5 ± 0.18 CFU/ml respectively in 50µg,100µg, 150µg and 200µg ethidium bromide added plates. The same species when treated with streptomycin, the bacterial count noticed was 110 ± 2.18 CFU/ml in control and 84 ± 2.51, 65 ± 1.17, 43 ± 1.13 and 26 ± 0.29 CFU/ml respectively in 50µg, 100µg,150µg and 200µg streptomycin added plates.

4. Effect of pH on cellulase enzyme production (U/ml) by UV mutated *Bacillus* sp APCMST10

Similar to that of *Pseudomonas aeruginosa* APCMST 11, UV mutated Bacillus sp APCMST10 also showed higher cellulase production (Table 3.3.3). Here, the maximum enzyme production of 57.76 ± 2.24 U/ml was observed in 15 mins UV mutated *Bacillus* sp APCMST10 at pH 6 than the wild type strains which showed only 49.33 ± 1.75 U/ml enzyme at the same pH. On the other hand the enzyme production decreased in 20 min UV mutated *Bacillus* sp APCMST10. All the UV mutated *Bacillus* sp APCMST10 showed more cellulase production than the wild type strain.

5. Effect of temperature on cellulase production (U/ml) by UV mutated *Bacillus* sp APCMST10

The effect of temperature on cellulase production by UV mutated *Bacillus* sp APCMST10 revealed that the mutation has enhanced the production of cellulase at almost all the tested temperatures (25ºC, 35ºC, 45ºC and 55ºC) when comparing it with the control (wild strain). Among the mutated strains, 15 minutes UV mutated *Bacillus* sp APCMST10 showed maximum production of 65.34 ± 1.06 U/ml at 35ºC. At the same temperature, the wild type showed only 49.33 ± 0.63 U/ml cellulase production. Further above and below this optimum temperature, the cellulase production was low but it was higher than control (Table 3.3.3).

6. Effect of various carbon source on cellulase enzyme production (U/ml) by UV mutated *Bacillus* sp APCMST10.

Table 3.3. 3 provide the data on cellulase production by the wild type and mutant strains of Bacillus sp APCMST10 grown on various carbon sources. In this experiment maximum enzyme production was observed in mutant strains than the wild type strains. In 15 minutes UV mutated strain grown in CMC medium the maximum enzyme production of 47.89 ± 1.27 U/ml was observed; whereas in control strain grown on the same medium registered only 40.27 ± 1.55 U/ml cellulase.

7. Effect of various nitrogen source on cellulase enzyme production (U/ml) by UV mutated *Bacillus* sp APCMST10.

The cellulase production by wild type and mutant strains of *Bacillus* sp APCMST10 in various nitrogen sources such as ammonium nitrate, ammonium chloride, ammonium sulphate and yeast extract were analyzed (Table 3.3. 3). Here, UV mutation exerted positive effect of cellulase production. Ammonium nitrate substituted medium showed a maximum production of 48.79 ± 0.98 U/ml cellulase by 15 mintues UV mutated *Bacillus* sp APCMST10, but the wild type produced only 34.81 ± 0.26 U/ml at the same medium. The cellulase production exhibited by UV mutated *Bacillus* sp APCMST10 in ammonium sulphate, ammonium chloride and yeast extract substituted medium was also more when compared with control.

8. Effect of various phosphate source on cellulase enzyme production (U/ml) by UV mutated *Bacillus* sp APCMST10

Table 3.3. 3 shows the data on the influence of phosphate sources on cellulase enzyme production by *Bacillus* sp APCMST10 cultured in various phosphate sources such as tricalcium phosphate, disodium hydrogen phosphate, dipotassium hydrogen phosphate and potassium dihydrogen phosphate. In all the tested phosphate sources higher cellulase production was achieved in mutant strains than the wild type strains. Accordingly in 15 minutes UV mutated *Bacillus* sp APCMST10, the maximum enzyme production of 47.72 ± 0.97 U/ml was observed when cultured in medium added with dipotassium hydrogen phosphate. In this experiment, the control strain (wild type) grown on the same medium produced only 44.26 ± 0.40 U/ml of cellulase. Further tricalcium phosphate, disodium hydrogen phosphate and potassium dihydrogen phosphate substituted medium were also favoured the cellulase production in mutant strains.

9. Effect of various surfactant on cellulase enzyme production (U/ml) by UV mutated *Bacillus* sp APCMST10.

The cellulase production by wild type and mutant strians of *Bacillus* sp APCMST10 cultured in medium with various surfactants such as tween 40, tween 60, tween 80 and tritonx100 were analyzed. Here, UV mutation excrted

positive effect of cellulase enzyme production. The maximum production of 48.19 ± 1.95U/ml cellulase was registered by 15 minutes UV mutated *Bacillus* sp APCMST10 when cultured in tritonx100 substituted medium. But in the same surfactant substituted medium the wild type (control) produced only 45.31 ± 1.44U/ml cellulase. The cellulase production exhibited by mutant strains of *Bacillus* sp APCMST10 cultured in tween40, tween 60 and tween 80 substituted medium was also more when compared with control (Table 3.3. 3).

10. Effect of various metal ions on cellulase enzyme production (U/ml) by UV mutated *Bacillus* sp APCMST10.

Table 3.3.3 provides the data on cellulase production by the wild type and mutant strains of *Bacillus* sp APCMST10 cultured in medium added with various metal ions such as calcium chloride, magnesium chloride, zinc sulphate and manganese sulphate. Here, maximum enzyme production was observed at mutant strains than the wild type strains and an enzyme production of 49.18 ± 1.91 U/ml was observed in 15 mintues UV mutated strains when grown on zinc sulphate. On the other hand, the control strain grown on the same medium exhibited only 46.16 ± 1.44 U/ml cellulase. Calcium chloride, magnesium chloride and manganese sulphate were also shown to influence cellulase production.

11. Effect of pH on cellulase enzyme production (U/ml) by chemically mutated *Bacillus* sp APCMST10.

Influence of pH on cellulase enzyme production by chemically mutated *Bacillus* sp APCMST10 was determined after treating them with selected chemical mutagens such as ethidium bromide and streptomycin. The mutated organisms were grown at various pH ranging from 5 to 8. The results indicated that the *Bacillus* sp APCMST10 mutated with 150 µg ethidiumbromide and streptomycin showed the maximum production of 58.67 ± 1.71 U/ml and 59.56 ± 1.86 U/ml cellulase at the pH6; whereas, at the same pH the wild type produced only 49.33 ± 1.95 U/ml cellulase. Further the maximum production was reserved in the pH range of 5 to 6 and thereafter the enzyme production started declining, also in all the tested pH higher production was registered by the mutant strains (Table 3.3.4).

12. Effect of temperature on cellulase enzyme production (U/ml) by chemically mutated *Bacillus* sp APCMST10.

Bacillus sp APCMST10 mutated with the two selected chemical mutagens such as streptomycin and ethidium bromide at various concentrations (50µg, 100µg, 150µg and 200µg) and also at varoius temperatures (25ºC to 55ºC with 10ºC intervals) was tested for cellulase enzyme production. The mutation inserted positive effect on cellulase production. In 150µg ethidium bromide, streptomycin mutated *Bacillus* sp APCMST10 at 35ºC, the cellulase production of 52.12 ± 1.69 U/ml and 53.23 ± 2.60 U/ml were registered respectively. The control strains at the same environment produced low level of 49.33 ± 0.63 U/ml cellulase. Here, more cellulase production was noticed in the temperature range of 25ºC to 35ºC and beyond this range the production declined. In all the tested temperatures more production was observed in mutant strains than that of registered in control strain (Table 3.3.4).

13. Effect of various carbon sources on cellulase enzyme production (U/ml) by chemically mutated *Bacillus* sp APCMST10.

The cellulase production under various carbon sources such as glucose, fructose, sucrose and CMC were analyzed with mutant and wild type strains of *Bacillus* sp APCMST10. Mutation with chemical agents such as ethidium bromide and streptomycin was found to positively influence the cellulase production. Among the tested carbon sources higher enzyme production (54.34 ± 2.24 U/ml and 50.01 ± 2.44 U/ml) was noticed in 150µg ethidium bromide and streptomycin mutated *Bacillus* sp APCMST10 strains on CMC substituted medium. Here, the wild type strain at the same medium produced only 40.27 ± 1.55 U/ml cellulase. Further the enzyme production in all the mutated strain was high when compared to wild type (Table 3.3.4).

14. Effect of various nitrogen sources on cellulase enzyme production (U/ml) by chemically mutated *Bacillus* sp APCMST10.

The cellulase production in various nitrogen sources such as ammonium nitrate, ammonium chloride, ammonium sulphate and yeast extract were analyzed with mutant and wild type strains of *Bacillus* sp APCMST10. Mutation with chemical agents such as ethidium bromide and streptomycin had excreted positive effect on cellulase production. Ammonium nitrate substituted medium showed the maximum cellulase production of 56.67 ± 1.45 U/ml and 49.56 ± 1.29 U/ml respectively by 150µg ethidium bromide, streptomycin mutated *Bacillus* sp APCMST10. At the same environment wild strain produced low level of 34.81±0.26U/ml cellulase (Table 3.3.4).

15. Effect of various phosphate sources on cellulase enzyme production (U/ml) by chemically mutated *Bacillus* sp APCMST10.

The cellulase production in medium added with various phosphate sources such as tricalcium phosphate, disodium hydrogen phosphate, dipotasssium hydrogen phosphate and potassium dihydrogen phosphate was analyzed in *Bacillus* sp APCMST10 mutated with 150µg ethidium bromide and streptomycin. Here, mutant strains grown in dipotasssium hydrogen phosphate substituted medium showed the maximum production of 48.35 ± 1.98 U/ml and 48.12 ± 1.89 U/ml cellulase respectively mutated in 150µg ethidium bromide and streptomycin. The wild type strain at the same medium produced only 44.26 ± 0.40U/ml cellulase. The other phosphate sources such as disodium hydrogen phosphate, tricalcium phosphate and potassium dihydrogen phosphate were also favoured the production of cellulase (Table 3.3. 4).

16. Effect of various surfactants on cellulase enzyme production (U/ml) by chemically mutated *Bacillus* sp APCMST10

The chemical mutated strains of *Bacillus* sp APCMST10 were analyzed for its cellulase enzyme production using various surfactants such as tween 40, tween 60, tween 80 and tritonx100 substituted in the cellulase production medium and the results obtained are shown in Table 39. Here, also mutated *Bacillus* sp APCMST10 showed higher production, when compared with wild type. Accordingly, the maximum enzyme production of 49.26 ± 1.93 U/ml and 48.29 ± 1.77 U/ml were noticed in 150µg ethidium bromide

and streptomycin mutated Bacillus sp APCMST10 on tritonx100 substituted medium. The wild type strain at the same surfactant produced only 45.31 ± 1.44 U/ml of cellulase (Table 3.3.4).

17. Effect of various metal ions on cellulase enzyme production (U/ml) by chemically mutated *Bacillus* sp APCMST10

The cellulase enzyme production by the wild type and mutant strains of *Bacillus* sp APCMST10 was assessed in medium substituted with various metal ions such as calcium chloride, magnesium chloride, zinc sulphate, and manganesh sulphate and the results are provided in Table 39. It inferred that, mutation with chemical agents such as ethidium bromide, streptomycin was found to have a positive effect on cellulase production. Here, zinc sulphate substituted medium showed maximum productions of 50.18 ± 1.76 U/ml and 49.97 ± 1.73 U/ml cellulase by 150µg ethidium bromide and streptomycin mutated *Bacillus* sp APCMST10. The wild type strain at the same medium produced only 46.16 ± 2.44 U/ml cellulase. The other metal ions such as magnesium chloride, calcium chloride and manganesh sulphate were also supported for the higher production of cellulase (Table 3.3.4).

Discussion

Many efforts were taken to generate microorganisms with high ability to produce cellulase that can degrade native cellulose reported by Aristidou and Penttila, (2000) [2]. Similarly, Gayal and Khandeparkar, (1998) [11] reported that, physicochemical factors influence the growth of the organisms and also the cellulase production. Efforts are going on throughout the world to enhance the production and purity of bacterial cellulases. Among the various factors that influence cellulase production during culture, the type of nutritional sources and inducers have a profound effect on production of cellulase. The present study was conducted to evaluate selected carbon sources, nitrogen sources, phosphate sources, surfactants and metal ions as substrates for cellulase production by *B.species* and *Pseudomonas aeruginosa*. The result revealed that the bacterial isolates utilized all the substrates supplemented with their source for their growth and cellulase production.

Table 1

Exposure to UV rays (Time in minutes)	Number of colonies (CFU/ml)
0	123 ±4.32
5	72 ± 2.72
10	45 ± 1.16
15	13 ± 0.86
20	8 ± 0.24

Table 2

Concentration of chemical mutagen (µg/ml)	Number of colonies (CFU/ml)	
	Etidium bromide	Streptomycin
0	78 ± 2.51	110 ± 2.18
50	69 ± 2.32	84 ± 2.51
100	34 ± 1.61	65 ± 1.17
150	12 ± 0.62	43 ± 1.13
200	5 ± 0.18	26 ± 0.29

Each value is the mean ± SD of three replicates

Table 3: Cellulase production (U/ml) by the physically mutated *Bacillus species APCMST*

Physically mutated *Bacillus species APCMST*	Cellulase production (U/ml) at $P^{H\,6}$	Cellulase production (U/ml) at temperature 35(C^{o})	Cellulase production (U/ml) by using carbon source CMC	Cellulase production (U/ml) by using nitrogen source NH4NO3	Cellulase production (U/ml) by using Phosphate source K_2HPo_4	Cellulase production (U/ml) by using surfactant Tritonx100	Cellulase production (U/ml) by using Metalions $ZnSo_4$
Wild strain	49.33 ± 1.95	49.33 ± 0.63	40.27 ± 1.55	34.81 ± 0.26	44.26 ± 0.40	45.31 ± 1.44	46.16 ± 1.44
	Physical mutation (PM)						
5 min UVmutant	50.89 ± 1.26	51.32 ± 0.76	41.43 ± 1.13	35.46 ± 0.73	45.78 ± 0.47	45.67 ± 1.55	46.98 ± 1.93
10 min UV mutant	55.43 ± 2.01	55.43 ± 0.81	43.23 ± 1.04	39.78 ± 0.79	46.84 ± 0.81	46.92 ± 1.79	47.28 ± 1.67
15 min UV mutant	57.76 ± 2.24	65.34 ± 1.06	47.89 ± 1.27	48.79 ± 0.98	47.72 ± 0.97	48.19 ± 1.95	49.18 ± 1.91
20 min UV mutant	52.34 ± 1.67	58.27 ± 0.98	43.23 ± 1.42	43.34 ± 1.17	42.45 ± 0.21	46.17 ± 1.63	46.28 ±1.69

Each value is the mean ± SD of three replicate

Table 4: Cellulase production (U/ml) by the chemically mutated *Bacillus species APCMST*

Chemically mutated *Bacillus species APCMST*	Cellulase production (U/ml) at $P^{H\,6}$	Cellulase production (U/ml) at temperature 35(C^{o})	Cellulase production (U/ml) by using carbon source CMC	Cellulase production (U/ml) by using nitrogen source NH4NO3	Cellulase production (U/ml) by using Phosphate source K_2HPo_4	Cellulase production (U/ml) by using surfactant Tritonx100	Cellulase production (U/ml) by using Metalions $ZnSo_4$
Wild strain	49.33 ± 1.95	49.33 ± 0.63	40.27 ± 1.55	34.81 ± 0.26	44.26 ± 0.40	45.31 ± 1.44	46.16 ± 1.44
Ethidium bromide (µg)							
50µg	50.46 ± 1.67	50.45 ± 1.34	41.34 ± 1.67	35.23 ± 0.39	45.69 ± 1.86	46.14 ± 1.79	46.97 ± 1.93

100µg	54.36 ± 1.73	51.65 ± 1.79	47.34 ± 1.20	45.98 ± 1.34	45.87 ± 1.67	47.29 ± 2.12	47.18 ± 1.24
150µg	58.67 ± 1.71	52.12 ± 1.69	54.34 ± 2.24	56.67 ± 1.45	48.35 ± 1.98	49.26 ±1.93	50.18 ± 1.76
200µg	53.12 ± 1.98	50.57 ± 1.95	48.87 ± 1.89	51.27 ± 1.84	45.12 ± 1.26	46.85 ± 1.95	47.69 ± 1.86
Streptomycin(µg)							
50µg	50.78 ± 2.93	50.88 ± 1.14	41.89 ± 1.56	34.92 ± 0.27	45.76 ± 1.12	45.97 ± 1.63	46.19 ± 1.42
100µg	52.43 ± 1.89	51.67 ± 1.46	43.86 ± 2.08	43.23 ± 1.27	46.36 ± 1.26	47.29 ± 2.69	46.79 ± 1.72
150µg	59.56 ± 1.86	53.23 ± 2.60	50.01 ± 2.44	49.56 ± 1.29	48.12 ± 1.89	48.29 ± 1.77	49.97 ± 1.73
200µg	50.68 ± 2.01	48.79 ± 1.55	45.65 ± 2.08	45.67 ± 1.30	45.12 ± 1.93	47.12 ±1.04	45.18 ± 1.63

Each value is the mean ± SD of three replicates

Further mutagenesis of bacterial strains is one of the most frequently employed methods of strain improvement (Chou *et al.*, 1999 a,b; Liu *et al*. 2000) [5, 6, 18]. In the present study, an attempt has been made to improve the cellulase enzyme production using physical agents such as UV irradiation and chemical agents such as streptomycin and ethidium bromide. Random mutagenesis was carried out through physical (UV, gamma etc) and chemical (EMS) agents which have been employed to obtain improved biological strains, including *Pantoea dispersa* (Gohel *et al.*, 2004) [12] and *Alcaligens xylosoxydans* (Vaidya *et al*., 2003) [26].

Conclusion

The bacteria as enzyme sources have many advantages that, the enzymes produced are normally extracellular, making easier for downstream process. The development of economically feasible technologies for cellulase production and for the enzymatic hydrolysis of cellulosic materials will enable to utilize the large quantities of biomass such as the residues of both food industries and agriculture. Thus the present investigation was selected to conduct an extensive study on cellulases from *Bacillus* sp. Present study aimed at isolation of promising cellulase producing *Bacillus* sp its identification, and optimization of cultural conditions for production of cellulolytic enzymes. Though we isolated the bacterial strain from coir retting effluent, the input of cellulase production by *Bacillus* sp. was attempted by the optimization and mutagenesis study. Present work suggests that retting effluent has an enhancing effect on cellulase production in this strain also and strengthening the hypothesis. Ethidium bromide and Streptomycin treated mutants showed higher cellulase production than wild type of Bacillus APCMST10 strain. The result concludes that chemical mutagens caused enhancement of cellulase production by mutation of regulatory genes or stability of mRNA of cellulase or by some other unknown mechanisms.

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