Isolation Of Bacterial Strains And Production of Enzymes For Biodegradation of Biomedical Cotton Waste

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Abstract

In recent times, biodegradation of used hospital cotton by using the bacterial strain is a promising and interesting field attracting worldwide researchers. It was observed that biodegradation of biomedical waste like cotton etc., is becoming a huge problem for both developed as well as developing countries. To overcome this problem, bacterial species were isolated using serial dilution and agar plating methods from different soil sources. Pure colony or single colony was isolated by the streak plate method. The bacterial species were identified with the help of various physical characteristics, staining, and biochemical activities. Strain improvement was done by UV and EtBr to know the best culture for amylase production. Purification of the crude amylase was done by dialysis, and its activity was calculated by DNS assay. Protein concentration was measured by Lowery's method. The purified enzyme was characterized for the effect of temperature, pH, activator, and inhibitors. The bacterial colonies were isolated and named as IS2015 01 to IS2015 16, the total enzyme activity of the purified enzyme was calculated to be 0.96 mg/ml, and the amount of protein in the purified sample was calculated to be 1.47 mg/ml. Amylase purified here was found stable in a pH range of 7 - 9 and temperature range of 28°C - 37°C. The activity was found to be enhanced under the influence of cations such as Ca^{2+}, Mg^{2+} and retarded under the influence of anions such as EDTA and SDS. The isolated bacterial strain and enzyme were found effective in degrading the biomedical cotton waste. In conclusion, the isolated bacterial strain and enzyme may be effectively used to degrade the biomedical cotton.

Keywords: Bacterial strain, Biomedical cotton, Biodegradation, enzyme

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1.Introduction

Medical waste management (MWM) has become a critical issue as it poses potential health risks and damage to the environment. It is also of greater importance due to potential environmental hazards and public health risks with a high propensity to result in epidemics [1]. It continues to be a major challenge, particularly in most healthcare facilities of developing countries where it is hampered by technological, economic, social difficulties and inadequate staff training for handling the waste [2]. Poor conduct and inappropriate management and disposal methods exercised during handling and disposal of medical waste (MW) are increasing significant health hazards and environmental pollution/hazards due to the waste's infectious nature and unpleasant smell. Even though, current medical waste management (MWM) practices vary from hospital to hospital, the problematic areas are similar for all healthcare units and at all stages of management [2].

In the process of healthcare delivery, medical waste is generated, which includes sharps, human tissues or body parts, and other infectious materials.Interestingly, there are reasonable ranges of technologies available for the treatment of healthcare wastes that may be appropriate for use in third-world countries [2]. The population of developing nations like India is increasing, and the amount of hospital waste generated is snowballing at alarming rates due to the growth of population and declining healthcare facilities [3]. However, there are some problems encountered with the management of MW, and they are improper storage, frequent dumping of infectious waste with municipal waste, no uniform definition, identification of hazardous waste, and low level of awareness about the management of medical waste. This has led to severe consequences of biohazards and excess of biomedical waste accumulation [2, 3].

Through this work, we have tried to resolve the issue of biomedicalwaste accumulation in clinical facilities of tertiary care hospitals in Meerut and Meerut. The preferable waste to be encountered here is cotton waste taken from general wards, pathology, and orthopedic wards of a general hospital in defined locations. Further, the work involves the study of pattern and analytical interpretation of utilizing the Acetogen bacteria to decompose the specified amount of cotton waste. Further, the work emphasizes the isolation of bacterial strains and synthesizing the enzyme responsible for the decomposition of biomedical cotton.

2. Material and methods

2.1 Sample Collection

In this study, four different samples were collected from different locations. Out of four, three samples were collected from the Bijnor, i.e., A) Soil of Biotechnology Department Vira Engg. College Dump area, B) Soil of Main Ground , C)College Gate pick up point D) College Fountain in Campus.

.Soil samples were collected randomly from 8-12 cm beneath the site surface by using a clean spatula, and afterward, the sample collected was put in sterile and labelled polybags.

2.2 Isolation

All the samples collected were serially diluted in saline followed by spreading over the respective solidified agar plates. Serial dilution of the samples was done to get the minimum isolated pure colonies. 50µl of the inoculums from the serially diluted test tube was taken and spread over the nutrient agar plates. Finally, plates were incubated in an upside-down position at 37^0C for 24 hours in the incubator [4].

2.3 Purification of the obtained mixed culture

In order to obtain single colonies, quadrant streaking is employed. This allows the sequential isolation of the original microbial material over the entire surface of the plate. As the original sample is diluted by streaking it over successive quadrants, the number of organism decrease. And finally, by the third or fourth quadrant, only a few organisms of the sample remain in the loop; as a result, we get pure isolated colonies. Briefly, 16 different colonies were differentiated on the basis of their colony morphology and named IS 01 to IS 16 were purified by quadrant streaking on the respective solidified NA

plates with the help of a sterile inoculation loop. All plates were incubated at 37^0 C for 24 hrs at the incubator [4].

2.4 Screening of pure culture

Screening is the procedure to check whether the selected microbe hasthe potential to secrete the desired secondary metabolite or not. Amylase-producing bacteria are detected with screening media in which carbon source is limited and additional substrate is provided in the form of SAM in order to check whether the bacteria are able to degrade the substrate we provided. The isolated pure strain was screened for the production of the extracellular amylase. This is visualized by the presence of a zone of hydrolysis on the plate after the treatment with gram iodine solution [5].

Briefly, 200ml of the minimal agar media was prepared supplemented with 1%Starch poured into the Petri plates and allowed to solidify. The plates were centrally streaked on respective plates with their respective isolate IS 01 to IS 16. Plates were incubated at 37^0C in the incubator. After incubation, screening was done by flooding plates with gram iodine solution for 15 mins. And finally, the plates were observed for the core zone of hydrolysis. On the basis of screening, the isolate number IS 16 was selected as an isolate having a maximum core zone of hydrolysis [5].

2.5 Identification

2.5.1 Gram Staining

Gram staining was done by the previously described method with little modification, a thinheat-fixed smear of bacteria was prepared, and crystals violet was added and left for one minute. After 1-minute slide was washed, and gram's iodine was added and left for one minute. Again washing was done, followed by adding 95% ethyl alcohol for 25 seconds. The slide was washed and counter-stain with safranine and left for 1 minute. Finally, the slide was washed, air-dried, and observed under a microscope [6].

2.5.2 Endospore Staining

A thin heat-fixed smear of bacteria was prepared, and malachite green dye was added. Afterward, the slides were kept in the boiling water bath for 20 mins. After 20minutes, the slide was washed and left for 1 minute after adding safranine. Again the slide was washed, air-dried, and finally observed under microscopy [6].

2.5.3 MR-VP (Voges Proskauer) Test

Gram staining was done by the previously described method with little modification, a 20 ml of VP broth was prepared, distributed into three test tubes equally, and autoclaved. Two test tubes were inoculated with the culture, and the third one was kept blank and incubated at 37^0c for 48 hrs. After incubation, 10-12 drops of the VP 1 reagent were added and incubated for 10 hrs. Afterward, 2-3 drops of VP 2 reagent were added to the test tube and incubated for 30 minutes. Followed by a gentle shaking after removing the caps to expose the media to oxygen,a change in colure was observed [6].

2.6 Strain Improvements

2.6.1By UV

Six plates of each, NA was prepared, and 20 µl of the inoculums was spread on the respected plate. The prepared plates were labeled as control, 2min, 4min, 6min, 8min, 10min, and the UV treatment was given for the specific time. Then the plates were incubated at 37ºC for 24 hrs, and the best source was identified [7].

2.6.2ByEtBr

Briefly, prepare 20 ml of NB media and transfer 3 ml of NB media ina test tube. Test tubes are mention as control, 1µg/ml, 2µg/ml, 3µg/ml, 4µg/ml, 5µg /ml, respectively. Briefly prepare sterile NB media in test tubes, add EtBr in each test tube, and mix properly. Then add 20µl of inoculation in each test tube and incubate NB plates for 24hrs [7].

2.6.3DNS Assay

3,5-dinitro salicylic acid (DNS assay) is an aromatic compound that reacts with the reducing sugars and other reducing molecules to form 3-amino-5-nitro salicylic acid; as the result of the reduction of the sugar, the pH of the mixture changes, which is seen as the change in the color the compound formed after the reduction of the DNS absorbs light of

the wavelength 540nm strongly. The enzyme activity of the enzyme is with the help of the DNS using maltose as the standard [8].
Briefly, 11 test tubeswere taken, and maltose from 0.1 ml to 1.0 ml was added in all tubes except the blank tube. The final volume makes up to 1 ml with distilled water. 1 ml of DNS was added to each test tube. All the test tubes were boiled in the water bath for 15 minutes, andOD was taken at 540nm. The standard graph was prepared between glucose concentration and OD [8].
Enzyme activity=(mg/ml of maltose released×0.360)÷0.5/incubation time.

2.7 Physical optimization of IS16 strain

pH prominent effect on the growth and survival of the bacteria, each bacterium has a particular range of pH at which it can function to the full of its potential. Briefly, 4 sterilized test tubescontaining 7 ml of NB were selected. All 4 test tube has different pH, i.e., 5, 7,9,11. After inoculation, the tube was incubated at a shaker for 24 hrs. Once the incubation time was over, the OD was measured at 600nm [9].
Temperature is another most important physical factor that directly affects the microorganisms' growth. So the effect of temperature was analyzedusing the previously described methods by some modifications [9]. Briefly, four sterile NA Petri plates were prepared and streaked with culture IS 16. Then the plates were incubated at different temperatures 22ºC, 28ºC, 37ºC, 50ºC. After the incubation time was over, the plate was examined for the culture's growth pattern [9].

2.8 Growth kinetics of IS 16

The growth curve of bacteria was analyzed by the previously described method, briefly sterile NB media was prepared, and fresh inoculums were added to every flask. Afterward, the flasks were incubated, and OD was measured at 600nm every 24 hrs till the decline phase reached [10].

2.9 Production of Amylase and determination of its activity

Submerged fermentation is the cultivation of microorganisms in liquid nutrient broth. The industrial enzyme can be produced using this process. Fermentation is a process of degradation of glucose/sucrose into alcohol with the help of microbes by using enzymes. The fermentation process was carried out in a flask containing sterile media. The media was inoculated with 1ml of IS 16 culture in the sterile condition, and the flask was incubated in a shaking incubator at 150rpm at room temperature for 4 days [11].
Test tube taken and one test tube kept as blank with 1 ml distilled water and other as a test.Briefly, 0.5 ml of starch and enzyme were added to the test tube. The test tube was incubated at room temperature for 15 min followed by the addition of 1 ml DNS to each test tube. Afterward, the tubes were kept in the water bath for 15 min. And finally, 5 ml of distilled water was added to each test tube, and OD was noted at 540nm. Finally, the enzyme activity was determined by comparing the OD with the standard graph [11].

2.10 Extraction of crude enzyme

2.10.1 Salt Precipitation

The crude we extracted is present in the solution, forming the homogenous mixture with the help of the hydrogen bonds, making it difficult for us to extract our protein from the mixture. In salt precipitation, we use another compound with a greater affinity to the hydrogen bonds with the water other than our protein. Briefly, a beaker containing the crude enzyme was kept on the magnetic stirrer, and washed magnetic bead was dropped for proper mixing under cold conditions. Ammonium Sulphate 44.85gm was added by pinch after 5 mins. When salt gets dissolved completely, the mixture is kept overnight at 4ºC. The next day the buffer was changed, and after 1 and ½ hours, the dialysis bag was opened, and protein was collected in the beaker [12].

2.10.2 Effect of temperature on purified enzyme

To check the activity of the enzyme at a different temperature, the enzyme was incubated at different temperatures, and then a DNS assay was performed. Briefly, 0.5 ml of 1% starch and 0.5 ml of the enzyme were added to the test tubes, followed by 1 ml distilled to the blank tube. Then the test tube

was incubated at different temperatures, i.e., room temperature, 22º C, 28°C,37 º C, and 50 º C for 15 mins. After incubation, 1 ml of DNS was added to all test tubes, and OD was recorded at 540 nm. And finally, the OD was determined by comparing the standard graph with the enzyme activity at different temperatures [13].

2.10.3 Effect of pH onenzyme activity

0.5 ml of 1% starch of different pH and 0.5 ml of the enzyme were added to the test tubes, followed by 1 ml distilled to the blank. The test tube was incubated at different temperatures at room temperature, 37º C, for 15 mins. After incubation, 1 ml of DNS was added to all test tubes, and the tubes were kept on the water bath for 15 mins. Afterward, 5 ml of distilled water was added to the test tube, and OD was taken at 540 nm. And finally, the OD was determined by comparing the standard graph with the enzyme activity at the different pH [13].

2.10.4 Effect of activator

0.5 ml of 1% starch and 0.5 ml of the enzyme were added to the 3 test tubes, followed by adding 0.2 ml of activator ($MgCl_2$, $CaCl_2$) to the three test tubes. The test was incubated at room temperature. After incubation, 1 ml of DNS was added to the test tubes, and the tubes were kept on the water bath for 15 mins, and OD was taken at 540 nm. And finally, the OD was determined by comparing the standard graph with the enzyme activity at all the activators [14].

2.10.5 Effect of Inhibitor

0.5 ml of 1% starch and 0.5 ml of the enzyme were added to the 3 test tubes, followed by the addition of 0.2 ml of inhibitor (SDS, EDTA) added to the three test tubes, and tubes were incubated at room temperature. After incubation, 1 ml of DNS was added to all five test tubes, and the tubes were kept on the water bath for 15 mins, and OD was taken at 540 nm. And finally, the OD was determined by comparing the standard graph with the enzyme activity at all the inhibitors [14].

2.11 Statistical analysis

Experiments (plate) were performed in triplicate. This helped to obtain the optimum range for everyparameter analyzed.All the statistical analyses were done by using GraphPad Prism. The results were presented as mean ± SEM values.

3. Results

3.1 Pure colony of bacteria

The bacteria from the soil were isolated by serial dilution, and nutrient agar plating technique fixed culture was obtained after spreading, as shown in the figure below (Figure 1).

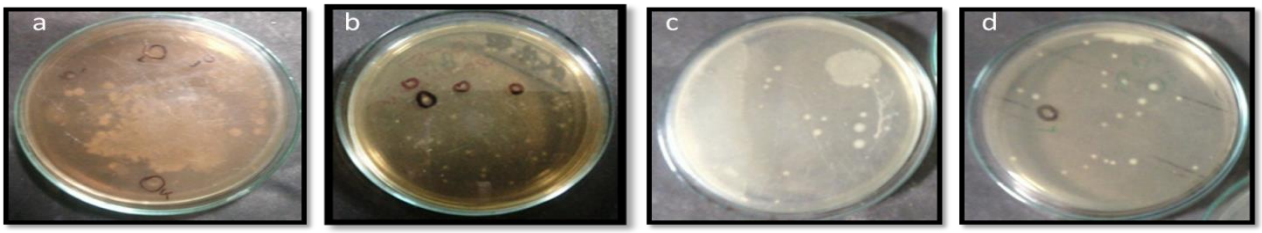


Figure 1: Plates showing the pure colony of bacteria isolated from soil from A) Soil of Biotechnology Department Vira Engg. College Dump area, B) Soil of Main Ground , C)College Gate pick up point D) College Fountain in Campus.

3.2 Colony morphology of bacteria

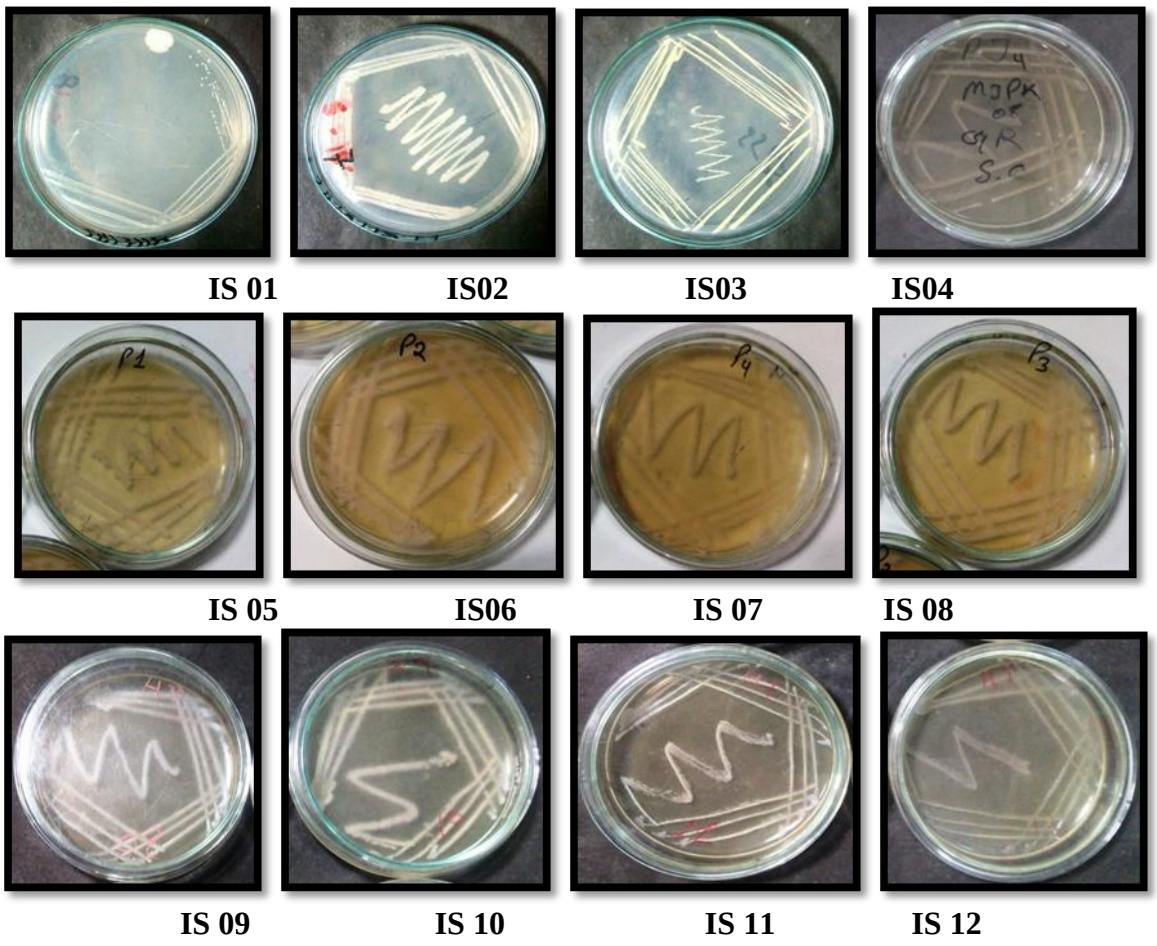
Colony morphology was differentiated on the basis of their morphology the physical characterizes of the colony such as colony shape color and were serially named as IS2015 01 to 16 (Table 1).

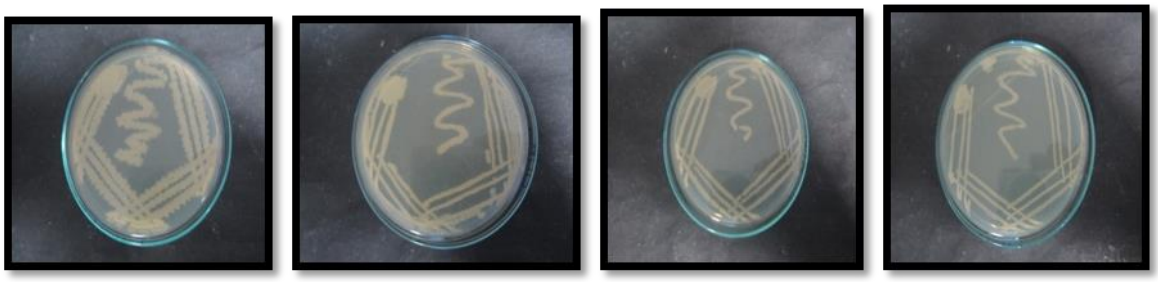
Table 1: A detailed description of the colony morphology of isolated bacteria

S.No.	Shape	Margin	Elevation	Pigmentation	Surface	Texture	Opacity
IS01	Circular	Discrete	Convex	White	Smooth	Soft	Opaque
IS 02	Circular	Entire	Convex	White	Smooth	Soft	Opaque
IS 03	Spindle	Entire	Flat	White	Smooth	Soft	Opaque
IS 04	Circular	Entire	Convex	White	Smooth	Soft	Transparent
IS 05	Spindle	Entire	Pulvonate	White	Smooth	Soft	Opaque
IS 06	Circular	Entire	Convex	White	Smooth	Soft	Opaque
IS 07	Circular	Discrete	Flat	White	Smooth	Soft	Opaque
IS 08	Spindle	Entire	Convex	White	Smooth	Soft	Opaque
IS 09	Circular	Entire	Flat	White	Smooth	Rough	Opaque
IS 10	Circular	Discrete	Convex	White	Smooth	Soft	Opaque
IS11	Circular	Entire	Flat	White	Smooth	Soft	Opaque
IS 12	Circular	Discrete	Convex	White	Smooth	Soft	Opaque
IS 13	Circular	Entire	Flat	White	Smooth	Rough	Opaque
IS 14	Spindle	Entire	Flat	White	Smooth	Soft	Transparent
IS 15	Spindle	Discrete	Convex	White	Smooth	Soft	Opaque
IS 16	Circular	Entire	Convex	White	Smooth	Soft	Opaque

3.3 Pure cultures of bacteria

A pure culture is obtained after quadrant streaking of the selected colony from the mixed culture plate, the figure of all pure cultures (Figure 2).





IS13 **IS14** **IS 15** **IS16**

Figure 2: Pure cultures of bacteria were isolated by streaking method

3.4 Screening of pure culture of bacteria

The pure cultures obtained were screened for the amylase activity. MAM (pH11) with 1% starch supplement was prepared and autoclaved, poured in sterile Petri plates, and allowed to solidify. The culture where centrally streaked in the solidified MAM plate. Plates were incubated at room temperature for 48 hrs to check the potential activity of the culture, which was subjected to secondary screening. Plate flooded with 1% iodine solution and zone of starch hydrolysis were examined. This table shows the remark of the culture after the secondary screening;the secondary screening result showed that the culture IS 16 shows the maximum zone of hydrolysis for the bacteria (Table 2 and Figure 3).

Table 2: Primary and secondary screening result of the isolated bacteria

SAMPLE. NO.	PRIMARY SCREENING	SECONDARY SCREENING
IS 01	+	-
IS02	-	-
IS 03	-	-
IS 04	++	+
IS 05	+	-
IS 06	-	-
IS 07	-	-
IS 08	+++	++
IS 09	++	-
IS 10	-	-
IS 11	++	-
IS 12	+++	++
IS 13	-	-
IS 14	-	-
IS 15	-	-
IS 16	+++	+++

(-) No hydrolysis, (+) Slight hydrolysis, (++) Moderate hydrolysis, (+++) Intense hydrolysis

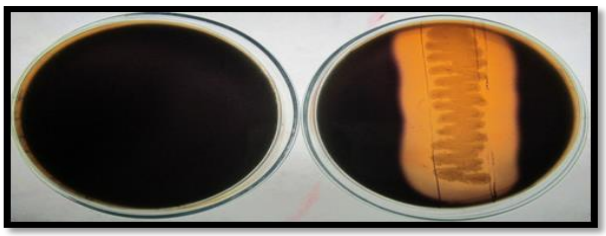


Figure 3: Plate of control and zone of Hydrolysis of IS 16

3.5 Identification

Theculture IS 16 showing maximum zone of hydrolysis was selected for further studies and was identified by performing and comparing various staining and biochemical test according to Bergey's manual. This table shows the list of all staining reactions and biochemical activity of the culture IS 16 (Table 3).

Table 3: Staining and biochemical activity of IS 16

S.No.	TEST	RESULT
1	Gram staining	+ve
2	Endospore staining	+ve
3	Catalase test	+ve
4	Mannitol test	+ve
5	VP test	-ve

Based on Bergey's manual of systematic bacteriology, the isolated strain was identified as*Bacillus megaterium.*

3.6 Strain improvement by UV on isolate IS 16

The isolate IS 16 was subjected to different UV treatment conditions to identify the most suitable condition for the growth of the isolated bacterial culture. The treatments are serially given as control, 2min, 4min, 6min, 8min, and 10min, respectively. This table shows the duration and isolated colony of the culture after UV treatment (Table 4 and Figure 4).

Table 4: Result of UV strain improvements

S.No.	Duration of UV	No. ofColony
1	Control	Lawn
2	2 min	46 colony
3	4 min	29 colony
4	6 min	9 colony and light lawn
5	8 min	Light lawn
6	10 min	Lawn

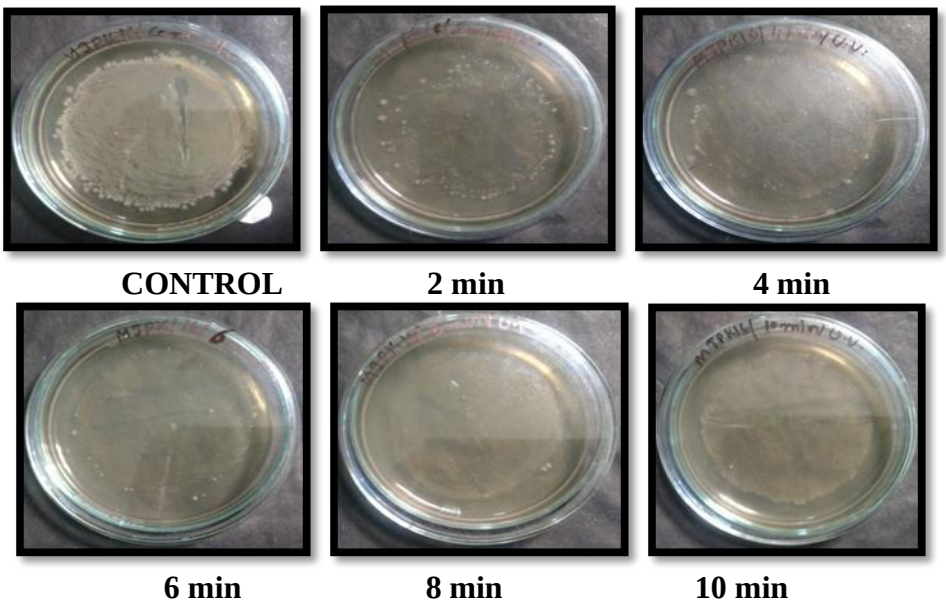


Figure 4: Plates showing the UV strain improvements result

3.7 Strain improvement by EtBr on isolate IS 16

The isolate IS 16 was subjected to different EtBr treatment conditions to identify the most suitable condition for the growth of the isolated bacterial culture. The treatments are serially given as control, 1ug/ml, 2ug/ml, 3ug/ml, 4ug/ml, and 5ug/ml. respectively. The table and figure shows the concentration of EtBr and isolated colony of the culture after EtBr treatment (Table 5 and Figure 5).

Table 5: Result of strain improvement by EtBr

S.No.	EtBr Concentration	Average Colony
1	Control	Lawn & colony
2	1 µg/ml	2 colony
3	2 µg/ml	8 colony
4	3 µg/ml	3 colony
5	4 µg/ml	3 colony
6	5 µg/ml	9 colony

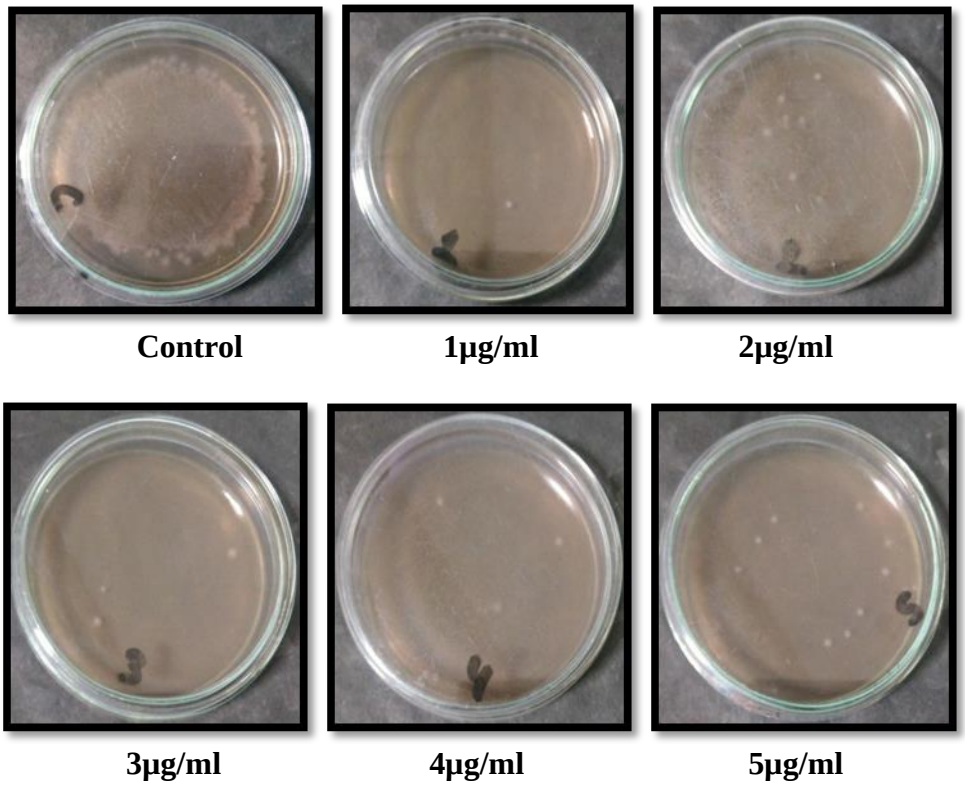


Figure 5: Plates showing theEtBr strain improvement results

3.8 DNS standard graphs

The standard graph was plotted to calculate the enzyme activity;with the help of the standard graph, we can calculate the amount of maltose released by comparing the OD. This table shows the different concentrations of starch (Table 6). With the help of these readings, we plotted the standard graph shown in the figure 6.

Table 6:DNS standard graph

S.No.	Maltose 0.5mg/ml	Distilled water	Conc. of maltose in mg/ml	DNS reagent (ml)	Distilled water
1	0.0	1.0	0.0	1	5
2	0.2	0.8	0.10	1	5
3	0.4	0.6	0.20	1	5
4	0.6	0.4	0.30	1	5
5	0.8	0.2	0.40	1	5
6	1.0	0.0	0.50	1	5

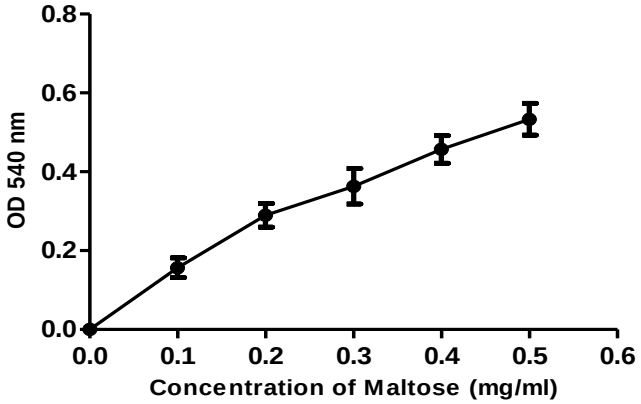


Figure 6: Standard graph plot between OD and concentration of maltose

3.9 DNS assay of UV strain improvement

Prepare the six test tubes of production media for the production of the best UV culture, and we find the best UV cultures for the production of Amylase are UV 8min. A detail description was mentioned in the table 7.

Table 7:DNS assay of UV strain improvement

S.No.	Duration of UV	OD (540nm)	Maltose released(mg/ml)	Enzyme activity(unit/ml/min)
1	Blank	0.0	0.0	0.0
2	Control	0.49±0.04	0.38	0.018
3	2 min	0.47±0.05	0.36	0.017
4	4 min	0.49±0.02	0.38	0.018
5	6 min	0.50±0.03	0.385	0.018
6	8 min	0.52±0.04	0.40	0.019
7	10 min	0.40±0.01	0.295	0.014

3.10 DNS assay of EtBrstrain improvement

Prepare the six test tubes of production media for the production of the best EtBr culture, and we find the best EtBr culture for the production of Amylase is 3µg/ml. A detail description was mentioned in the table 8.

Table 8:DNS assay of EtBrstrain improvement

S.No.	EtBr Concentration	OD 540nm	Maltose released(mg/ml)	Enzyme activity(unit/ml/min)
1	Blank	0.0	0.0	0.0
2	Control	0.55±0.06	0.425	0.024
3	1µg/ml	0.56±0.04	0.43	0.026
4	2µg/ml	0.56±0.03	0.43	0.026
5	3µg/ml	0.59±0.01	0.435	0.028
6	4µg/ml	0.56±0.02	0.43	0.026
7	5µg/ml	0.55±0.02	0.425	0.024

3.11 Optimization

3.11.1 Effect of different physiological condition on isolate IS 16

The isolated IS16 was further subjected to different physiological conditions to identify the most suitable condition for the growth of the isolated bacterial culture.

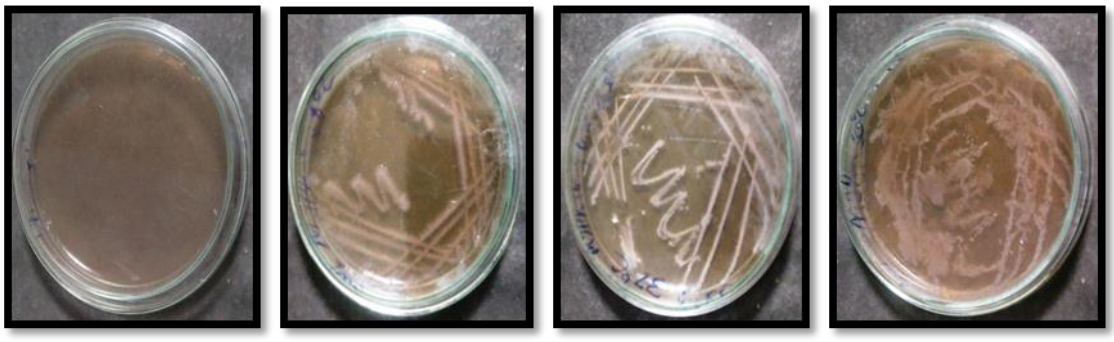
3.11.2 Effect of temperature on isolated IS16

The isolated IS16 was streaked on four sterile NAM plates were kept at four different temperatures, 22°C, 28°C, 37°C, 50°C. The table 9 shows the result of the different temperatures on isolated IS16 (3ug/ml).

Table 9: Effect of temperature on culture IS16

S.NO.	Temperature	Remarks
1	22°C	-
2	28°C	++
3	37°C	+++
4	50°C	+

(-) No growth, (+) Slight Growth, (++) Moderate Growth, (+++) Intense Growth



22ºC **28ºC** **37ºC** **50ºC**

Figure 7: Effect of temperature on culture IS16

NAM Plates in refrigerator, room temperature, incubator, hot air oven.

3.11.2 Effect of pH on isolated IS16

The isolate IS 16 was in four test tubes with different pH as 5, 7, 9, 11; after incubation, the OD was taken, which is given in the table. The result showed the culture had maximum growth at pH 7 (Table 10).

Table 10: Effect of pH on isolated IS16

S.No.	pH	OD at 600 nm
1	5	0.20±0.02
2	7	0.54±0.06
3	9	0.05±0.01
4	11	0.15±0.02

3.12 Growth Curve

The growth curve of the isolated bacterial strain IS 16was analysed by using spectrometry method. The result reveals that after reaching 1.13 OD on day 2 the bacterial growth starts declining as shown in the figure 8.

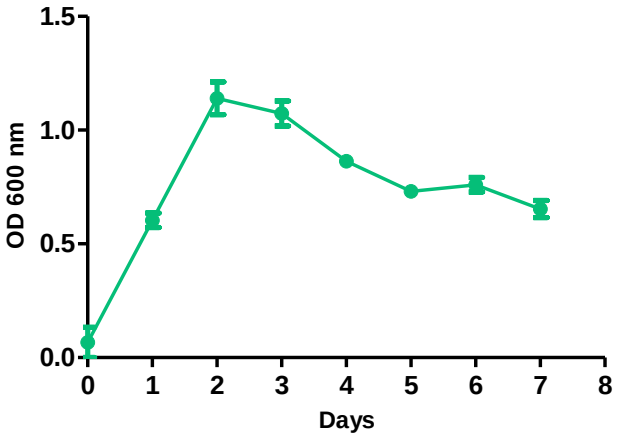


Figure 8: Growth curve analysis of isolated IS 16 bacterial strain

3.13 Lowry's standard graph

A standard graph was plotted to calculate the protein activity with the help of a standard graph. We can calculate the amount of protein released by comparing the OD, table 11 shows the OD at different concentrations of starch. With the help of these readings, we plotted the standard graph shown in the figure 9.

Table 11: Lowry's standard Graph

BSA (0.2mg/ml)	Distilled Water	Conc. of BSA (mg/ml)	Reagent C	Reagent D
0.0	1.0	0.0	5ml	0.5ml
0.1	0.9	0.5	5ml	0.5ml
0.2	0.8	1.0	5ml	0.5ml
0.3	0.7	0.15	5ml	0.5ml
0.4	0.6	0.20	5ml	0.5ml
0.5	0.5	0.25	5ml	0.5ml
0.6	0.4	0.30	5ml	0.5ml
0.7	0.3	0.35	5ml	0.5ml
0.8	0.2	0.40	5ml	0.5ml
0.9	0.1	0.45	5ml	0.5ml
1.0	0.0	0.50	5ml	0.5ml

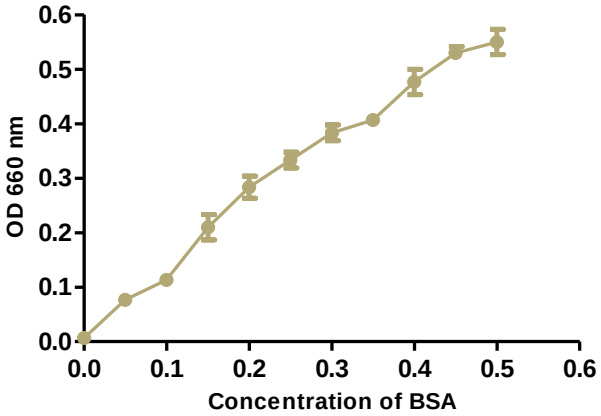


Figure 9: Lowry's standard Graph plot

3.14 Downstream processing

3.14.1 Extraction of crude amylase by centrifugation

After 3 days of incubation, the fermented media from both processes (solid-state and submerged) were centrifuged, and the supernatant was collected and subjected to DNS assay and Lowry's assay to find out the specific activity of the product crude enzymes (Table 12)

3.14.2 DNS and Lowry's assay ofcrude enzyme

Table 12: DNS &Lowry's assay of the Crude Enzyme

S.No.	Sample	OD at 540nm	Maltose released (mg/ml)	Enzyme activity (unit/ml/min)
1	Blank	0.0	0.0	0.0
2	DNS	1.62	1.24	0.059
3	LOWRY'S	1.82	1.39	0.066

3.14.3 Dialysis

Dialysis was performed to purify the enzyme from contamination, like traces of salt present in the crude enzyme. The dialysis bags are suspended in the Tris buffer in the picture below (Figure 10). The bags filled with white fluid in the salt precipitated enzyme obtained from submerged fermentation. The obtained data was shown in the table 13.

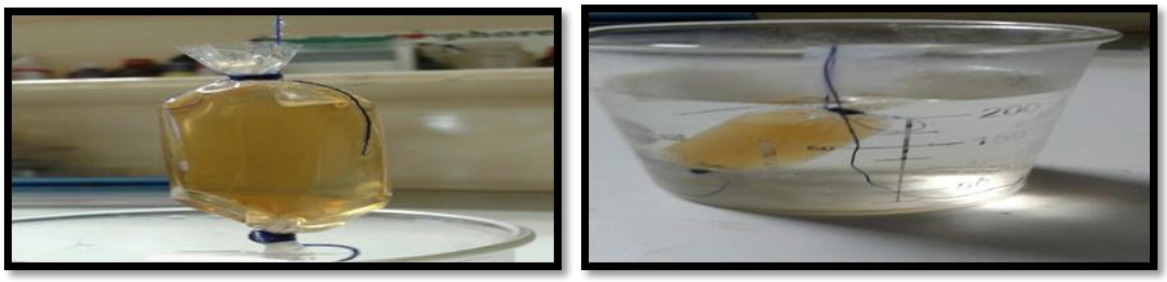


Figure 10: Image showing the purification of enzyme via dialysis bags

Table 13: DNS and Lowry's assay of the Purified Enzyme

S.No.	Sample	OD at 540nm	Maltose released (mg/ml)	Enzyme activity (unit/ml/min)
1	Blank	0.0	0.0	0.0
2	DNS	1.37±0.08	1.02	0.048
3	Lowery's	0.82±0.03	0.61	0.029

3.15 Effect of temperature on purified enzyme

To check the activity of the purified enzyme, it was incubated at different temperatures with the substrate. The results show that as the temperature increases (higher than 28ºC) the enzyme activity starts decreasing (Table 14).

Table 14: Effect of Temperature on the Purified Enzyme

S.No.	Temperature	OD (540nm)	Maltose released(mg/ml)	Enzyme activity(unit/ml/min)
1	Blank	0.0	0.0	0.0
2	22ºC	0.90±.07	0.70	0.033
3	28ºC	1.0±.05	0.775	0.037
4	37ºC	0.96±0.07	0.745	0.035
5	50ºC	0.90±0.03	0.70	0.033

3.16 Effectof pH on purified enzyme

DNS assay of the purified enzyme was carried out with different pH of tris containing 1% starch. The enzyme showed a consistent activity on a wide range of pH. It was also consistent at awide range of pH (Table 15).

Table 15: Effect of pH on the purified enzyme

S.No.	pH of starch	OD (540nm)	Maltose released(mg/ml)	Enzyme activity(unit/ml/min)
1	Blank	0.0	0.0	0.0
2	5	1.10±0.05	0.78	0.037
3	7	1.18±0.06	0.88	0.042
4	9	1.16±0.05	0.87	0.041
5	11	1.14±0.09	0.86	0.041

3.17 Effect of activator

Tocheck the activity of the purified enzyme, it was incubated at different activators with the substrate as shown in the table 16.

Table 16: Effect of activator on enzyme activity

S.No.	Activator	OD (540nm)	Maltose released(mg/ml)	Enzyme activity(unit/ml/min)
1	Blank	0.0	0.0	0.0
2	WA	1.03±0.05	0.77	0.036
3	MgCl2	1.17±.04	0.88	0.042
4	CaCl2	1.10±0.05	0.78	0.037

3.18 Effect of inhibitor

Tocheck activity of the purified enzyme, it was incubated at different inhibitor with the substrate as shown in the table 17.

Table 17: Effect of inhibitoron enzyme activity

S.No	Inhibitor	OD 540nm	Maltose released(mg/ml)	Enzyme activity(unit/ml/min)
1	Blank	0.0	0.0	0.0
2	WI	0.76±0.07	0.595	0.028
3	EDTA	0.76±0.07	0.60	0.028
4	SDS	0.68±0.08	0.53	0.025

3.19 Degradation of cotton waste

Finally the degrading activity of the isolated bacterial strain and enzyme was assessed and interestingly the obtained result reveals that both of them has biodegrading activity as shown in Figure 11 and 12).

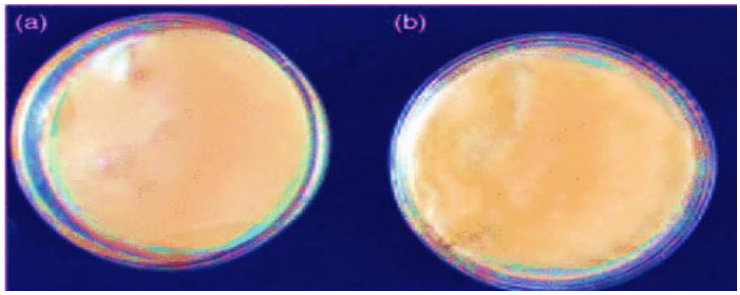


Figure 11: Degradation of cotton **a:** degradation of cotton waste through purified enzyme **b:** degradation of cotton through cellulose-producing bacteria.

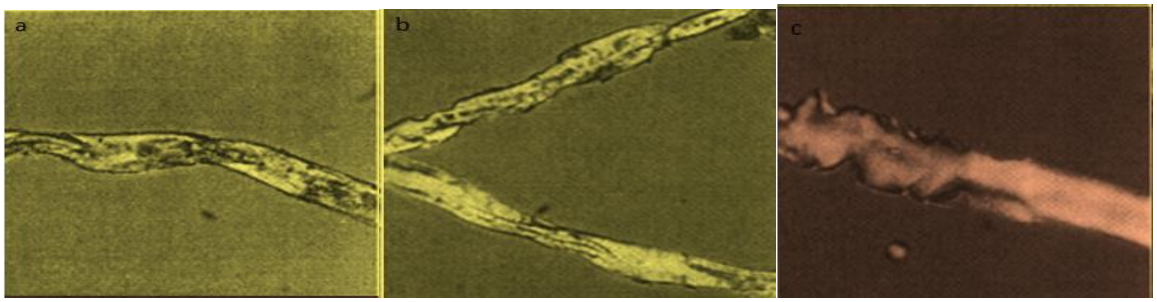


Figure 12: A: optical micrograph of cotton fiber, B: Optical micrograph of cotton fiber shredded through purified enzyme C: Optical micrograph of cotton fiber shredded with bacteria D: Optical micrograph of cotton fiber shredded with fungus.

4. Discussion

Due to improper handling and decomposing of hospital wastes like used cotton, which is generally very common worldwide, it might badly disturb the hospital atmosphere and public and pose a severe threat to community health [2]. Bacterial colonies were isolated from the soil by serial dilution and agar plating methods [4]. Isolates were further purified, which were named IS2015 01-IS2015 16 for bacteria. Screening of purified culture was done on MAM (Minimal agar medium) supplement with 1% starch. The culture growing in MAM was flooded with Iodine solution, and the zone of hydrolysis was obtained in the plate showing starch hydrolysis in order to screen the microorganisms for amylase production [5]. Strain improvement was done by UV and EtBr to know the best culture for amylase production [7]. The bacterial species were identified with the help of various physical characteristics, staining, and biochemical activities as done earlier [15]. Submerged fermentation for the production of amylase was done earlier [11]. Partial purification of the crude amylase was done by dialysis[16]. The reducing sugar was measured by adding DNS reagent, using maltose as standard, and the total enzyme activity of the purified enzyme was calculated to be 0.96 mg/ml. Protein concentration was measured by Lowery's method [17]. Using Bovine serum albumin as standard and the amount of protein in the purified sample was calculated to be 1.47 mg/ml [12, 18]. The purified enzyme was characterized for the effect of temperature,

pH, activator, and inhibitors; 37°C was found to be optimum temperature, pH 9 [13, 14].

5. Conclusion

This study was performed with the aim of isolating the bacterial strains and further investigating the biomedical cotton biodegradation potential of the isolated strains and producing the enzymes from them. And it was carried out by serial dilution, plated on nutrient agar, colonies obtained were characterized morphologically, biochemically and enzyme isolation was done by the fermentation process. Finally, based on the above study, it can be concluded that bacterial species can be a good source for the production of enzyme amylase being used in industries. Amylase purified here was found to be stable in a pH range of 7 to 9 and a temperature range of 28 to 37°C. The activity was found to be enhanced under the influence of cations such as Ca^{2+}, Mg^{2+} and retarded under the influence of anions such as EDTA and SDS. The isolated bacterial strain and enzyme were found effective in degrading the biomedical cotton waste. In the future, further purification of enzymes should be done in order to attain higher specific activity.

Conflict of interest: None

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