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ORIGINAL ARTICLE

ISOLATION AND CHARACTERIZATION OF BACTERIAL STRAINS FROM SOLID WASTES FOR THE PRODUCTION OF EXTRACELLULAR ENZYMES AND DEGRADATION OF ENVIRONMENTAL WASTES

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Abstract:

A large amount of agricultural, municipal, domestic and industrial solid wastes are generated in the field agriculture, municipality and industry. These wastes saturate in the environment and form again the problem of utilization; here rapid utilization of solid waste is important to cultivate economical agriculture crop plants. Some microorganisms have the ability to degrade solid wastes and produce economical useful of enzymes through the process of fermentation. In this paper 12 isolates of bacteria were isolated from different sampling of solid wastes of Solapur region, using Nutrient Agar medium. Among twelve isolates seven bacterial isolates from waste dump sites of Solapur Municipality and five isolates from waste dump sites of Solapur industrial region. The optimal microbial cultural conditions, biochemical characters, susceptibility of antibiotics and production of extracellular enzymes of the bacterial isolates were documented. Among twelve isolates four isolates were capable of producing protease and rest isolates were producing amylase.

KEYWORDS:

Solid wastes, Susceptibility of antibiotics, Biochemical characters, Extracellular enzymes.

I. INTRODUCTION

Solid wastes are generated from many ways in the environment, and causes the problem of pollution. Solid waste is the unwanted or useless solid materials generated from various activities of residential, industrial and commercial in the specific region. Solid wastes may be categorized into different types according to its origin or its source like agricultural, municipal, domestic, industrial and institutional, further its waste divided into its contents, like organic, inorganic, metal, plastic paper and glass or it may be according to its hazard potential like toxic, non-toxic etc. It's very difficult to manage through reduction or elimination of solid waste from the environment and causes the adverse effects on the environment and human health. Various processes are involved in the effective management of solid wastes for a municipality. In which biological degradation facilitates the reduction of 90 percent of solid biomass from the waste. Thousands of species of bacteria are residing in the waste due to presence complex nutrients substances complete the demands of all kinds of bacteria. Those bacteria are having the ability to degrade the specific complex substances means the degradation ability because of the enzymes induce by the substrate molecules. Here is the conversion or biotransformation of large complex unwanted wastes into the simple useful products due to bacteria and their metabolic enzymes. Converted products may be different according to the origin of compounds for the degradation for e.g. complex polysaccharides

converted into the products useful for the fermentation having enough amount of sugar concentration for the production of alcohol. From the above introduction our present study was aimed to isolate bacterial isolates from the solid waste may be municipal, agricultural, domestic, industrial and residential etc. characterization of the isolated species for the identification and further the study of the production of extracellular enzymes with the ultimate objective to apply the same isolates on the solid waste to facilitate the biodegradation process.

II. MATERIAL AND METHODS:

Present study sampling region

For the present study sampling was done from the waste disposal site of Solapur Municipality and Solapur Industrial region, Chincholi in Solapur, Maharashtra State, India.

Collection of samples

For the isolation of bacterial isolates, 10 waste samples were collected, 5 from disposal site of Solapur municipality, another 5 from Solapur Industrial region of Chincholi, Solapur. Sample consists of soil mixed with solid waste was collected in sterile zip-lock plastic bag in aseptic conditions, stored at 4^0 C and labelled them accordingly to their source and place where we have sampled. The collected samples were transported to the microbiology laboratory for the isolation of bacterial isolates and the water activity or moisture of samples and pH of samples were analyzed.

Physico-chemical Analysis of waste

The chemical properties of solid waste are composed of moisture content, volatile solid, ash content, Carbon, Hydrogen, Nitrogen, Sulfur, Potassium, Phosphorous in and heavy metals.

Collected samples were allowed to calculate Moisture content of waste- Initially samples were kept in filter paper and initial weight was recorded in grams. After initial weight samples were exposed in hot air oven at 115^0 C. After particular time samples were weighed until a constant weight of samples were achieved. Waste samples moisture content was calculated by using the following formula (3)

$$\text{Moisture Content (MC)} = \frac{W-w}{W} \times 100$$

Where,
-'W' is the initial amount weight
-'w' is the final or constant weight after heat exposure.
The temperature values were recorded of the solid wastes ranged from 26^0C to 30^0 C.

Chemical Analysis

pH of waste sample was determined by using digital pH meter, the mean pH values were ranged from pH 5.5 to 7.8.

Collected samples were analyzed for the chemical properties: Total organic matter in (%), Total Nitrogen (%), Phosphorous (%), Potassium (%), Sulfur (%) and heavy metals.

- Total organic carbon was determined by rapid titration method (4)
- Total nitrogen content was determined by Kjeldahl procedure.(1)
- Total phosphorous was determined by Spectrophotometric method
- Total Sulfur was determined by Spectrophotometric method
- Potassium was determined by Spectrophotometric method

Heavy metal contents are mostly composed of Arsenic (As), Cadmium (Cd), Cromium (Cr) Copper (Cu), Mercury (Hg) and Lead (P) by using inductive coupled plasma Spectroscopy (6).

Isolation of bacterial isolates from collected solid waste samples

For the isolation of bacterial isolates Serial dilutions technique was used in laboratory. In this

technique culture suspension was prepared by soil sample of solid wastes (Municipal and industrial regions both waste samples). Culture suspensions were prepared separately according to the type of wastes .1gm of soil sample of waste was added to the 10 ml of sterile saline (stock) and allowed for mixing on a shaker for 2-3 minutes. An aliquot 1ml was transferred into new dilution tube (containing 9 ml of sterile saline solution for dilution) labelled 10^{-1} dilution, and further diluted serially into next new dilution tubes (containing 9 ml of sterile saline solutions for dilutions) by transferring 1ml from previous dilution, prepared 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} dilutions. The dilution of 10^{-4} was used for inoculation in plating for bacterial isolates, because the dilutions of 10^{-2} and 10^{-3} produce more confluent or merging growth while 10^{-5}, 10^{-6} and 10^{-7} produce small number of colonies. Inoculation is done by streak plate method. After inoculation inoculated plates were incubated in inverted position at 37^0 C for 24- 48 hrs. After incubation plates were observed for the growth of bacterial colonies and counted average number of colonies and recorded total viable count of bacterial isolates. After successful growth of bacteria the pure culture of isolates were enriched and sub-cultured in NA slants.

Culture media was used for the isolation

Nutrient Agar medium (NA):
Composition for 1000 ml

Ingredients	Gms/litr
Peptic digest of animal tissue or Peptone	5
Sodium Chloride	5
Beef extract	3
Agar	15
Distilled water	1000ml
pH (at 25^0C)	6.8 ± 0.2

IDENTIFICATION OF BACTERIAL ISOLATES

For identification of bacterial strains gram staining procedure was performed to observe the bacterial morphology and gram nature of bacteria. Biochemical test were performed by standard biochemical procedure based on the Bergey's Manual. The biochemical analysis used for following tests: - Sugar Utilization by bacteria, IMViC test for differentiation of members of Enterobacteriaceae family, Amino acid decarboxylation, Oxidase and catalase production by bacteria, Nitrate reduction, Hydrogen sulfide production test, Coagulase production test, starch, casein and urea hydrolysis test were performed.

Preparation of pure culture and Maintenance of bacterial isolates

After complete study of biochemical characterization bacterial isolates were identified and re-streaked as a primary inoculant on the surface of a nutrient agar plate and incubated at 37^0 C for 24 hrs. After incubation pure cultures were checked by well isolated colonies on nutrient agar plates, further Gram Staining procedure was performed to re-check the bacterial cells morphology and gram nature of bacterial isolates were compared to original isolates. After studying the all biochemical characters cultures were re-streaked on fresh nutrient agar plates. Finally we received pure cultures of bacterial isolates were streaked on Nutrient agar slants. All the bacterial isolates were maintained at 4^0 C on Nutrient agar slants. All the bacterial isolates were sub-cultured at15 days intervals.

Effect of various growth conditions on growth of bacteria

Bacterial isolates were studied for the optimization of culture media, the pH values were adjusted to 4.5, 5, 5.5, 6.5, 7.2, 8.5, 9.5 and 11 in Nutrient agar medium. The temperature values of incubation period were optimized at 24, 26, 28, 30, 32, 34, 36, 38, 40 and 42^0C for 8 to 72 hours and recorded the results.

Effect of various antibiotics on growth of bacteria

Disc diffusion assay was performed to determined antibiotic sensitivity of bacteria. Use of

antibiotic discs to test the extent to which bacteria are affected by those antibiotics. For this assay sterilized special nutrient agar medium (for 1000 ml- Meat extract-10 gm, Peptone- 10gm, Sodium chloride- 5gm, Glucose-1gm, Agar-20 gm, Distilled water- 1000 ml, pH-7.5) was prepared for bacterial growth. Nutrient agar plates were inoculated with test bacterial strains by spread plate technique. Filter paper discs already impregnated with various antibiotics, for e.g. (Penicillin, Streptomycin, Ampicillin, Gentamycin and Tetracycline) each disc was carrying 100 µl of antibiotics. Antibiotic discs were placed on inoculated plate at centre of plate. Each bacterial isolate inoculated on separate nutrient agar plate with separate disc of antibiotic solution. All plates were incubated at 37+ 2 ^{0}C for 24 to 48 hours. Zone of inhibitions were observed around the disc and recorded the results.

Analysis of the heavy metal tolerance of the bacterial isolates with study of their Minimum Inhibitory Concentration (MIC)

Bacterial isolates were studied for the tolerance to heavy metals e.g. Arsenic (As), Zinc (Zn), Cadmium (Cd), Mercury (Hg) and Lead (Pb), with determination of Minimum Inhibitory Concentrations (MIC) by Tube dilution method (13). Various concentrations of heavy metals were prepared in tubes with appropriate volume of metal solution and 200 µl of standard culture

With a final volume 10 ml of Nutrient broth (7). Nutrient broth and metallic solution were sterilized separately for 15 min at 110^0 C. In this experiment three tubes were prepared for each metallic solution. A positive control tube was having lack of metal in medium inoculated with isolated bacteria and a Negative control was having metal supplemented medium without inoculation of bacteria. All tubes were incubated at 370 C for 3- 4 days. After incubation observed the tolerance to heavy metals and lowest concentration of metal completely inhibited growth of bacteria known as Minimal Inhibitory Concentration (MIC) and recorded the results.

Study for extracellular enzymes production and activity

Bacterial isolates were studied qualitatively for the production of extracellular enzymes such as Amylase, protease and lipase. For this study each bacterial isolate was streaked on sterile starch nutrient agar plate, sterile nutrient agar plate containing casein and sterile nutrient agar plate containing olive oil respectively. All plates were incubated at 37^{0}C for 24 hours. After incubation plates were flooded with indicator solution and observed the development of clear zone around the growth of bacteria. Clear zone around the growth of bacteria indicated positive test for the enzyme and studied enzyme activity.

Analysis of degradation of environmental wastes by bacterial isolates

Bacterial isolates were studied for their waste degradation potential. In this test 5ml of 24 hours old bacterial culture was mixed or inoculated with sterile garbage (solid waste). Along with this set up negative control (sterilized garbage without bacteria) was present. Mixture was allowed for 15 days of incubation at 37^{0}C. After 15 days we observed the waste degradation potential of bacterial isolates by weight reduction of garbage, and determined the difference between initial and final weight of garbage and recorded the results.

III. RESULTS AND DISCUSSION

Isolation of bacterial isolates from solid wastes, sampling was done from waste disposal site of Solapur Municipality and Solapur industrial region, Chincholi. Total 10 wastes samples were collected. While sampling soil sample depth in soil was from 50 to 100 centimeters used for isolation of bacterial isolates. Among the 10 samples 5 from disposal site of Solapur municipality and another 5 from waste site of industrial region, Chincholi. Soil samples were having moisture content calculated by routine method and formula used in this experiment and values of moisture content in percentage shown in table 3.1. Along with moisture content quality of soil and specific gravity of soil samples were checked of both the sampling sources. Permeability of soil samples measured to know the property of soil to transmit water or holding capabilities in terms of moisture contents of soil and pH values of soil of waste samples were measured by digital pH meter instrument. Both the sources of soil samples were used in measurement calculated the average range of pH value of both sources, and came to know the property of soil in terms Acidity or alkalinity. Collected soil sample were not much acidic and alkaline it's slight near to neutral value of pH. Temperature is another parameter of soil measured the values of temperature of soil samples of both the sources. Calculated the range of temperature of soil with the help of standard thermometer and recorded the

results. All the values of parameter of soil samples were recorded and shown in table 3.2.Collected samples were analyzed for the chemical properties. Calculated values of chemical content in soil samples by various methods of chemical analysis.

Table 3.1 Quality of soil with values of moisture content

Sr. No.	Source of soil sampling	Moisture content in (%)
1	Soil sample from solid waste site of Solapur Municipality. (SAM-1)	12.5
2	Soil sample from solid waste site of Solapur Municipality. (SAM-2)	11.8
3	Soil sample from solid waste site of Solapur Municipality. (SAM-3)	12.9
4	Soil sample from solid waste site of Solapur Municipality. (SAM-4)	11.4
5	Soil sample from solid waste site of Solapur Municipality. (SAM-5)	12.3
6	Soil sample from solid waste site of Solapur industrial region, Chincholi. (SAM-7)	14.1
7	Soil sample from solid waste site of Solapur industrial region, Chincholi. (SAM-7)	14.3
8	Soil sample from solid waste site of Solapur industrial region, Chincholi. (SAM-7)	14.6
9	Soil sample from solid waste site of Solapur industrial region, Chincholi. (SAM-7)	14.7
10	Soil sample from solid waste site of Solapur industrial region, Chincholi. (SAM-7)	13.9

*SAM = Soil Sample

Table 3.2 Quality of soil parameters

Sr. No.	Soil Parameters	Measurement values
1.	Average specific gravity for Soil Samples from waste site of Solapur Municipality, Solapur.	1.456
2.	Average specific gravity for Soil Samples from waste site of Solapur industrial region, Chincholi	2.344
3.	Average Permeability of Soil Samples from waste site of Solapur Municipality, Solapur.	0.42 Cm/S
4	Average Permeability of Soil Samples from waste site of Solapur industrial region, Chincholi	0.55 Cm/S
5.	pH range value of Soil Samples from waste site of Solapur Municipality, Solapur.	5.5 to 7.8.
6.	pH range value of Soil Samples from waste site of Solapur industrial region, Chincholi	5.7 to 7.6.
7.	Temperature range of Soil Samples from waste site of Solapur Municipality, Solapur.	27-30
8.	Temperature range of Soil Samples from waste site of Solapur industrial region, Chincholi	26-29

Cultural characteristics of isolated of bacterial isolates from soil samples of solid wastes

In our study MC1, MC2, MC3, MC4, MC5, MC6, MC7, IA1, IA2, IA3, IA4 and IA5 these bacterial strains were isolated on nutrient agar medium. Bacterial isolates were identified by visual colony morphology and microscopic observations. Gram staining was performed to check the gram nature of bacterial isolates. Details of colony morphological features and gram nature of bacterial strains are noted in table 3.3. Various biochemical tests were performed for 12 isolates and to know their biochemical nature of bacterial strains. Details of the biochemical characters of 12 bacterial strains were recorded and shown in table 3.4. The above results shown the morphology, colony characteristics and biochemical characteristics of the bacterial isolates help in identification and characterization of the isolates.

Based on biochemical and morphological characters bacterial isolated strains identified. With the help of Bergey's Manual of microorganisms that cleared the bacterial identities. Few species were belonging to the Bacillus Genus- *Bacillus licheniformis, Bacillus megaterium* and *Bacillus substilis. Another bacterial isolates* include *Arthrobacter spp., Escherichia coli, Klebsiella spp., Micrococcus spp., Proteus spp., staphylococcus aureus, Pseudomonas* spp. and *streptococcus* spp. had largest frequency in isolation.

Table 3.3: Colony Morphology and Gram nature

Sr. No.	Strain No.	Colour of Colony	Nature of Colony	Gram Nature	Shape
1.	MC1	Cream	Regular , opaque	Gram Positive	Short Rods (Bacilli)
2.	MC2	Cream	Regular, opaque	Gram Negative	Rods (Bacilli)
3.	MC3	Yellow	Irregular, opaque	Gram Negative	Rods (Bacilli)
4.	MC4	Cream	Regular, opaque	Gram Positive	Short Rods (Bacilli)
5.	MC5	White	Regular, transparent	Gram Negative	Short Rods (Bacilli)
6.	MC6	Cream	Irregular Opaque	Gram Positive	Rods (Bacilli)
7.	MC7	Cream	Regular, opaque	Gram Positive	Short Rods (Bacilli)
8.	IA1	White	Regular, transparent	Gram Negative	Short Rods (Bacilli)
9.	IA2	White	Regular, transparent	Gram Positive	Coccus
10.	IA3	Cream	Regular, opaque	Gram Negative	Rods (Bacilli)
11.	IA4	White	Regular, transparent	Gram Positive	Short Rods (Bacilli)
12.	IA5	White	Regular, transparent	Gram Positive	Rods (Bacilli)

Table 3.4: Biochemical Characterization of bacterial isolates

Tests	MC1	MC2	MC3	MC4	MC5	MC6	MC7	IA1	IA2	IA3	IA4	IA5
Indole production	+	+	-	-	-	+	-	-	-	+	+	+
Methyl Red	+	-	+	+	+	+	-	-	+	+	+	-
VP	-	+	-	-	-	-	+	+	+	-	-	-
Citrate Utilization	-	-	-	-	-	-	+	+	-	-	-	-
Amino Acid decarboxylation	+	+	-	-	-	+	+	-	+	-	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	-	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate Reduction	+	-	-	-	+	-	-	-	-	+	-	-
H_2S Production	-	-	-	-	+	-	-	+	-	-	+	+
Starch Utilization	+	-	-	+	-	-	+	+	+	+	+	+
Casein Hydrolysis	+	+	+	+	+	+	-	-	+	+	+	+
Urea Hydrolysis	+	+	-	+	-	-	+	-	+	-	+	+

(+ = Positive) (- =Negative)

EFFECTS OF GROWTH CONDITIONS ON GROWTH OF BACTERIA

Identified bacterial strains were maintained at 4^0 C for further study for the extracellular production of enzymes and for the study of degradation potential of isolated bacterial strains.
Also studied the effects of environmental parameters on growth of identified bacterial isolates with optimization of culture medium. In this study pH values of culture medium were adjusted to range from 4.5 to 11 and observed effect of pH on identified isolated *Bacillus* were strongly showing growth at near acidic environment, while other species of bacteria were showed maximum growth at 5.6 to 7.2 pH range. At pH 11 maximum bacteria were inhibited means our studied species of bacteria were not tolerating high alkaline pH. Temperature was another parameter we studied here, in this experiment we exposed the bacterial isolates at various temperature ranges from 24 to 42^0C and we observed maximum growth of isolates at the range of 29 to 36^0 C from these observations indicated the bacterial isolates were belong to the mesophilic group of bacteria, e.g. *Pseudomonas spp., Escherichia coli, Micrococcus spp., Proteus spp*. All bacterial strains showed their growth within 24 to 48 hrs period of incubation for growth we also studied.

EFFECT OF VARIOUS ANTIBIOTICS ON GROWTH OF BACTERIA

We studied the effect of various antibiotics on growth of bacteria. Antibiotic test helps to

determine the sensitivity of bacteria for antibiotic. In this experiment we have used disc diffusion assay, special nutrient agar medium was prepared for growth of bacteria. Nutrient agar plates were inoculated with test bacterial culture by spread plate technique and paper disc (already impregnated with various antibiotics) placed on nutrient agar plate at the centre of a plate, and incubated at 37+ 2^0 C for 24 to 48 hours. We observed the zone of inhibitions were around the antibiotic disc and recorded the results for sensitivity towards different antibiotics by various isolates of bacteria shown in table 3.5. Most of antibiotics were inhibited growth of bacterial isolates or strains only few strains were shown resistance against antibiotic compounds.

Table 3.5: Antibiotic sensitivity test

Antibiotic concentration (µg or units /ml)					
Strain No.	**Penicillin**	**Ampicillin**	**Gentamycin**	**Streptomycin**	**Tetracyclin**
MC1	+	+	+	+	+
MC2	+	+	+	+	+
MC3	-	+	+	+	+
MC4	+	-	+	+	+
MC5	+	+	-	+	-
MC6	-	+	+	+	+
MC7	+	+	+	+	+
IA1	-	+	-	+	+
IA2	+	-	+	+	-
IA3	-	+	+	-	+
IA4	-	-	+	+	+
IA5	+	-	+	+	+

(+ = growth inhibited, - = growth not inhibited)

HEAVY METAL TOLERANCE

Bacterial isolates were studied for the tolerance to heavy metals with determination of Minimum Inhibitory Concentrations (MIC) by Tube dilution method (13). Various concentrations of heavy metal solutions were prepared in tubes. With appropriate volume of heavy metal solution and 200 µl of standard culture in final volume 10 ml of Nutrient broth. All tubes were incubated at 37^0C for 3-4 days. After incubation we observed heavy metal tolerance and calculated MIC of Heavy metals with bacterial strains, and recorded the results shown in table 3.6. Total five heavy metals were present for the determination of heavy metal tolerance ability. Among the all heavy metals, maximum tolerance or resistance by bacterial strains was shown to As and Pb and minimum tolerance or resistance shown to Cd, Hg and Zn. In this experiment we observed the MIC of each heavy metal and calculated the lowest MIC or most toxic heavy metal compound is Cd, whereas the least toxic metal compounds are Pb, As, Zn and Hg.

Table 3.6: Heavy metal tolerance assay

Heavy metal inhibitory concentration µg/ml					
Strain No.	**As**	**Zn**	**Cd**	**Hg**	**Pb**
MC1	450	375	115	375	550
MC2	470	355	100	355	560
MC3	430	380	80	370	580
MC4	460	400	85	380	570
MC5	480	360	90	365	590
MC6	475	400	95	360	595
MC7	460	395	90	370	580
IA1	430	375	80	380	560
IA2	430	380	95	390	570
IA3	470	385	100	390	565
IA4	460	395	110	365	580
IA5	430	365	90	385	590

Enzyme Production

Bacterial strains were studied qualitatively for the production of extracellular enzymes. In this experiment total 12 isolates were subjected to qualitative analysis for the production of three different extracellular enzymes such as Protease, Amylase and lipase. Among the all bacterial isolates we observed the four bacterial strains were strongly producing protease and rest of the all bacterial strains were satisfactory produced amylase. None of any isolate could produce lipase. Data for the qualitative production of extracellular enzyme shown in table 3.7.

Table 3.7: Qualitative production of extracellular enzymes

Strain No.	Protease	Amylase	Lipase
MC1	+	+	-
MC2	-	+	-
MC3	+	-	-
MC4	+	+	-
MC5	+	+	-
MC6	-	+	-
MC7	-	+	-
IA1	Growth absent	-	Growth absent
IA2	-	+	-
IA3	-	+	-
IA4	Growth absent	-	Growth absent
IA5	-	+	-

(+ = production of Enzyme) (- =No production of enzyme)

Analysis of degradation of environmental wastes by bacterial isolates

Bacterial isolates were studied for their waste degradation potential. In this test 12 isolates were subjected to analysis waste degradation of waste by waste degradation efficiency test. We observed MC1, MC4 and MC5 were waste degrader while other isolates were not degraded wastes satisfactorily. We studied this assay by weight reduction of waste garbage shown in table 3.8. Garbage reduction it's all because of the degradation of waste garbage by the production of extracellular enzymes. As the weight loss of garbage were increased with progression of decomposition and production of extracellular enzymes were studied. Activity of amylase and protease studied for the quantification of enzymes shown in table 3.9.

Table 3.8.: Waste degradation potential by bacterial isolates.

100gm weight of waste garbage mixed with bacterial isolates				
Strain No.	Incubation after 3 days	Incubation after 7 days	Incubation after 10 days	Incubation after 14days
MC1	98.3gms	96.1gms	93.8gms	91.1gms
MC2	99.7gms	99.2gms	98.8gms	98.3gms
MC3	99.7gms	99.2gms	98.8gms	98.3gms
MC4	97.9gms	95.7gms	93.2gms	90.3gms
MC5	98.1gms	95.8gms	93.5gms	90.6gms
MC6	99.7gms	99.5gms	99.1gms	98.8gms
MC7	99.9gms	99.9gms	99.8gms	99.7gms
IA1	99.9gms	99.9gms	99.8gms	99.7gms
IA2	99.9gms	99.9gms	98.8gms	98.3gms
IA3	99.9gms	99.7gms	99.5gms	98.4gms
IA4	100gms	100gms	100gms	100gms
IA5	99.8gms	99.6gms	99.4gms	99.4gms

Table 3.9.: Activity of enzymes

Strain No.	Activity of Protease in IU/ml	Activity of Amylase in IU/ml
MC1	688.3	309.3
MC2	-	470.3
MC3	320.1	-
MC4	780.3	333.2
MC5	730.2	240.8
MC6	-	450
MC7	-	467.3
IA1	-	-
IA2	-	240.3
IA3	-	344.5
IA4	-	-
IA5	-	240.3

CONCLUSION:

From the above study we isolated 12 strains from the 10 waste samples, morphologically observed and characterized for the identification of isolates with the help of Bergey's manual. Among the all isolates few isolates were able to produce extracellular enzymes and having ability to degradation of wastes. These isolates were strongly helpful in the environmental waste degradation, which reduces the pollution by wastes of municipality and industrial regions.

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