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Screening of Microbes producing extracellular hydrolytic enzyme from corporation waste dumping site and house hold waste for the enhancement of bioremediation methods

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Abstract: *Search for microorganisms capable of biodegradation is one of the extensive areas of research. Screening and Identification of extra hydrolytic enzyme producing microbes from soil samples of landfill area and households of Trivandrum city was carried out. The ability of the bacterial isolates to produce various hydrolytic enzymes was determined using the plate assay. Result showed that the collected sample contains microbes capable for producing such enzymes and those strain can be further utilised for the enhanced bioremediation methods.*

Key words: *Bioremediation, microorganisms, extracellular hydrolytic enzymes, enzyme assay*

I. Introduction

The pollution of soil and water by organic and inorganic contaminants is a serious issue of the modern world. Due to their extensive use, they are found as environmental contaminants in numerous aquatic and terrestrial ecosystems. Management and removal of such pollutants are facing a crucial state, due to the unavailability of suitable strategies for the treatment and waste disposal. Unscientific disposal can cause an adverse impact on all components of the environment and human health. (Rathi., 2006; Gupta *et al*., 1998)

The use of bioremediation technologies for removing these contaminants provides a safe and economic alternative to commonly used physical-chemical treatment. Bacterial extracellular enzyme mediated activity is the major process involved in the hydrolysis of organic pollutants. Bacterial communities contain a broad range of genetic information to build up specific enzymes for the biodegradation.Extracellular hydrolytic enzymes thus produced can disrupt major chemical bonds in the toxic molecules and results in the reduction of their toxicity. (Sharma and Shah., 2005; Lal and Saxena., 1982)

A great deal of research has been devoted to finding the organisms, usually bacteria that are capable of altering or degrading pollutants to environmentally tolerable forms. Bacteria are particularly suitable for biodegradation application because of the wide variety of carbon sources or electron acceptors used by various strains. The exploitation of the metabolic versatility of microorganisms is advantageous in bioremediation but the actual number of degraders of a target compound may only 5-10% of the total microbial community (Chandrakant and Shwetha, 2011). To understand how microorganisms can be manipulated and exploited to reduce the frequency of such breakdowns and shorten start-up times of biological waste treatment, key bacterial strains actively involved in the degradation is important. Keep that in mind an attempt was made to screen the major microbes present in the corporation landfill site and house hold wastes having the ability of easy breakdown of major organic material mediated by extra cellular hydrolytic enzyme.

Objectives

1. Collection of soil samples
2. Isolation of Potential Microbes
3. Screening and Enzyme analysis

II. Methodology

Soil samples collected aseptically from a depth of 10cm from 10 different areas of corporation landfilling sites of Attakulangara Thiruvanathapuram city, were pooled. Two set of household vegetable compost samples collected from Thiruvananthapuram were also screened.

Isolation of Microbes

Landfill soil samples and vegetable compost were plated on nutrient agar plate up to 10^{-4} dilutions. Different bacterial colonies were selected and sub cultured and colony characteristics were studied. 17 types of colonies varying in colony morphology were selected and characterised as per Bergey's manual of Systematic

Bacteriology. Colonies were in inoculated in broth for microscopic analysis by staining methods and biochemical analysis. These organisms were further screened for the production of extracellular hydrolytic enzymes.

Screening for extracellular protease production

Screening of the best isolate with reference to their proteolytic activity in 10% milk agar were done. Nutrient agar containing 10% v/v milk boiled and filtered was sterilised, poured into sterile petridishes. These were inoculated with young inoculum and incubated for 24-48 hrs at 37° c. Zones of clearance indicating hydrolysis were measured. The experiments were repeated at various temperatures 30-40ºC

Screening for extracellular lipase production

Nutrient agar was supplemented with 0.01% $CaCl_2.H_2O$. Tween 80 sterilized for 20 minute at 120°C was added to give a final concentration of 1%. Medium was shaken until the Tween 80 had dissolved completely and then was streaked onto petridishes. Cultures were incubated at temperatures varying from 30-40ºC. For positive test an opaque halo occurred around colonies.

Screening for extracellular amylase production

Nutrient agar was supplemented with 1% starch and sterilised. After inoculating the test organisms and incubating for 48 hrs at various temperatures 30-40ºC, plates were flooded with iodine solution. Hydrolysis of starch was visualised as clear zones around the colonies against deep blue brown staining for starch.

III. Results and discussion

Isolation and characterisation of organisms

Heavy growth of organism was observed from colonies both the soil samples. Total number organism observed by pour plate method and spread plate methods are given in table1. After 24 hrs. of incubation at 37°C, were a total of 17 morphologically different colonies were selected (9 from sample 1) and (8 from sample 2). They were coded as A_c-H_C (compost isolates) and A_S-H_S (soil isolates). The results of colony characteristic study was given in table 2.Cultures were maintained on sterile nutrient agar slants at 4°C. Colony no 1-15 were gram positive rods and spore bearers and was observed through staining method. Further biochemical analysis confirmed that organism these organisms were belongs to bacillus group. Colony morphology and presence of gram positive filamentous rods confirmed the identity of organisms 16 and 17 as Actinomycetes.

Screening of isolates for proteases, lipase and amylase

The isolated organisms were identified and coded. A large number of hydrolytic enzymes from bacteria, and fungi, have been reported to be involved in the biodegradation of toxic organic wastes (Buzzini and Martini, 2002). The role of microbial extracellular enzymes for the biodegradation of pollutants was reported by Chadrakant and Shwetha (2001); Dang *et al* (2008) stated that extracellular enzymes produced by sediment bacteria play important roles in deposited and buried organic matter decomposition and nutrient recycling. To explore such potentials microbial isolates were screened for extracellular activities. Results of Proteolytic activity are given in table 4 and figure1.Their growth and hydrolysis zone were monitored at 24, 48 and 72 hours. Zones of hydrolysis varied from 2-35 mm in diameter. The highest zones of clearance were shown by H_C and I_S respectively. The isolates were screened for lipase production and lipase production was visualised as opaque halo around the colonies (Table5 and Figure 2).Results of amylase production was shown in table 5 and figure3. Starch utilisation was visualised as clear zones against dark blue brown staining for starch when flooded with iodine solution.

Soil sample	**Bacteria CFU/gm or ml of sample on NA**	
	Spread plate	Pour plate
1	$9.6x10^4$	$9.1x10^4$
2	$8.8x10^4$	$8.2x10^4$

Table1:Total plate count (TPC) of bacteria

Sl. No.	Colony code	Colony characters
1	A_C	Medium sized (1-2 mm) convex, opaque, cream coloured, circular and mucoid colony with smooth and entire margin.
2	B_C	Small pin tip sized, slightly convex, opaque, cream coloured and mucoid colonies with circular entire margin.
3	C_C	Large sized (3-4 mm), slightly convex, opaque, white coloured colonies without mucus or pigmentation.
4	D_C	Fungus like morphology
5	E_C	Medium sized, convex, opaque, yellow coloued circular mucoid colonies with smooth entire margin.
6	F_C	Medium sized, convex, opaque, circular non-mucoid white coured colonies with irregular margin.
7	G_C	White fungus like star shaped colonies.
8	H_C	Medium sized, slightly convex round mucoid colonies with red pigmentation and circular entire margin.
9	A_S	Small pin tip sized, convex, opaque, white round colonies with smooth entire margin.
10	B_S	Small, round opaque non-mucoid cream coloured colonies with irregular margin.
11	C_S	White, convex, non-mucoid, opaque colonies with irregular margin.
12	D_s	Small, round, cream, mucoid, opaque colonies with smooth margin.
13	E_S	Large, round, opaque, creamish white, non-mucoid colony with irregular margin.
14	F_S	Large, round, opaque and mucoid colonies with smooth and entire margin.
15	G_S	Small pin tip sized, opaque and mucoid colonies with round smooth and entire margin.
16	H_S	Fungus like appearance
17	I_S	Large round and fungus like colonies.

Table3: Colony Characteristics of the microbes isolated from landfill soil

Sl. No.	Isolate	Zone size 24 hrs	Zone size 48 hrs	Zone size 72 hrs
1	A_C	4	9	15
2	B_C	1	3	13
3	C_C	2	6	14
4	D_C	4	9	17
5	E_C	2	3	11
6	F_C	1	4	13
7	G_C	5	2	9
8	H_C	6	15	25
9	A_S	1	3	12
10	B_S	2	5	14
11	C_S	3	6	13
12	D_S	3	5	11
13	E_S	3	7	17
14	F_S	4	7	15
15	G_S	2	6	14
16	H_S	4	8	15
17	I_S	7	16	30

Table 4: Comparative analysis of protease hydrolysis zones at various time intervals.

Sl. No.	**Isolate code**	**lipase**	**amylase**
1	A_C	+ve	+ve
2	B_C	+ve	+ve
3	C_C	-ve	+ve
4	D_C	+ve	+ve
5	E_C	+ve	-ve
6	F_C	+ve	+ve
7	G_C	+ve	+ve
8	H_C	+ve	-ve
9	A_S	-ve	-ve
10	B_S	+ve	-ve

11	C_S	+ve	+ve
12	D_S	+ve	+ve
13	E_S	-ve	+ve
14	F_S	+ve	+ve
15	G_S	+ve	+ve
16	H_S	+ve	+ve
17	I_S	+ve	+ve

Table:5 Lipase and Amylase analysis of various microbes isolated.

Fig1:Trivandrum corporation landfill area a potential source of microbial resources

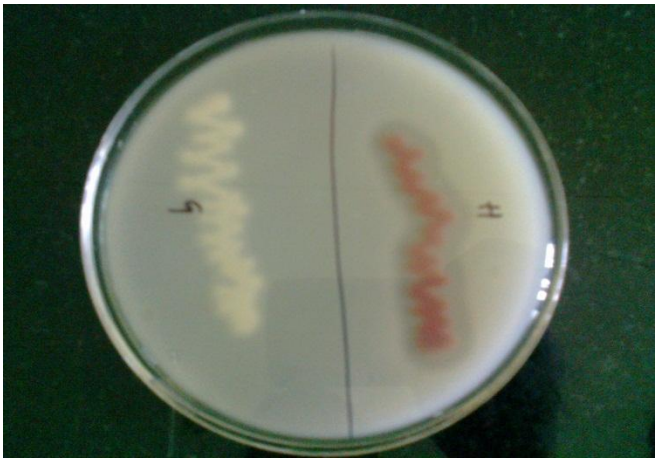

Fig 2: Screening of isolates for protease activity G (-) & H(+) for Protease Activity

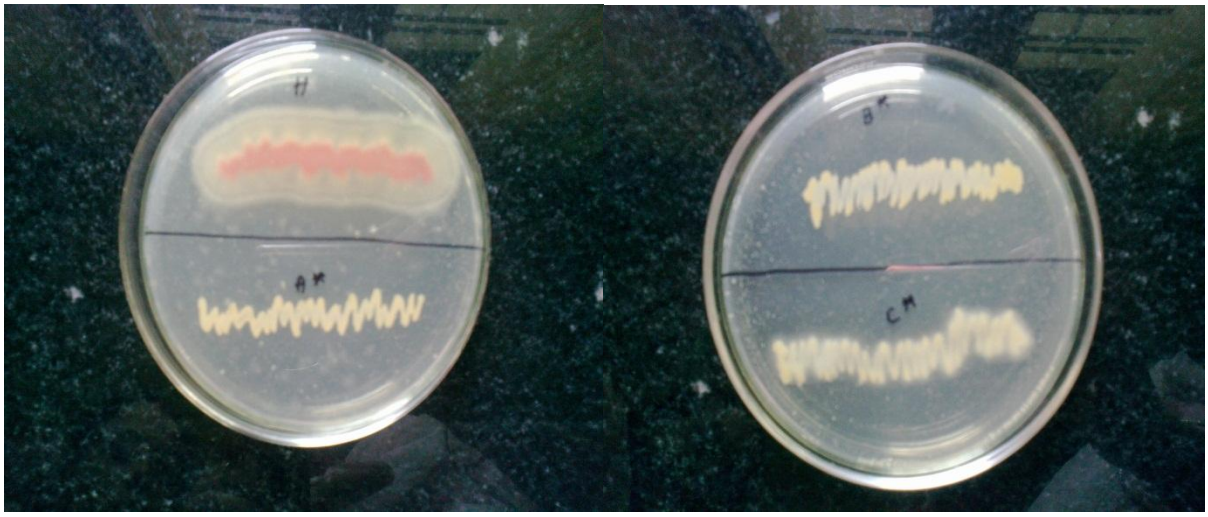

Fig 3 Screening of isolates for lipases activity plate 1 shows clear zone around the colonies(+) were plate2 show no clear zone(-)

Fig 4 Screening of isolates for amylase indicates Clear zone Plate1 (+) Plate 2 (-)around the organism

The study clearly recorded the occurrence of bacterial strain capable of producing industrially important extracellular enzymatic activities. Consortium of these microbes can be utilized for the evaluation of biodegrading ability in *exsitu*. Further refinement can also be made in the standardisation of condition for maximum enzyme production. In addition to that extracellular hydrolytic enzymes such as amylases, proteases, lipases, and xylanases have quite diverse potential usages in different areas such as food industry, feed additive, biomedical sciences, and chemical industries (S´anchez-Porro *et al.*, 2003). Scot *et al* (2010) reported the use of such enzymes as free-enzyme bioremidiant in herbicide biodegradation. All these organism produced enzymatic activities under 30-40 ºC hence can be used as candidate for commercial application. However, the efficiency for the industrial application should be much explored. Kelly and Fogarty (1983) reported that there is always constant search for better performers in these aspects.

IV. Conclusion

Since, waste disposal based pollution at landfilling areas are one of the hottest issues of Thiruvananthapuram city of Kerala the methodology can be further refined and implement for the large scale application of enhanced biodegradation of organic waste. The same strategies can be extended to the management of pollution due to organic waste in households and waste disposal sites of rural areas.

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