Microbial Consortium: A New Approach in Effective Degradation of Organic Kitchen Wastes

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***Abstract*— Present study was taken up to prepare efficient microbial consortia with concomitant enzymatic activity for the effective degradation of organic kitchen waste.**

Eleven different consortia were prepared and the compatibility of the bacterial strains within the consortia was checked by gram staining and enzyme production. Seven successful microbial consortia were selected in which all the bacterial strains concomitantly produced all these enzymes (amylase, protease, lipase, cellulose) in a specialized media that are responsible for the degradation of kitchen wastes. Three consortia (3, 5 and 12) were best producers of all the enzymes required for kitchen wastes degradation and it was monitored for 30 days by gradual decrease in the volume of the kitchen wastes. The maximum reduction observed was 65% in consortia no. 12 and 55% in consortia no 7 in just 21 days without any foul smell.

The degradation of organic wastes by the bacterial consortia is highly significant. It reduces the time span of degradation and produces no foul odor. Pretreatment of the kitchen wastes can also be for mineralization of garbage wastes and further as biomanure which is a novel approach.

***Index Terms*—Degradation, Kitchen wastes, Microbial consortia, Waste management.**

I. Introduction

An Indian city produces about 0.8 to 1 kg solid wastes per capita per day (waste management at military station, 2009). These wastes are collected and dumped into the landfills, causing major pollution [1-3]. This results in loss of potentially valuable materials that can be processed as fertilizer, fuel and fodder [4]. The bulk of organic kit comprising mainly carbohydrates, amino acids, peptides and proteins, volatile acids, fatty acids and their esters are easily biodegradable.

The biological treatment of these wastes appears to be most cost effective and carry a less negative environmental impact [5]. This process of biological treatment of wastes is also known as Composting. It is a self-heating, aerobic solid phase biodegradative process of organic materials under controlled conditions, which distinguishes it from natural rotting.

It has clearly been established that composts have the potential to protect the soil against erosion [6] , to enhance the soil water retention [7], to reduce soil compactibility, to decrease soil acidity [8], to enhance soil biochemical [9] and biological activity [10] and to establish a sound soil ecological equilibrium [11]. Additionally, composts can protect plants from soil [12] or seed borne pathogens [13]. Hence, compost can be considered as a much-needed soil conditioner [14] with generally positive crop yield effects [15].

The exploitation of the metabolic versatility of microorganisms is advantageous in biological waste treatment but the actual number of degraders of a target compound in a mixed culture may only represent 5-10% of the microbial community [16]. To understand how microorganisms may be manipulated and exploited to reduce the frequency of such breakdowns and shorten start-up times of biological waste treatment, the important bacterial strains actively involved in the degradation of food waste were isolated and screened.

Thus, the main aim of this study is to develop some successful bacterial consortium that can concomitantly degradation different components of the kitchen wastes with the help of their enzymes in less span of time under natural conditions without producing any foul odour.

Manuscript received on April1, 2011.

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II. Materials and Methods

A. Isolation of Bacteria

Soil samples were collected from different five different areas (Nursery, Agricultural firm, Petrol pump, Oil rich soil from temples and Areas dumped with kitchen wastes) in New Delhi and Noida, India. The different bacterial strains were isolated using standard serial dilution procedure. The isolated strains were further characterized on the basis of their substrate specificity and gram character. They are maintained on nutrient agar slants at 4°C and with 50% glycerol at - 20 °C for future use.

B. Determination of Metabolic Characteristics

The isolated strains were individually inoculated by single streaking on selective media such as Starch agar (2% starch), Skim milk agar, Czapek-mineral salt agar and nutrient agar plates with 1% tributyrin to isolate amylase, protease, cellulase and lipase producers respectively. The inoculated plates were incubated overnight at 37^{o}C and checked for a zone of clearing around each bacterial isolate. For starch agar, the zone of clearing was observed after flooding the plates with iodine. The strains showing the positive results

were subjected to gram staining and their morphology was determined by light microscopy under oil immersion.

C. Preparation of Bacterial Consortia

To prepare successful microbial consortium, bacterial cultures must be compatible with each other in order to concomitantly produce all these enzymes required for the degradation of kitchen wastes. 15 different consortia were prepared and incubated overnight at 37 ^{O}C in 120 rpm. The compatibility of the bacterial strains within the consortia was checked by gram staining. Microbial consortium was prepared by inoculating 5 over night grown bacterial strains in 20ml of nutrient.

D. Media Optimisation

Modified Czapek-mineral salt broth per litre supplemented with 0.5% starch, tributyrin and milk powder each was used. 1% of each successfully compatible consortium was inoculated separately in 250 ml of specialized media and incubated at 37°C in 120 rpm till 5 days. After every 24 hours, 5 ml of each consortium was taken out to check the production of amylase, protease, lipase and cellulase that are responsible for the degradation of kitchen wastes.

E. Different Enzymatic Assay of Consortia

For enzymatic assays the bacterial consortia were centrifuged at 10,000 rpm for 10 min. The supernatant was used for enzymatic assay. The experiments were carried out in duplicates and standard error was calculated.

Lipase assay: Lipase activity was assayed titrimetrically at pH 8.0 with a standard tributyrin as substrate. 1ml tributyrin was mixed with 3ml of Tris HCl (pH 8.0) to form emulsion. 1 ml of the enzyme was added to the emulsion. The mixture was incubated at 50°C for 30 min. The liberated fatty acids were titrated with 50mM NaOH. One unit of activity was defined as the amount of enzyme which liberated 1µM butyric acid per min under standard conditions.

Protease assay: The enzyme extract suitably diluted, was mixed with 50mM glycine - NaOH buffer (pH 9) to make 1 ml volume. 1ml of 1% casein (substrate) was added and incubated for 10 min at 60°C. The reaction was stopped by addition of 0.5 ml TCA (20%, w/v). The mixture was allowed to stand at room temperature for 30 min and filtered. 1 ml of the filtrate was mixed with 5 ml of 0.5M Na2CO3 solution. 0.5 ml of Folin & Ciocalteu's (phenol reagent) reagent was added and kept in dark to develop the blue color. It was estimated spectrophotometrically at 660nm against tyrosine as standard. One unit of protease activity was defined as the amount of enzyme required to liberate1 g tyrosine per milliliter in 1 min under the experimental conditions used.

Amylase assay (DNSA 3, 5 dinitro salicylic acid methods): One ml of 1% starch was incubated with different dilutions of the enzyme extract and 1ml of citrate-phosphate buffer (pH 6.0) and was incubated at 50°C for 30 min. The reaction was stopped by adding 2 ml of DNS and kept in boiling water bath for 10 min and absorbance was recorded at 540nm against glucose as the standard. One unit of enzyme activity is defined as the amount of enzyme, which releases 1µmole of reducing sugar as glucose per minute, under the assay conditions (U/ml/min).

F. Determination of Food Waste Degradation (Lab Trial)

The consortia capable of producing all these enzymes concomitantly were further selected for laboratory trials. Laboratory trials were carried out in 5 kg small heaps of kitchen wastes collected from Amity University's different canteen. Each heaps were inoculated with 5% of consortium by evenly mixing the inoculum with the wastes and kept under natural condition for 15 days to observe the visual rate of degradation. The heaps were periodically altered and water was sprinkled for proper aeration and moisture.

G. Lab Trials with 25kg of the kitchen waste

After the successful degradation of kitchen wastes in lab trials by the bacterial consortia large scale trials were also set up in closed container with their mouth partially open for adequate aeration and moisture. Each container was 2/3 filled with 25 kg of kitchen wastes and was inoculated with 5% consortia by evenly mixing the inoculam with the wastes. It was kept under natural condition for 25 - 30 days to observe the visual rate of degradation by gradual decrease in the volume of the waste pile. The heaps were periodically altered and water was sprinkled for proper aeration and moisture.

III. Results

A. Isolation and determination of the metabolic characteristics of bacteria

About 80 bacterial cultures were isolated from the above mentioned sites of which 35 cultures produced the desired enzymes required to degrade the kitchen wastes. Clear zone of hydrolysis along the line of streaking were produced by the bacterial strains that can degrade cellulose, pectin and lipid in CMC agar, Skim milk agar and tributyrin supplemented agar plates respectively. Amylase producers showed clear zone of hydrolysis along the line of streaking in starch agar plates when flooded with gram's iodine. Table 1 summarizes the results of all the metabolic characteristics

TABLE I: Physicochemical And Biochemical Characterization Of Strains.

SAMPLE	MORPHOLOGY	CMC	AMYLASE	PROTEASE	LIPASEE
A*	(-)	+	+	++	++
Ac2	(+) rods	++	-	++	+++
A8	(+) rods	+++	+++	+++	++
A25	(+) rods	+++	+++	+++	+
SAMPLE	MORPHOLOGY	CMC	AMYLASE	PROTEASE	LIPASEE
A29	(+) rods	+	+++	++	+
A35	(+) rods	+++	++	+++	++
Ac5	(+) cocci	+++	-	++	++
Ad4	(-)	++	+	+	+++
Ad10	(-) small rods	+	-	++	+
B 56	(+) rods	++	+	+++	+

B59	(+) rods	+	++	+++	+++
B69	(+) rods	++	+++	+++	+
Ba2	(-) small rods	+++	-	++	++
Ba2g	(-) big rods	+++	+	+	+++
B92	(+) rods	+++	++	++	++
B93	(+) rods	+++	+	+++	+
C107	(-)	++	+++	++	-
Bb1	(+	+	-	-	+
E151	(+) rods	++	++	+	-
E162	(+) rods	++	+++	++	+
Bb3	(-	++	-	+	++
Bb4	(+)cocci	+++	-	++	+++
Bb5	(-	++	+	+	+++
Bd2	(+)cocci	+	-	++	+++
C4	(-	+	-	+	++
E163	(+) rods	+	+++	+++	++
E164	(+) rods	++	++	+++	-
Bb6	(+	++	-	-	+
Bb7	(+)cocci	+	-	+	++
Bc1	(-	+	-	+	++
C7	(+)cocci	++	+	++	+++
D10	(+	++	+	+	+++
D11	(+	++	-	++	+++
DW	(-)	+	+	+	++

B. Preparation of bacterial consortium

These 35 different enzyme producing bacterial strains were combined with each other by permutation combination in order to make different microbial consortia. Table 2 shows that 11 different bacterial consortia were prepared of which 7 consortia showed the best compatibility when gram staining was performed. Fong and Tan [17] have also reported the use of bacterial consortia for degradation of food wastes.

TABLE II: DIFFERENT COMPOSITION OF THE BACTERIAL CONSORTIA

CONSORTIA	COMPOSITION
1	C7, B56, C4, E162, Bb6
2	C7, B56, A8, Ac5, DW
3	Bb5, B59, Ba2g, B92, Bb3
4	Bb3, E151, Ad11, C107,
5	D10, B93, Bb3, E151, Ad11
6	D10, B93, C107, Bb1, A8
8	E163, Bb7, E164, D11, B69
9	A25, Bc1, Bb7, E164, D11
10	A35, Bb4, A25, Bc1, A29
11	Ad11, B92 A35, Bb4, E162,
12	C107, Bb1, A8, Ac5, DW

C. Concomitant production of various enzymes by bacterial consortia in the production media

These 7 consortia were further inoculated in modified Czapek-mineral salt broth to check the concomitant production of all the four enzymes (amylase, protease, lipase and cellulase). The consortia utilized cellulase in a slow pace within 5 days of incubation. Rest all the components in the media were utilized by the consortia within 24-48 hours of incubation as can be noted from table 3.

TABLE III: CONCOMITANT PRODUCTION OF VARIOUS ENZYMES BY BACTERIAL CONSORTIA IN THE PRODUCTION MEDIA.

CONSOR -TIA	LIPASE	CELLULASE	AMYLASE	PROTEASE
1	+++	++ (5 days)	+++	++ (4 days)
8	+++	+++ (5 days)	+++	+++ (4 days)
3	+	++ (48 hours)	+++	+
10	+++	++ (48 hours)	-	+++
5	+++	+++ (,,)	++	+
12	+++	+++ (,,)	-	+++
7	++	++ (,,)	+++	+++

D. Enzymatic Assay

All the 7 consortia produced all the three enzymes. Figure 3 shows that Consortia no 10 was the best lipase producer followed by consortia no 5 and 12.While Consortia no 12 was the best protease (figure 2) and amylase (figure 1) producer followed by consortia no 7 and 3.

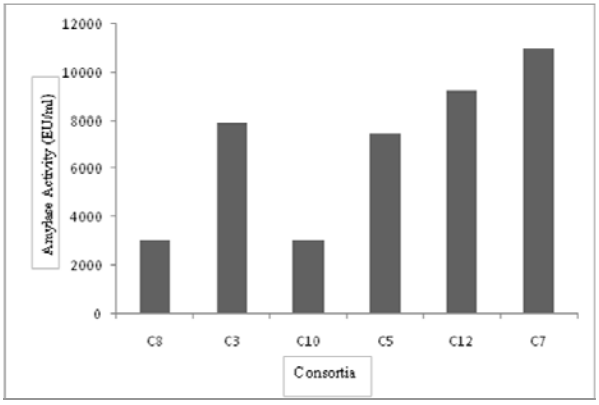

Fig. 1. Amylase production by different consortia.

These 7 consortia showed desired enzyme production. They were further used for lab trial with 5 kg of kitchen wastes. Degradation of the kitchen wastes were monitored by gradual decrease in the volume for 15 days. The maximum reduction observed was 50% in consortia no. 12 and 5. Rest showed the reduction less than 50%.

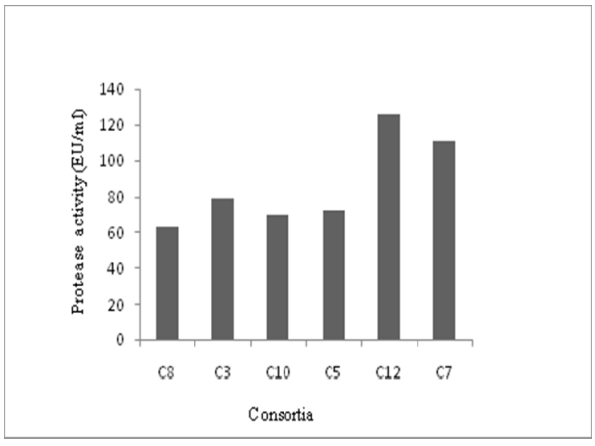

Fig. 2. Protease production by different consortia.

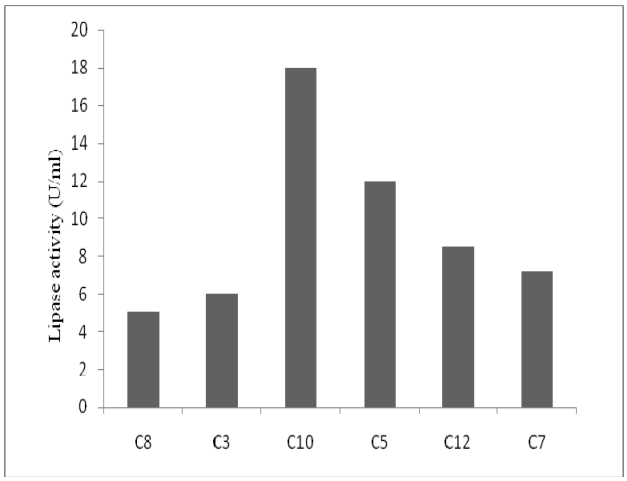

Fig. 3. Lipase production by different consortia.

E. Lab trials with 25 kg kitchen wastes

The best 3 consortia (3, 5 and 12) were further selected for large scale trial with 25 kg of kitchen wastes. Degradation of the kitchen wastes were monitored by gradual decrease in the volume for 30 days. All the 3 consortia showed more than 50% degradation in 21 days time while control with no inoculation showed only 36 % degradation in same time. Results in figure 4 suggest that the maximum reduction observed was about 65% in consortia no. 12 and 55% degradation was exhibited by consortia no.5. All the experiments were carried out in duplicates.

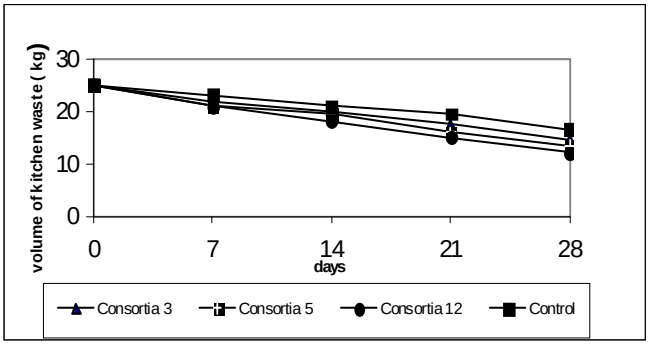

Fig.4. Large scale degradation of kitchen wastes by best three bacteria

F. Different enzymatic assay of the best degrader

During the lab trials with 25 kg of organic kitchen wastes consortium 12 was the best degrader among the three consortia. This consortium was selected to further assess its enzymatic activity. Enzymatic assay of consortium 12 at a constant interval of 24 hours till 5 days showed that amylase production continued to increase till fifth day. Both protease and lipase showed same pattern of activity. The activity of these two enzymes reached their respective peaks on the 3rd day and then there was a gradual decrease in the enzyme activity.

Though the enzymatic assay of the all consortia reveals that consortium no 12 was not the best producer of all the enzymes but the degradation capability of this consortium was best in 5 kg and 25kg organic kitchen wastes lab trials as shown in figure 5.

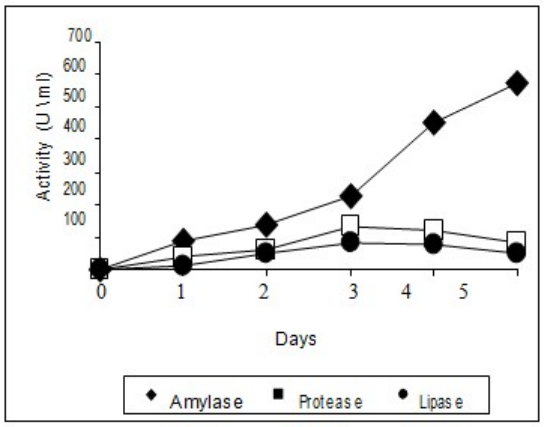

Fig. 5. Different enzyme production by consortium 12

IV. Conclusion

The degradation of organic wastes by the bacterial consortia is highly significant. It reduces the time span of degradation and produces no fowl odour. The use of microbial consortium generated through natural selection or improvement of the performance of these microorganisms in organic kitchen waste degradation through genetic manipulation, may be the best option for the efficient treatment of organic kitchen waste or domestic wastewater in the near future. The pretreatment of food waste can be used for biological solubilization and mineralization in garbage disposal system which is a novel approach.

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