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Development of Augmented Microbial Consortium for Kitchen Waste Composting

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A B S T R A C T

The present study focuses on Home Composting as a sustainable approach for Solid Waste (SWM) Management of kitchen waste at source. Five bacterial isolates were selected for microbial consortia preparation from amongst 15 isolates based on enzyme production (viz. Cellulase, Amylase, Protease, Pectinase, and Lipase) and their mutual compatibility as analyzed by the antagonistic assay. Two consortia Type 1 and Type 2 augmented along with an activator like cow dung were prepared and their potential for kitchen waste degradation was monitored in comparison with control for two weeks. The organic manure generated in consortium type 2 was greater than the amount generated by consortium type 1. The manure generated was further tested for plant growth-promoting ability and was found to be effective. This study signifies the scope of the developed consortia, to promote home composting in providing a solution to tackle the solid wastes at the immediate source of generation without producing foul odor, thereby contributing to sustainably reducing the burden on the Municipal dumping grounds.

***Key words*:** Home composting, Microbial consortium, Organic kitchen wastes, SWM, Augment

Solid Waste Management (SWM) is becoming very essential for maintaining the sustainability and health of cities and communities. Mismanagement of this solid waste can have adverse effects on human health and be disastrous for the hygiene of local environments. According to the World Bank 2018 report, it is disheartening to know that India disposes of 77% of the waste generated in open dumpsites [1]. India has 3,159 operational dumpsites and according to the Central Pollution Control Board (CPCB's) Annual Report 2018–19 on SWM, 23.35 million tons of waste was dumped in these dumpsites. Maharashtra has 327 dumpsites and the Deonar dumpsite located in Mumbai is India's oldest (started in 1927) and the largest dumpsite (over 326 acres). This dumpsite received 3,000 metric tons of waste every day in November 2019 [2]. Solid waste comprises two fractions viz. Biodegradable and Non-biodegradable. As per the estimations, at least 60% of domestic solid waste is biodegradable being derived majorly from kitchen waste or green waste. According to the 2016 rules of SWM, the disposal of biodegradable waste should be tackled by the society within its premises by adopting efficient composting methods or by employing bio-methanation processes in its vicinity. Thereafter, as per the directives of the local authority, the society should be discarding only the residual untreated waste to the waste collectors [3].

The pressing problem and challenge to society is the collection and disposal of Municipal Solid Waste (MSW). One of the major fractions of this waste is organic household waste (OHW) or kitchen waste. To reduce the load on the MSW which has become an acute problem, the best solution would be treating the kitchen waste at homes. The proper disposal of this waste is not only needed for the improvement of public health but also has resource recovery [4]. One of the practical solutions to treat the biodegradable portion of household kitchen waste at the source is Composting. Composting is the process of biological treatment of solid wastes during which the organic waste is converted into a crumbly soil-like material called "compost" by biological agents (such as microorganisms) in the presence of air and limited amounts of water [4]. The product of composting i.e., 'Compost', contains plant nutrients that can amend the soil by improving soil texture and fertility thus improving plant growth. The microbial composting works on the aerobic decomposition of complex organic matter into a simpler form by utilizing the degradation potential of microbes such as bacteria, actinomycetes, fungi, etc. Microbial composting is most convenient and reliable as it requires low maintenance, produces no foul odor, and carries a less negative environmental impact [5].

The use of Efficient Microorganisms (EM) consortium is made by combining microorganisms with selected properties to make a consortium that will stimulate plant growth and fertility, a concept which was developed in 1971[6]. The development of a microbial consortium has great potential to increase the

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efficiency of waste degradation. The organic wastes contain a variety of compounds which are needed to be degraded. A single culture may not give the expected results in a short time. However, if a consortium is prepared, the organisms in it would provide an array of enzymes, thereby leading to efficient degradation [7]. The main aim of this study was to develop an efficient bacterial consortium that can concomitantly degrade kitchen wastes with the help of their enzymes under natural conditions without producing any foul odor.

MATERIALS AND METHODS

Sample collection

For the isolation of different microorganisms, samples were collected from various locations across Mumbai. The samples consisted of soil samples collected from local community waste dump sites from Panvel, Deonar dumping ground, garden from Matunga, and compost samples from V.E.S College composting pit. All the samples were collected in clean Ziplock polythene back and transported to the laboratory for immediate processing (Table 1).

Table 1 Sites for sample collection

Sites of collection of soil samples	Soil sample name
Five Gardens, Matunga, Mumbai	G
Soil beside Municipal Dustbins, Panvel	P
Compost pits, V.E.S College, Chembur	C
Deonar dumping ground, Mumbai	DG
▪ Dry Soil	DG1
▪ Wet Soil	DG2

Enrichment and isolation of bacteria

The enrichment medium used was sterile Bushnell and Hass minimal medium (BH) with 1g of autoclaved bio-waste (dried and pulverized kitchen wastes) as the sole carbon source. The samples were processed by vortexing 1g of collected samples in 10ml of saline and 1 ml of this supernatant was used as inoculum, which was aseptically inoculated in 5ml of sterile enrichment medium. An uninoculated control tube consisting of 5 ml with 1g of similarly treated bio-waste was also set up [8]. Both test and control tubes were incubated at 32ºC for 48 hours. After 48hours, the loopful of the growth from the 'test' test tube was transferred onto Nutrient Agar media and incubated at 32ºC for 24 hours. The different isolates obtained were subjected to re-purification on the same medium. Pure cultures obtained were sub-cultured on Nutrient Agar slants and preserved at 4ºC and were maintained/preserved further on Nutrient Agar slants. Gram stain was performed to determine the gram nature and morphology of bacterial isolates.

Screening of isolates for enzyme production

All the isolates were tested for their ability to produce various hydrolytic enzymes like cellulase, pectinase, amylase, protease, and lipase. For screening of cellulase producers, isolates obtained were spot inoculated on sterile Carboxymethyl Cellulose medium and incubated at 32ºC for 1 week. To check the cellulolytic activity of isolated strain, the plates were flooded with 1% Congo red and incubated for five minutes at room temperature [9]. Screening for pectinase producers was carried out by spot inoculating the isolates on the sterile Pectinase screening agar medium (PSAM) and incubated at 32ºC for 24hours. The plates were flooded with Gram's iodine solution [10]. Amylase producers were screened by spot inoculating the isolates on sterile Starch agar plates and incubation at 32ºC for 24-48 hours, plates were flooded with Gram's iodine to produce a deep blue colored starch-iodine complex all over the plate except around the colonies showing a halo zone of degradation with the absence of blue color, which is the basis for the detection and screening of amylolytic strain [11]. For screening of lipase producers, the isolates were spot inoculated on sterile Gorodkowa's Tributyrin agar plates and incubated at 32ºC for 2 days. A halo zone of clearance surrounding the colony indicates positive lipolytic activity [12]. Screening of protease producers was carried out by spot inoculating the isolates on sterile Skimmed milk agar plates and incubating at 32ºC for 48 hours. A halo zone of clearance surrounding the colonies indicates proteolytic activity [13]. The isolates were named according to the source they were obtained from as Cd, DG1, etc.

Selection of isolates for preparation of bacterial consortia

For the preparation of a successful consortium, the selection of the isolates plays an important role, and hence isolates selected were the ones that possess a diverse array of hydrolytic enzymes. Also, the effect of one culture on the other in terms of their compatibility and effect on their degradative capacity was considered for the selection of the isolates. The consortium compatibility was checked by the Antagonism Assay. For this, an individual selected strain with the desired characteristic was inoculated as a wide streak diametrically across on a 14 mm sterile Nutrient agar plate, and the remaining selected strains to be used in the preparation of the consortium were streaked at right angles to the original diametrically streaked culture (4 strains per plate). The plates were incubated at 32ºC overnight, and inhibition if any caused by central strain was recorded. The procedure was repeated independently for each of the bacterial strains [8].

Fig 1 Kitchen waste and its processing before the experimental setup

Set-up for kitchen waste degradation

Composting baskets were set up as described in (Table 2). The kitchen waste was categorized as fruit waste (apple core, banana and orange peels, etc.), vegetable waste (leftover wastes of coriander leaves, Cabbage**,** Cauliflower, etc.) and garden waste (used flowers, dried leaves, etc.). Further the contents in the baskets also included sterile soil, cocopeat, saw dust and dried cow dung besides spraying the respective consortia as described in (Table 2). The use of coconut coir or coco peat has proven to have several advantages as a soil amendment component with respect to the water retention capacity of the soil/compost, increasing the nutritive content and water/nutrient absorption [14]. Saw dust helps in fixing nitrogen and is an excellent energy source during composting. If cow dung and sawdust are mixed, there is greater mineralization of Nitrogen

and can act as a good conditioner and is known to augment biodegradation [15]. The kitchen waste was sun dried for 4 days and coarsely ground using a kitchen grinder before adding to the composting baskets (Fig 1). The baskets were kept under natural conditions for 2 weeks. During the incubation, the compost pile was turned 4 times with an interval of 3 to 4 days to ensure proper aerobic condition. A small amount of water was sprayed onto the composting material as required to maintain the moisture. At the end of two weeks, the compost was observed visually for degradation by gradual decrease in the volume of the waste pile [16]. The extent of degradation of waste by the two different developed consortia was checked by the sieving method in which the degraded waste was sieved through a sieve of 2 mm mesh size to separate fine compost. The sieved compost was weighed using analytical balance and stored in containers for further experiments.

Organic fertilizers are derived from animal waste matter, human excreta or vegetable waste matter which can be generated from various sources like kitchen waste, domestic waste, etc. (e.g., compost, manure). A sieve analysis is a preliminary practice or process which is used to assess the particle size distribution (*gradation*) of a granular material. The size distribution of the particle is often of critical importance to the way the material performs in use. The amount of waste degraded can be assessed from the size of the particles. A sieve analysis can be performed on any type of non-organic or organic granular material. The properties of the compost depend on the particle size of the compost. Thus, this can prove to be a method to check the degradation efficiency. Fine fractions (<2mm) show better quality, higher maturity, and cleanness but low nutrient contents. The particle size of more than 2 mm was rich in fertilizing elements [17-18]. The formula used to calculate the percentage of waste degraded is:

$$\text{Percent retained} = \frac{\text{Soil retained}}{\text{Total weight}} \times 100$$

$$\text{Soil retained} = \text{Weight of the empty sieve + Soil retained} - \text{weight of the empty sieve}$$

Table 2 Set-up of composting basket

Contents	Waste	Waste + Activator	Waste + Activator + Consortia		Waste + Consortia	
Kitchen waste	300 gm	300 gm	300 gm	300 gm	300 gm	300 gm
Sterile Soil	50 gm	-	-	-	50 gm	50 gm
Cocopeat	10 gm	10 gm	10 gm	10 gm	10 gm	10 gm
Sawdust	10 gm	10 gm	10 gm	10 gm	10 gm	10 gm
Consortia	-	-	Consortia 1	Consortia 2	Consortia 1	Consortia 2
Dry cow dung	-	50 gm	50 gm	50 gm	-	-
Distilled Water	Distilled water	-	-	-	-	-

To check the efficiency of the compost for its plant growth promotion activity

The compost obtained from the consortia 2 treatment was tested for its plant growth promotion activity. For this, 20 grams of compost was mixed with 180 grams of sterile soil in a clean plastic pot. A pot containing 20 grams of cocopeat mixed with 180 grams of sterile soil was kept as control. Both pots were seeded with 10 green gram (*Vigna radiata*) seeds each. The pots were sprayed with water and observed for 15 days for germination and development of plantlets [19].

RESULTS AND DISCUSSION

The enrichment of bacterial isolates from different samples was successful in Bushnell and Hass medium incorporated with Kitchen waste. After 48hrs of incubation at 32ºC, visible turbidity was seen in all tubes except the uninoculated control. Further, the color of the medium changed from dark brown to pale yellow (Fig 2). This indicated that the bacteria from the soil samples can degrade kitchen waste.

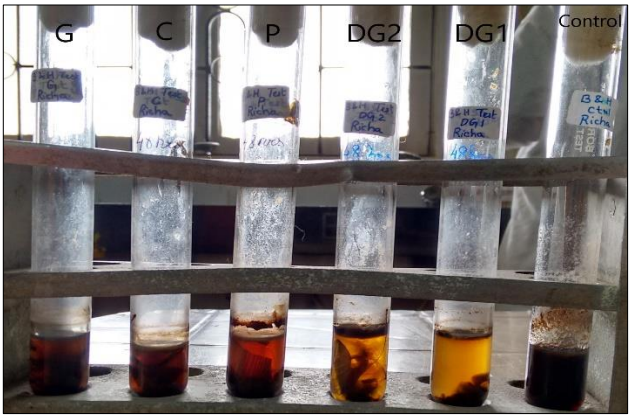

Fig 2 Results of enrichment of bacterial isolates in BH medium incorporated with kitchen waste

The enriched samples were isolated on St. Nutrient agar plates. All 15 different isolates were isolated from four different sampling sites as listed in (Table 3).

Table 3 Isolates and their source with typical colony characteristics and Gram nature

Sample source and code	Isolate codes	Colony characteristics	Gram nature	Morphology and arrangements
Five Gardens, Matunga, Mumbai (G)	G	Colorless, Pinpoint, Circular, Translucent	Gram Negative	Coccobacilli
Soil beside Municipal Dustbins, Panvel (P)	Pa	Cream, Large, Circular, Translucent, Bluish-green color on media	Gram Negative	Short rods
	Pb	Cream, Small, Circular, Translucent	Gram Negative	Short rods
Compost sample, VES College, Chembur (C)	Ca1	Cream, Small, Circular, Translucent	Gram Positive	Cocci in chains and clusters
	Ca2	Colorless, Pinpoint, Circular, Translucent	Gram Positive	Short, fat rods
	Cb	Cream, Large, Circular, Opaque	Gram Negative	Coccobacilli
	Cd	White, Large, Circular, Opaque	Gram Positive	Cocci in pairs
	DG1a	Cream, Small, Irregular, Translucent	Gram Negative	Short rods

Deonar dumping ground, Mumbai (DG) (DG1-Dry Soil DG2-Wet Soil)	DG1b	White, Small, Circular, Opaque	Gram Positive	Cocci in singles
	DG1c	Cream, Large, Circular, Translucent	Gram Negative	Short rods
	DG1d	Cream, Large, Irregular, Translucent	Gram Negative	Short rods
	DG2a	Yellow, Small, Circular, Opaque	Gram Negative	Coccobacilli
	DG2b	Colorless, Pinpoint, Circular, Translucent	Gram Negative	Rods
	DG2c	White, Large, Circular, Translucent	Gram Negative	Short rods
	DG2d	White, Large, Circular, Opaque	Gram Positive	Cocci in pairs

Table 4 Results of screening for enzyme activity

Isolates	Cellulase	Pectinase	Amylase	Lipase	Protease
G	-	+	++	-	-
Ca1	-	-	+	-	-
Ca2	-	-	+	-	-
Cb	-	+	++	-	-
Cd	+	-	++	-	-
Pa	-	-	-	++	++
Pb	-	-	-	-	-
DG1a	-	-	+	+	+
DG1b	-	-	+	-	-
DG1c	-	-	-	-	+
DG1d	-	-	++	+	+
DG2a	-	-	-	-	-
DG2b	-	-	-	-	-
DG2c	-	-	++	++	++
DG2d	-	-	++	-	-

++ = Large zone of hydrolysis; + = Small zone of hydrolysis; - = No zone of hydrolysis

Screening for enzyme activity

As seen in (Table 4), all 15 isolates were tested for their ability to produce cellulase, pectinase, amylase, protease and lipase enzymes. Ability to produce amylase was seen in most isolates followed by protease and lipase. Pectinase and cellulase production ability was seen in two and one isolates respectively.

The total of five isolates viz. G, Cb, Cd, DG1d, and DG2 were selected from a semi-quantitative enzyme assay. Isolates G and Cb produced Pectinase, Cd produced Cellulase, and DG1d and DG2c produced Amylase, Lipase, and Protease. The organisms producing respective enzymes were selected and their suspension was made in the mentioned proportions (Table 5). This mixture was sprayed on the kitchen waste.

Table 5 Ratio of the isolates for the preparation of consortium

	Cellulase	Pectinase	Amylase + Lipase + Protease
Consortium 1 Ratio (2:1:2)	Cd (10 mL)	G (2.5 mL) & Cb (2.5 mL)	DG1d (5 mL) & DG2c (5 mL)
Consortium 2 Ratio (2:2:1)	Cd (10 mL)	G (5 mL) & Cb (5 mL)	DG1d (2.5 mL) & DG2c (2.5 mL)

The results of the antagonistic assay indicated that the strains did not show antagonism against each other and therefore could be used for preparing a consortium (Fig 3). The two different consortia were prepared with different proportions depending on the kitchen waste components.

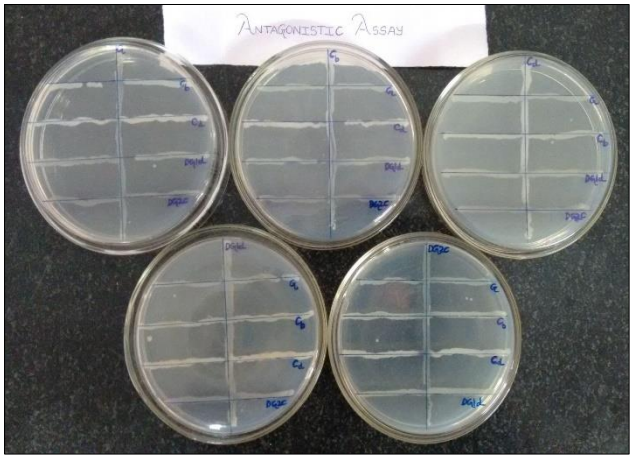

Fig 3 Antagonism assay indicating that all 5 isolates did not inhibit the other 4 isolates under investigation

The efficiency of kitchen waste degradation by each consortium

The kitchen waste treated with both consortia 1 and consortia 2 was degraded within two weeks. After 15 days of composting, the compost had reduced in size and had turned visibly dark brown. The color of the ripe compost is blackish brown. If the color is similar to its raw material, it means that the compost has not been well-processed or immature [20]. It had a fresh earthy odor (Fig 4a-b). The organic manure was separated from each compost basket using a sieve and collected in different containers and weighed (Fig 4c).

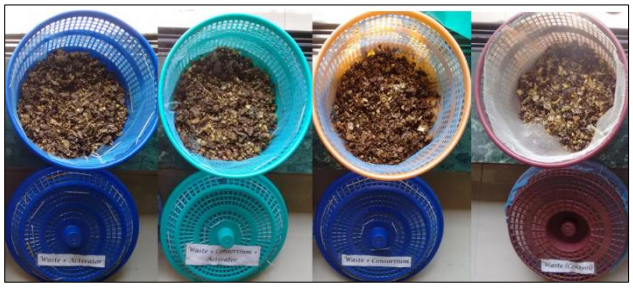

Fig 4(a) Kitchen waste in the composting basket at day 0

Fig 4(b) Dark brown compost obtained after 15 days of composting

The entire content of the composting baskets was sieved through a 2 mm sieve to recover compost generated in each basket. Greater amounts of compost were generated in waste

treated with a mixture of activator + consortium 2 as compared to the similar set up with consortium 1, thus indicating that the isolates in consortium 2 and the organisms in the activator work well in synergism. This compost generated from consortium 2 was tested for its plant growth-promoting abilities. It was observed that the organic manure was efficient in promoting seed germination and promoted plant growth (Fig 6).

Fig 4(c) Organic manure obtained after sieving compost after 2 weeks of composting

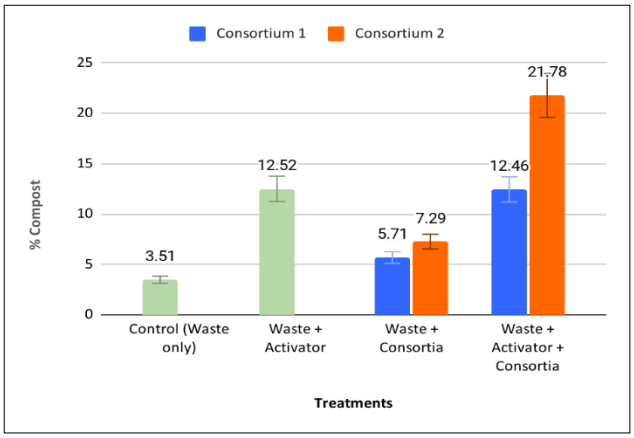

Fig 5 Percent (%) compost generated after various treatments

The isolates obtained from soil samples were enriched using Sterile Bushnell and Hass minimal medium incorporated with kitchen waste in it. The turbidity was observed after incubation indicating the presence of microorganisms that are capable of degrading kitchen waste. The 15 isolates were characterized macroscopically and microscopically (Gram staining) and it was found that 5 of the isolates were gram-positive and 10 were gram-negative.

All the isolates were tested for their ability to produce different hydrolytic enzymes namely, cellulase, pectinase, amylase, lipase, and protease. The five potent enzyme-producing isolates were identified namely 'Cd (Cellulase)', 'G and Cb (Pectinase)', 'DG1d and DG2c (Amylase + Lipase + Protease)'. Food waste mainly consists of carbohydrates, proteins, and fats. Bacteria possessing various hydrolytic enzymes such as cellulase, amylase, pectinase, protease, and lipase can break the bonds in these macromolecules and are thus beneficial in the waste conversion process [21]. The antagonism assay indicated that all the five isolates were compatible with each other.

Fig 6 Moong seeds germination in (a) Sterile soil only (b) Sterile soil amended with organic manure

The two types of bacterial consortia were prepared depending upon the enzymatic activity they possessed. The compost baskets were kept under natural conditions for two weeks and the degradation with consortium type 1 and type 2 were analyzed. The organic manure generated in consortium type 2 was more as compared to consortium type 1. The proportion of pectinase-producing isolates in consortium type 2 was higher as compared to that in consortium type 1, whereas the proportion of amylase + lipase + protease proportion was decreased in the latter, which can be attributed to having resulted in better degradation activity by consortium type 2. Thus, our observation suggests that more proportion of pectinase along with cellulase are needed for efficient degradation of the kitchen waste into organic compost.

The production of good quality dark brown-black compost is also dependent on other factors such as temperature, aeration, moisture content, and composition of the organic waste materials used in composting [22]. The cow dung (activator) facilitated the composting process and contributed to the early maturity of the compost. This was concluded on the basis that more organic manure was generated when waste was treated with a mixture of consortia and activator as compared to the consortia or activator alone [19]. They reported increased humic fraction in the MSW compost obtained from the composting of MSW with a mixture of cow dung slurry and cellulolytic bacteria.

It is important to evaluate the quality of compost, and in fact, the seed germination test is a powerful tool to examine the toxicity of compost [23]. Studies on moong seed germination indicated that compost was not toxic to seeds and facilitated seed germination as more plantlets germinated in the test as compared to control. In general, the use of good mature compost helps to increase soil nutrients and to improve some properties such as the pH of the soil, texture, soil aggregation, and chemical composition of the soil, thus helping seed germination and plant growth.

CONCLUSION

Solid Waste Management has become an issue of utmost concern in our society in the present times. India's largest landfill, the Deonar dumping ground, Mumbai, is currently facing complete closure with incidents like fire breakouts, a saturation of land in accepting wastes, and causing problems that are seriously affecting public health and aesthetics. Thus, there is an urgent need to find solutions to treat the wastes that we generate. The present study treats organic kitchen waste that can be decomposed at the source of generation itself by using the Developed Augmented Microbial Consortia (also termed as Efficient Microorganism-EM) in the 'Home Composting' method shows great promise to degrade the waste within two weeks. Composting with microbial consortia (EM- Efficient Microorganism compost) has proven to be better than other natural methods of composting available. EM compost replenishes the soil's biological activity and also may neutralize the toxicity of chemical fertilizers. The use of EM has been

shown to increase soil biomass, respiration, and enzymatic activity. All of this leads to efficient nutrient recycling increasing the overall potential of the soil. This method can be improvised to a greater extent by consistent and continuous maintenance of the ideal composting conditions and appropriate addition of waste to enhance the efficiency of the composting which can also enhance the ability to degrade waste and generate manure more efficiently in a shorter period as the consortia increase their numbers in the compost with time**.** This study has thereby successfully developed an augmented microbial consortium which has the potential to increase its numbers excessively and can be used in the effective treatment of domestic organic wastes efficiently promising therein a sustainable approach in finding solutions to SWM and reducing the load on landfills and dumping grounds in the future.

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