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Isolation and characterization of microbes from waste dumping for organic manure

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Introduction

ABSTRACT

Composting is a preferred and environmental sound method in which organic waste is reduced to organic fertilizer and soil conditioners through biological processes. Microorganisms are able to convert organic waste into micro and macro nutrients to be utilized by plants and reduce C:N ratio to support soil productivity and to minimize ecological imbalance. The parameters including C:N ratio, composting temperature, pH of the finished product and moisture content are used to assess the quality and stability of the compost. In the present investigation, the isolated microbes were assessed for their composting abilities using kitchen and garden wastes. About 42 morphologically distinct bacteria and fungi were isolated from different sources using serial dilution method on Nutrient Agar and Potato Dextrose Agar media. These isolates were further screened for cellulase, pectinase and amylase activities and four isolate in which two bacterial (BA1 and BC6) and two fungal (FB4 and FD5) were selected. These isolates were having better enzyme activity as compared to other isolates. Hence, identified isolates may be a boon for commercial units of compost making.

1. Introduction

Composting is a preferred environmental method in which organic waste is reduced to organic fertilizer and soil conditioners through biological processes (Gautam *et al.*, 2010). It is an aerobic process where complex degradable materials are degraded and transformed by microbe into organic and inorganic by-products (Chaher *et al.*, 2021). To enhance the yield of crop, farmers adopt the strategy by applying large amount of chemical fertilizers and pesticides (Piqueres *et al.*, 2006). However, the continuous use of fertilizers is creating environmental problems. The practice and rampant application of these chemical fertilizers, pesticides and insecticides degraded the quality of soil and also contributed largely in the deterioration of the environment (Indumathi, 2017). After the green revolution, the rate of nitrogen, phosphorus, and potassium (NPK) based chemical fertilizer application has increased tremendously. Wastes are unwanted materials which have lost their utility and are discarded by people. The household waste constitutes approx 70-90% of biodegradable waste which include kitchen waste, paper waste, clothes and 10-15% is non-degradable waste which are like glass, polythene bags, plastic, rubber and leather. Nowadays garbage is becoming more complex as different types of materials are thrown together as garbage. It is very important to understand the waste, their nature and how to dispose them in a hygienic manner.

Waste generation is steadily growing in developing countries including India due to the continuous growth of industrialization, urbanization, and population (Malinauskaite *et al.*, 2017). Similarly, tons of kitchen wastes are produced daily in highly populated areas (Sarkar *et al.,* 2011). There is a clear connection between the problematic of kitchen waste and the three sustainability dimensions: environmental, economic, and social (Salihoglu *et. al.*, 2018). The Food and Agriculture Organization (FAO) has estimated that at least one-third of the food produced in the world (estimated as 1.3

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billion metric tons) is lost and wasted every year (FAO, 2014). In India, it is estimated that the total solid waste available is about 10^6 million MT per annum and in the Bengaluru, generation of Solid Waste is about 3000- 3500 MT per day that leads to challenges for its safe disposal, with the waste being usually either burned or land filled (Patil *et al.,* 2018). Another is garden waste which consists of tree trimmings, garden litter, grass, leaves and other similar constituents (Hernandez *et al.,* 2014). These wastes are collected and dumped into the landfills, causing major pollution (Bouallagui *et al*., 2005). Burning of garden residues in the field affects soil C: N ratio and biota; on the other hand, burning of waste leads to emission of greenhouse gases such as CO_2, CH_4 and NO (Chatterjee, 2010). Besides that, the garden waste is either dumped into landfill or incinerated. Composting has several advantages over incineration and land filling and it is an effective solution to recycle such wastes. It is now increasingly realized that for successful survival on the earth, recycling of both organic and inorganic materials is essential. There are several methods for decomposition of waste being currently in use but all of these depend on microbial conversion of organic materials into inorganic compounds.

In nature's laboratory there are many microbes such as bacteria, fungi and mesophilic (*Streptomyces rectus)* andthermophilic *Actinomycetes (Actinobifida chromogena)* that have ability to convert organic waste into valuable resources containing plant nutrients and organic matter. These enhance the rate of lingo cellulose degradation due to their synergistic activity through utilization of intermediate degradation products. This technique not only reduces the amount of waste damped to landfills, but also contributes to social, ecological and economic improvement, being the best alternative for the management and transformation of organic waste (Fernandez- Brana *et al.,* 2020). Indigenous microorganisms are a microbial inoculants produced at home and can be produced from kitchen, fruit or vegetable wastes all geared towards increasing speed of compost maturity or shortening the duration of composting time. Therefore, present paper has been prepared to isolate and characterize microbes from waste dumping which will provide to manage the waste through recycling at larger extent.

2. Materials and methods

Collection of Samples

Kitchen waste samples (1kg) were collected from various dumping sites such as Ajanta Hostel CCS HAU, Hisar (29.150156, 75.704298), Azad Nagar (29.120503, 75.703023), Chandan Nagar (29.139951, 75.679419), Hisar and Garden waste samples were collected from waste dumping sites in the vicinity Collage of Basic Sciences & Humanities, CCS HAU, Hisar (29.143155, 75.707799).

Isolation and screening of microbes

Serial dilution technique was used for isolation of kitchen waste and garden waste degrading microbes (Johnson and Curl, 1972). For this, about 10 gram of sample was added in 90 ml sterilized distilled water and shaken properly. For isolation of bacteria, serial dilutions were made upto the level of 10^{-3} to 10^{-7} and0.1 ml of each dilution was spread on sterilized plates containing 20 ml of sterilized nutrient agar (NA) medium. For fungi, 0.1 ml of suspension from the dilution of 10^{-3}, 10^{-4}, 10^{-5} was spread on the sterilized plates containing 20 ml sterilized potato dextrose agar (PDA) medium. Plates were incubated at room temperature (25-30 ^{0}C) for three to seven days with periodic observation for growth of bacterial and fungal colonies. After isolation, pure fungal and bacterial cultures were maintained at 4 ^{0}C on PDA and NA slants respectively. Bacterial and fungal isolates were screened for the production of lignolytic enzymes such as pectinase, cellulase and amylase on the basis of zone of hydrolysis on various media such as pectinase screening agar medium, carboxymethyl cellulose (CMC) medium and starch agar medium, respectively.

Characterization

Gram's reaction, Indole production, Methyl red test, Voges - Proskauer reaction, Citrate utilization test, Oxidase test, Catalase production and Motility test were carried out for characterization of bacterial isolates.

3. Results and discussion

Bacteria and fungi were isolated from soil samples collected from various waste dumping sites of Hisar. A total of 42 isolates (25 bacterial and 17 fungal) were obtained on NA medium and PDA medium (Table 1).

The morphological characteristics of the bacterial isolates revealed that the colony color varied from white to yellow and brownish. The appearance of colonies was smooth and round, lobbed and raised (Table 2 & Fig 1).

Table 1. Bacterial and fungal isolates obtained from different samples

Sample No.	Site(s)	Number of isolates	Isolates
A	Hostel No. 3, CCS HAU, Hisar (Kitchen waste)	7	BA1, BA2, BA3, BA4, BA5, FA1, FA2
B	Azad Nagar, Hisar (Kitchen waste)	11	BB1, BB2, BB3, BB4, BB5, BB6, BB7, FB1, FB2, FB3, FB4
C	Garden waste, behind COBS&H, CCS HAU, Hisar	13	BC1, BC2, BC3, BC4, BC5, BC6, BC7, FC1, FC2, FC3, FC4, FC5, FC6
D	Chandan Nagar, Hisar (Garden waste)	11	BD1, BD2, BD3, BD4, BD5, BD6, FD1, FD2, FD3, FD4, FD5

*B Bacteria, *F Fungus

Table 2. Morphological characteristics of bacterial isolates

Sample No.	Site(s)	Number of bacterial isolates	Isolate	Characteristics
A	Hostel No. 3, CCS HAU, Hisar (Kitchen waste)	5	BA1	Creamish, smooth, round
			BA2	Milky white, smooth, round
			BA3	White, smooth, round
			BA4	White, smooth, round
			BA5	Brownish, smooth, round
B	Azad Nagar, Hisar (Kitchen waste)	7	BB1	White, smooth, round
			BB2	Transparent white, smooth, round
			BB3	Brownish, gummy, shiny
			BB4	Creamish, smooth, round
			BB5	Creamish, rough, raised
			BB6	White, smooth, round
			BB7	White, smooth, large
C	Garden waste, behind COBS&H, CCS HAU, Hisar	7	BC1	Milky white, smooth, round
			BC2	Brownish, dry, irregular
			BC3	Brownish, gummy, raised
			BC4	Yellowish, smooth, round
			BC5	White, flat, irregular
			BC6	White, smooth, lobbed
			BC7	Brownish, rough, irregular
D	Chandan Nagar, Hisar (Garden waste)	6	BD1	Creamish, smooth, round
			BD2	Yellowish, lobbed, round
			BD3	White, gummy, shiny
			BD4	Creamish, gummy, raised
			BD5	Milky white, smooth, round
			BD6	White, rough, irregular

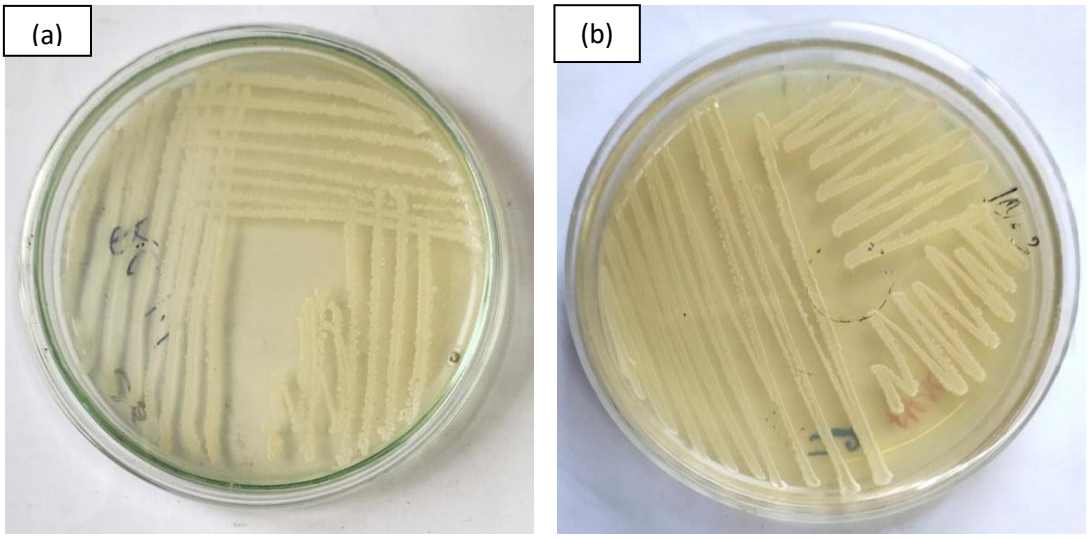

Fig 1. Morphological characteristics of bacterial isolates (a) BC6 and (b) BA1

Table 3. Morphological characteristics of fungal isolates

Sample No.	Site(s)	Number of fungal isolates	Isolate	Characteristics
A	Hostel No. 3, CCS HAU, Hisar (Kitchen waste)	2	FA1	White cottony, filiform, filamentous
			FA2	Black, undulate, powdery
B	Azad Nagar, Hisar (Kitchen waste)	4	FB1	White, filiform, filamentous
			FB2	Dirty white, undulate, powdery
			FB3	Green, filiform, powdery
			FB4	Olive green, undulate, powdery
C	Garden waste, behind COBS&H, CCS HAU, Hisar	6	FC1	Black, undulate, powdery
			FC2	White, filiform, filamentous
			FC3	Light green, undulate, rhizoid
			FC4	Brown with white margin, thick mycelia
			FC5	Cottony white, filiform, filamentous
			FC6	Dirty white, undulate, powdery
D	Chandan Nagar, Hisar (Garden waste)	5	FD1	Green with white margin, powdery
			FD2	White colony, wave like appearance
			FD3	Cottony white, thick mycelia,
			FD4	Black, undulate, powdery
			FD5	Light green, undulate, powdery

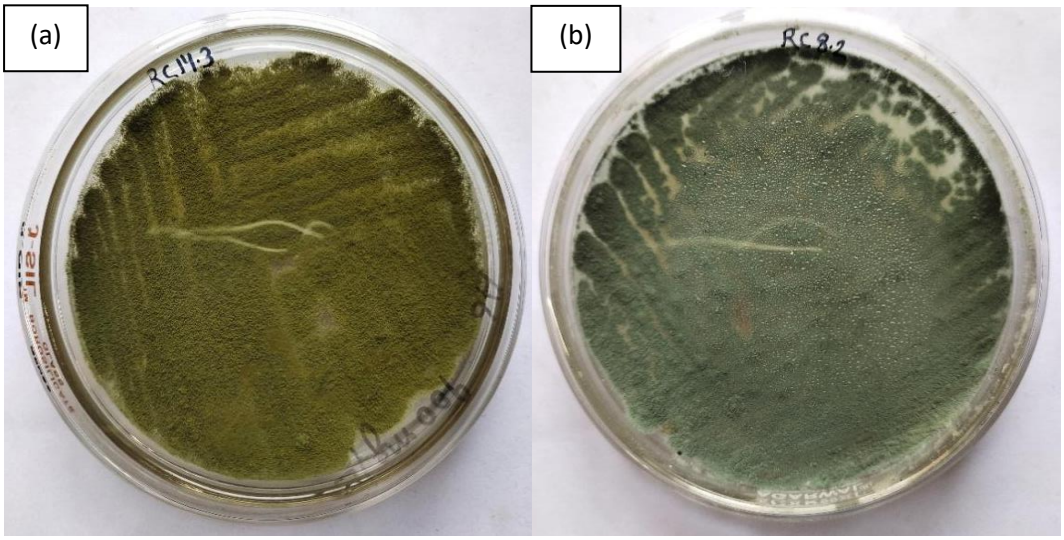

Fig 2. Morphology of fungal isolates (a) FB4 and (b) FD5

Screening of bacterial and fungal isolates was done for the production of lignolytic enzymes including cellulase, amylase and pectinase qualitatively (Table 4 & Fig 3).

Table 4. Screening of bacterial and fungal isolates for various enzyme activities

Sr. No.	Isolates	Cellulase activity	Amylase activity	Pectinase activity
1	BA1	-	+	-
2	BA2	+	+	-
3	BA3	-	+	+++
4	BA4	-	+	++
5	BA5	-	++	-
6	FA1	-	-	+
7	FA2	-	-	-
8	BB1	-	+	-
9	BB2	-	-	+
10	BB3	-	+	+
11	BB4	-	++	-
12	BB5	-	-	+
13	BB6	+	-	-
14	BB7	-	+	+
15	FB1	-	-	+
16	FB2	-	-	+
17	FB3	-	+	+
18	FB4	+++	-	+
19	BC1	+	-	++
20	BC2	-	-	+
21	BC3	-	+	-
22	BC4	++	-	-
23	BC5	+	-	-
24	BC6	+++	++	++
25	BC7	-	-	-
26	FC1	-	++	+
27	FC2	-	+	-
28	FC3	++	+	+
29	FC4	+	-	+

30	FC5	-	-	+
31	FC6	+	+	-
32	BD1	-	+	-
33	BD2	-	-	++
34	BD3	++	+	-
35	BD4	-	-	-
36	BD5	-	++	+
37	BD6	+	+	-
38	FD1	-	+	-
39	FD2	+	-	+
40	FD3	+	-	-
41	FD4	-	++	++
42	FD5	++	-	-

+++ high, ++ moderate, + low, - no activity

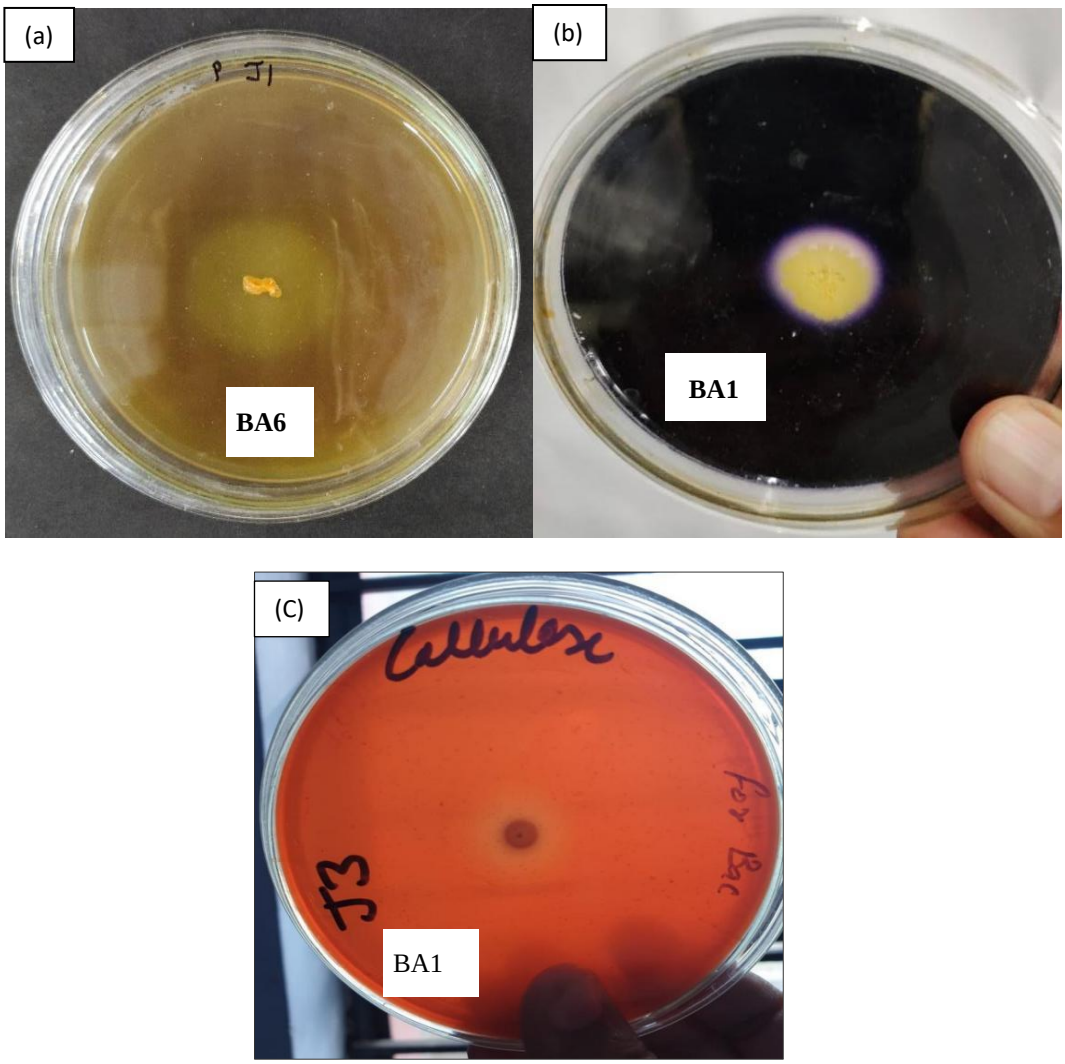

Fig 3. Screening of isolates (a) Pectinase activity, (b) Amylase activity and (c) cellulase activity

Selection of promising isolates for decomposition of kitchen and garden waste

A total of forty-two isolates have been retrieved from different samples. Out of which two bacterial isolates (BA1 and BC6) and two fungal isolates (FB4 and FD5) were selected on the basis of cellulase, amylase and pectinase activity. Three isolates (BC6, FB4 and FD5) were found to be cellulase producers. Isolates BA1 and BC6 were found amylase and pectinase positive and out of them, BC6 was found to be better amylase and cellulase producer whereas BA1 was better pectinase producer (Table 4).

Biochemical characterization of bacterial isolates

Various biochemical tests were performed such as Indole, Methyl red, Vogous-Proskauer, Citrate utilization, Oxidase, Catalase and Gram's reaction (Table 5 & Fig 4).

Bacterial isolates were identified on the basis of their morphological and biochemical properties. Both the isolates were found to be rod shaped. BA1 was found to be Gram negative and BC6 was Gram positive. Fungal isolates were identified on the basis of their morphological characteristics. Isolate FB4 was found to exhibit clear and septate hyphae with long unbranched conidiophores and isolate FD5 was found to have smooth and septate hyphae with highly branched conidiophores (Fig 5).

On the basis of these morphological characteristics and biochemical properties, the bacterial isolates BA1 and BC6 were tentatively identified as *Pseudomonas* sp. and *Bacillus* sp. Respectively and the fungal isolates FB4 and FD5 may be identified as *Aspergillus* sp.

Table 5. Biochemical characterization of selected bacterial isolates

Tests	BA1	BC6
Indole test	-	-
MR test	-	-
VP test	-	+
Citrate utilization test	+	+
Oxidase test	+	+
Motility test	+	+
Catalase test	+	+
Gram Reaction	-	+

+ positive reaction, - negative reaction

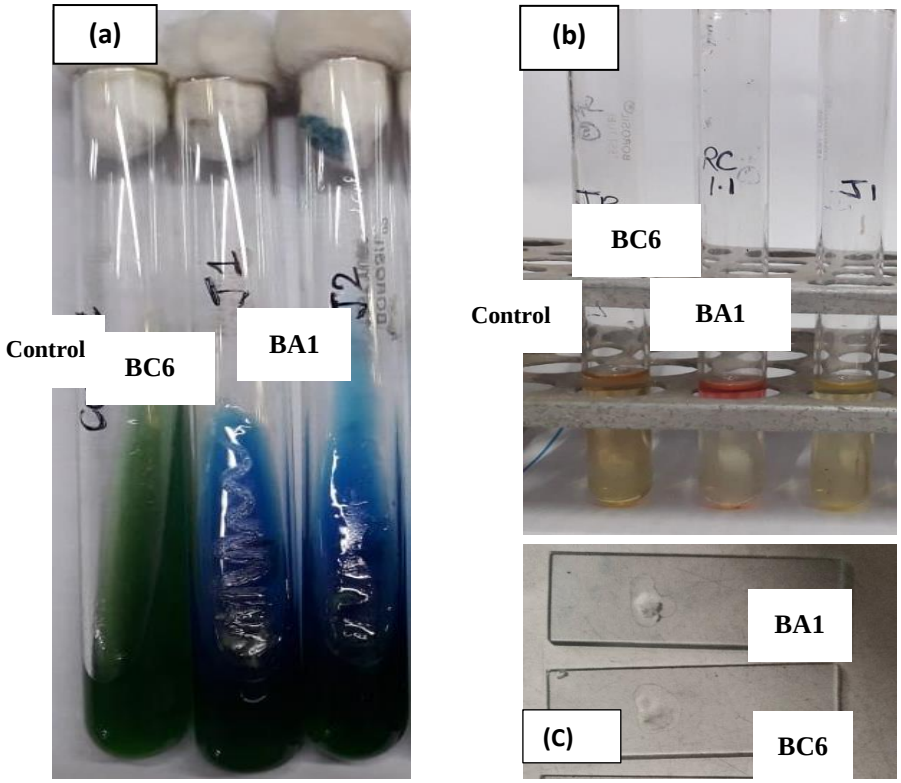

Fig 4. Biochemical test of bacterial isolates: (a) Citrate utilization test, (b) Indole test (c) Catalase test

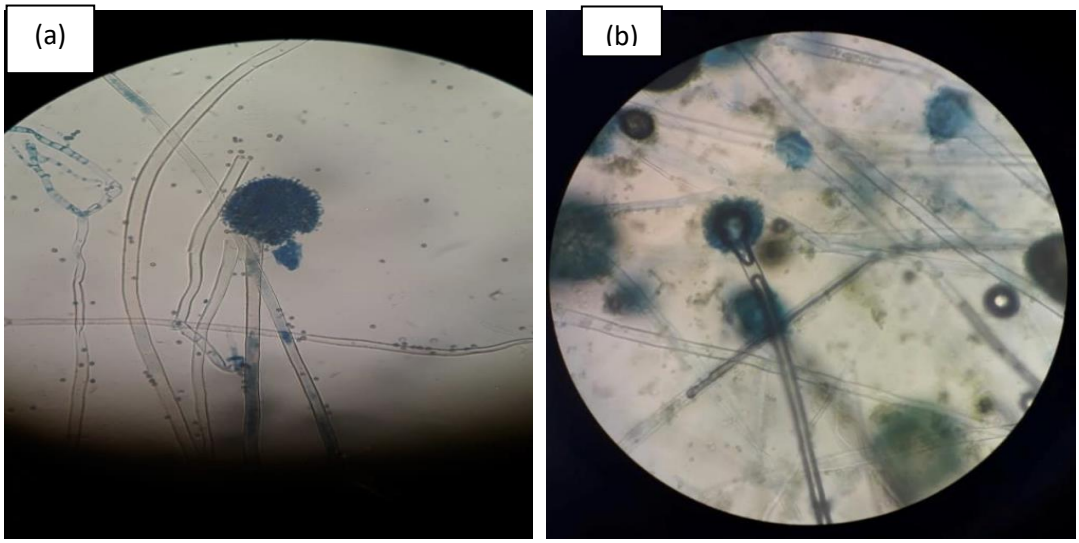

Fig 5. Microscopic characteristics of fungal isolates were viewed at 40X magnification (a) FB4 & (b) FD5

Hence, a total of forty-two isolates were obtained from kitchen waste and garden waste. On the basis of various enzymes activities (cellulase, pectinase and amylase), four isolates (two bacterial and two fungal) were selected for co-composting of kitchen waste and garden waste. Promising bacterial isolates were found to be *Bacillus* sp. (BC6) and *Pseudomonas* sp. (BA1) on the basis of Bergey's Manual of Systematic Bacteriology. Fungal isolates were identified as *Aspergillus* sp. (FB4 & FD5) according to morphological characteristics. The co-composting of kitchen and garden waste using these four isolates as inoculants was carried out under 1.5×1.5×1.5 ft size cemented pit house conditions and @ 2% inoculam was added for 90 days. Composting process lasted for 90 days and parameters like organic C, total N, available phosphorus and available potassium contents were analyzed. Similar to our reports, Sahu *et al.* (2017), isolated microbial decomposers from kitchen waste such as *Pseudomonas sp., Trichoderma viride,* 1, 2, 3 and *T. harzianum* and studied their effect on kitchen waste decomposition.

4. Conclusion

A total number of 42 bacterial and fungal isolates were obtained from kitchen waste and garden waste samples on Nutrient agar and Potato dextrose agar media respectively. The isolates were screened for various enzyme activities such as cellulase, pectinase and amylase by plate assay. Out of 42 isolates, four isolates (BA1, BC6, FB4 & FD5) showed better enzyme activity as compared to other isolates. The selected bacterial and fungal isolates were characterized morphologically. Bacterial isolates (BA1 and BC6) were creamish to white color having smooth margin with convex to raised colony elevation while fungal isolates (FB4 and FD5) were olive to light green color with powdery appearance. On the basis of these morphological characteristics and biochemical properties, the bacterial isolates BA1 and BC6 may be identified as *Pseudomonas* sp. and *Bacillus* sp. respectively. According to morphology, the fungal isolates FB4 and FD5 have been tentatively identified as *Aspergillus* sp.

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