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Research paper

Role of Actinomycetes in Turning Waste to Resource

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Abstract: Bioremediation techniques, which use naturally occurring microorganisms to eliminate hazardous organic chemical residues and contaminated areas, are constantly evolving. Actinomycetes have gained importance as they play a significant role in the recycling of organic matter and the production of novel pharmaceuticals, cosmetics, enzymes, antitumor agents, enzyme inhibitors, immune modifiers, and vitamins. Diverse Actinomycetes genera have the potential to be utilized in the bioconversion of underutilized urban and agricultural waste into high-value chemical compounds. This study was conducted to isolate actinomycetes capable of producing waste-degrading enzymes from floral waste vermicompost. In this study, floral waste was decomposed using the technique of vermicomposting. The floral wastes and cow dung in a ratio of 1:1 (50% each) were fed to *Eisenia fetida* earthworms for two months. Twenty actinomycetes were isolated and characterized morphologically using the soil dilution technique on starch-casein agar media. The amylase, protease, and peptonization-coagulation activities were determined through a screening procedure. Almost every isolating organism demonstrated maximum enzyme production. Actinomycetes growth patterns and mycelial coloration were documented. The cultural and morphological analyses identified the actinomycetes genera as *Streptomyces*.

Keywords: Floral Waste, Ecofriendly waste treatment, Actinomycetes, Enzyme production

Introduction:

Waste disposal is a major concern around the world. Diversity in the content of waste creates problems in its reduction. Safe disposal of floral waste has been a cause of concern for the temple management. The floral waste is directly disposed of into rivers, oceans, etc., which has a bad impact on the water quality as well as the living organisms present in the waters. Flowers come as waste from

hotels, wedding ceremony gardens, worship places, and various civil and sacred ceremonies, which make them a usual source of floral waste. Flowers are considered holy entities and are hence offered by pilgrims to their idols. Every day, these flowers offered by the devotees in temples are left unused and therefore become waste. This floral waste gets accumulated at religious sites like temples, mosques, and Gurudwaras due to a number of religious practices and is also generated in places like residential areas, community centers, and communities, etc.

The Latin word 'Vermi' means worm," and thus 'Vermicomposting' refers to composting with worms. In vermicomposting, various organic waste materials are broken down by worms, bacteria, and fungi. These organisms are nature's vitally useful tools to decompose organic materials. So, vermicomposting is a process that boosts nature's process of decomposing organic waste materials and produces a very useful end product. Vermicompost, the end product of the vermicomposting process, is a heterogeneous mixture of decomposed organic wastes, bedding materials, worm castings, decomposed worms, as well as other decomposing organisms, worm cocoons, etc. The worm castings, one of the major components of vermicompost, contain lower levels of contaminants and higher levels of nutrients than organic wastes do before vermicomposting. Vermicompost is rich in many water-soluble nutrients, which makes it an excellent organic fertilizer. It is a biological technique for converting organic wastes into a rich soil amendment. In a vermicomposting system, both earthworms and microorganisms play important roles in the decomposition and conversion of sludge (Yami et al., 2003). Relative to microbes, earthworms make a larger contribution to sludge stabilization through gut digestion, mucus production, and then casting. This makes earthworms significant in vermicomposting. Accordingly, many environmental factors, such as temperature, moisture, noise, and light, may also influence the growth of earthworms and thus modify the properties of the final products (Singh et al., 2011, Lee et al., 2018). Among these variables, temperature is considered one of the most critical factors affecting the growth and reproduction of earthworms (Marhan et al., 2015, Hackenberger et al., 2018). For instance, *Eisenia fetida*, a ubiquitous epigenetic species capable of vermicomposting, exhibited wide tolerance to a broad temperature range (15–25ºC) for their growth (Edwards, 1977). Previous studies also indicate high variability in the temperature used for vermicomposting, with most using room temperature, also suggesting a wide range of temperatures useful for treating sludge (Fu et al., 2015, Fu et al., 2016, Haung et al., 2018). Only a few publications clearly reported that 20°C was the optimal temperature condition for treating activated sludge (Hait et al., 2011). Given that the growth of earthworms is strongly associated with temperature, different regimes may also affect the quality of the vermicomposting product and its agricultural value. Hence, understanding the optimal temperature condition to operate vermicomposting of sludge is vital to driving the development of the vermicomposting industry. Although the use of earthworms is popular in vermicomposting systems, microorganisms also play important roles in decomposing and converting the organic matter present in waste into valuable resources. These include mainly bacteria, fungi, and actinomycetes. Actinomycetes are noteworthy as antibiotic producers, making three-quarters of all known products (Waksman, 1961). *Streptomyces* species are the most common genus in soils, accounting for up to 90% of the populations. However, new approaches for the isolation of actinomycetes from banana waste compost and their enzyme activity are significant during the thermophilic

composting of banana waste. Recently, the rate of discovery of new compounds from existing genera obtained from terrestrial sources has decreased, while the rate of re-isolation of known compounds has increased.

Thus, it is critical that new groups of microbes from unexpected habitats, particularly actinomycetes, be introduced during the vermicomposting of floral waste. Keeping this point in mind, the present study has been undertaken to isolate, screen, and evaluate the enzyme activity of mesophilic actinomycetes from floral waste vermicompost.

Materials and methods:

The floral waste (6kg) was collected from nearby temples in Ujjain (M.P.). The 6kg of cattle dung was obtained from a nearby local cowshed. The moisture content was maintained at about 70–80% so as to remove excess heat before preparation of vermicompost; otherwise, it can lead to the death of earthworms. The earthworms (*Eudrilus eugeniae*) were purchased from the local organic farming supplier, Agar, Ujjain. For the present study, separate vermicompost was made using 15 days old cow dung and floral waste. The flower waste was pre-composted to remove the aromatic compounds that might harm the survival of the earthworms, as suggested by Singh et al. (2011). Collected floral wastes, after being chopped into small pieces, were put into plastic cylindrical bins (27h x 21r) in the following ratio: 1:1 (50% FW: 50% CD (1kg FW+1kg CD + Earthworms) in duplicates labeled as A and B. The windrow compost method was used, in which it was not covered and no ventilation was provided with pipes. The physical parameters such as temperature (25-30^{o}C), pH (6-8) and moisture content (60–70%) were maintained throughout the study by regular sprinkling of water so as to provide optimum conditions for earthworms to survive and grow (Shouche et al., 2011). The study was carried out during the winter season. The experiment was maintained for 45–60 days until finely granular vermicompost was prepared. When the floral vermicompost was ready, 100g of sample from each bin was collected in sterile plastic bags, labeled, and kept for further study until pretreatment. The rest of the floral vermicompost was also labeled separately and stored in clean, dried, airtight containers at 4^{o}C.

Pretreatment: Collected samples from both bins A and B were air dried for 2 days and then heated in a hot air oven for 2 hours at 50ºC prior to isolation (Arifuzzaman et al., 2010). This helps in decreasing the population of gram-negative bacteria (Jeffrey, 2008).

Isolation of actinomycetes

Ten grams of each of the pretreated samples were suspended in 90 ml of sterile 0.9% normal saline separately in 100 ml conical flasks and shaken well in a vortex mixture to allow spore detachment. From each of these stock solutions, samples were serially diluted up to 10^{-4} and were used to spread on sterilized starch casein agar (SCA) medium (HiMedia) by using L-shaped glass rod and incubated at 30°C for 1–2 weeks. Itraconazole (75 µg/ mL) and chloramphenicol (50 µg/ mL) were added to both media to inhibit fungal and bacterial contamination, respectively. The media was procured from HiMedia (Mumbai). After 7 days, the plates were observed for actinomycetes colonies based on color, presence, and absence of aerial and substrate mycelium and subcultured on starch-casein agar plates and stored at 4°C for further studies.

Macroscopic Characterization

Morphological characteristics of isolates, such as colony color, size, shape, opacity, pigment production, and the presence or absence of aerial and substrate mycelium were observed (Duddu et al., 2016).

Microscopic characterization

Microscopy was performed with a simple stain technique using methylene blue (Kahasabuli and Kibera, 2014). A mass of colony-forming isolate was picked or

scooped using a sterile wire loop, spread on a clean glass slide, air dried, and fixed by passing it gently over a flame. The slide was flooded with methylene blue stain, and after a few seconds, excess stain washed off in running tap water. The slide was dried using blotting paper and observed using a light microscope under 40X and also under 100X using immersion oil. The nature of the hyphae and spore chain was observed and identified according to Shirling and Gottlieb (1966), and a photograph was taken.

Enzymatic screening

Amylase Production: The isolates were cultured on sterile starch media (HiMedia) for 5 days at 30ºC, and then they were tested for amylase production by flooding bacterial growth with iodine solution. The positive result was represented by the appearance of a clear zone around the isolates, which were surrounded by a purple background. **Protease Production:** Actinomycetes isolates were grown on casein agar media The plates were streaked by isolates and incubated at 30ºC for 4 days. The formation of a clear zone around Actinomycetes colonies represented positive results (Kavya et al., 2012). Peptonization and coagulation of milk: The milk coagulation and peptonization tests were carried out with skim milk. The skim milk containing test tubes were inoculated with isolates and incubated at 30ºC for 4 days. The extent of coagulation and peptonization was recorded on the fourth day.

Results and discussions:

From the two vermicompost beds A and B, 20 colonies were obtained on starch-casein-agar plates. The isolates from each sample collected and their codes are depicted in Table 1. A similar study was carried out by Pattnaik and Reddy (2012), where actinomycetes were isolated from floral waste vermicompost.

Table1: Actinomycete isolates from each soil sample collected from floral waste

Sample No.	Sample Code	No. of isolates	Isolates codes
1	VFWCD-A	11	ACT1-ACT11
2	VFWCD-B	9	ACT12-ACT20

Out of 20 isolates with distinct shape, size, color, and dominance, their morphological characteristics were recorded (Table 2). Macroscopic characters include colony characteristics such as size, shape, color, the absence or presence of aerial mycelium, etc. (Burkholder et al., 1954; Anusuya and Geetha, 2014). Thus, a total of eight out of 20 isolates were selected for further microscopic study and enzymatic screening. All eight actinomycetes isolates selected were subjected to microscopic study and characterized based on their type of spore chain morphology. All 8 isolates were identified as *Streptomyces* species (Table 3), as recommended in the International Streptomyces Project (Shirling and Gottileb, 1966). Spore chain morphology is one of the most important features in actinomycetes identification, and it varies by genus and species (Meena et al., 2013). All eight actinomycetes isolates were positive for amylase, protease production and showed peptonization and coagulation of milk (Table 4). Jeffrey (2008) also showed that approximately 98.4% of the total isolates produced one or more enzymatic activities. The potential of actinomycetes to secrete broad-range enzymes may have resulted from natural selection in order to survive in a competitive environment (Boroujeni et al., 2012).

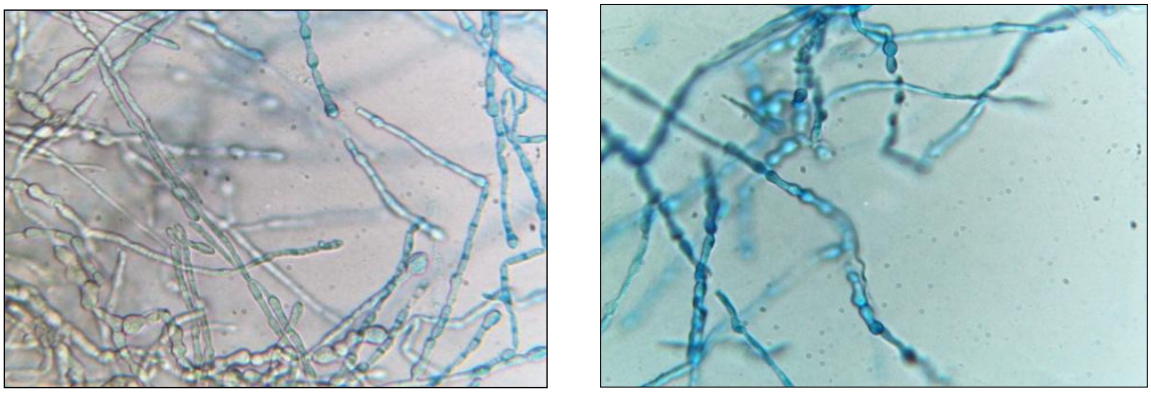

Fig.1. Spore chain morphology of some of the actinomycetes isolates

Table 2: Macroscopic characteristics of actinomycetes isolates

S. No.	Isolate	Colony Cultural characteristics	Mycelium
1	ACT1	Round smooth brownish white cottony colonies	Aerial & Substrate
2	ACT2	Bluish white, hard, bluish at centre ,	Aerial & Substrate
3	ACT3	Bright white small size cottony colonies	Aerial & Substrate
4	ACT4	Brownish white cottony colonies,	Aerial & Substrate
5	ACT5	Grayish black hard , grey at centre	Aerial & Substrate
6	ACT15	Round smooth white cottony colonies	Aerial & Substrate
7	ACT18	Small bluish white colonies	Aerial & Substrate
8	ACT20	Greenish white velvety colonies	Aerial & Substrate

Table3: Type of spore chain morphology of actinomycetes isolates

Isolate Code	Type of spore chain morphology	Species identified
ACT1	Rectus flexibilis (RF)	*Streptomyces* sp.
ACT2	Rectus flexibilis (RF)	*Streptomyces* sp.
ACT3	Straight Rectus (SR)	*Streptomyces* sp.
ACT4	Rectus flexibilis (RF)	*Streptomyces* sp.
ACT5	Spira Type	*Streptomyces* sp.
ACT15	Rectus flexibilis (RF)	*Streptomyces* sp.
ACT18	Rectus flexibilis (RF)	*Streptomyces* sp.
ACT20	Rectus flexibilis (RF)	*Streptomyces* sp.

Table4: Enzymatic production ability of Streptomyces isolates

Test	ACT1	ACT2	ACT3	ACT4	ACT5	ACT15	ACT18	ACT20
Amylase	+	+	+	+	+	+	+	+
Protease	+	+	+	+	+	+	+	+
Peptonization &coagulation of milk	+	+	+	+	+	+	+	+

Conclusion:

Vermicomposting technology could be broadly used for the management and recycling of nirmalaya or floral wastes, lowering the bulk and level of pollution at the generation site. It could be the best organic fertilizer for producing organic vegetables, organic fruits, and ornamental plants. Since vermicompost produces a high population of beneficial microflora, its application to cultivated land will also increase their population, increasing soil fertility and reducing pathogenic microorganisms, and the analysis will let us know the microbial diversity mainly involved in the decomposition process, which will be beneficial to prepare a microbial consortium culture that can be used to achieve even faster decomposition in a short period of time. Apart from bioremediation, actinomycetes also play an important role as biocontrol agents. At present, there is a need to find novel antimicrobial-producing strains as the pre-existing drugs have failed due to the development of resistance among the microorganisms. The present study is also a small contribution towards meeting this need. The study showed that actinomycetes are important agents in turning waste into a resource that can be used to produce antibiotics of medicinal value and enzymes of commercial importance.

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