Rapid Detection of Thermophilic Actinomycetes in Agaricus bisporus Compost Using Foldscope

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Abstract

Foldscope is the ultra-affordable, paper microscope designed to be extremely portable, durable, and to give optical quality similar to conventional research microscopes. However it's potential as in tool in the area of agricultural sciences is still not known. In this study, how foldscope can be used as an efficient tool to detect actinomycetes in the compost preparation process of mushroom cultivation. Foldscope images revealed white colored actinomycetes. Based on data the use of foldscope was recommended.

Keywords: Foldscope, Compost, Mushroom cultivation.

Introduction

Composting is a multistep process by a consortium of microorganisms, especially fungi, bacteria and actinomycetes, which inhabit the compost mass in a well defined succession for creation of a selective substrate for button mushroom cultivation. Under optimum conditions, composting proceeds through three phases: moderate temperature phase which last for few days; high temperature phase which last for about one month and last cooling or maturation phase which last for some days. Mushroom substrate preparation comprises chemical, biological and physical processes in which micro flora present in the substrate initially takes an active part [1]. During composting process, turnings of substratematerial is key step to maintain internal micro-environment of the substrate conducive for microbes, which last for about 28 days in long term process of compositing (LTC).

Due to aerobic fermentation brought about by microbes the temperature of the compost rises. It was reported that during composting ammoniagas is produced due to decomposition of protein further rises the temperature of compost formation to 65-70^{o}C and softens the straw [2].

A population of differentmicroorganisms and thermophilic flora are involved in composting process, however, the actinomycetes, especially thermophilic species, are well known components of the microflora of composts and give rise to whitish grey "fire gang" [3]. Actinomycetes are gram positive bacteria that mostly posses mycelium. Thermo actinomycetes inparticular is very important as it plays a leading role in decomposition of plants residues and converting soluble forms of nitrogen into microbial biomass. This microbial biomass and decomposed straw in the form humus-lignin complex both become a source of organic and inorganic nitrogen for mushroom cultivation. During composting process degraded straw is seems to be coated with microbial cell substances/biomass and their matrix [1]. This material regarded as nutrient rich substrate selective for mushroom mycelium.

Foldscope, is a paper based, portable microscope with magnification power of 140 X. it was invented by Prakash lab at Stanford University of America in 2014 [4]. Thereafter, Department of Biotechnology (DBT), Government of India and Prakash

Lab at Stanford University, USA earlier signed an agreement to bring the Foldscope to India to encourage curiosity in science. However the role of foldscope as an efficient tool in plant biology is not known worldwide so far. With this background, the present study was carried out to assess the usefulness of portable foldscope for in–situ detection of actinomycetes in mushroom composting. Due to its less cost, environmental friendly, small size, and portable in nature, can be widely used even by Plant Biologists in the field conditions.

Material and methods

Plant materials

Collection of samples

Mushroom compost was prepared according to various formulationsas described previously [5]. Compost was prepared at mushroom farm [under Unnat Bharat Abhiyan (UBA)] located in the vicinity of Lyallpur Khalsa College. Samples of compost to study microbial population were taken randomly as per different turnings as indicated earlier [3].

Image detection

Foldscope was used in current study, was supplied by Department of Biotechnology (DBT), Government of India [4]. Thin section of samples were taken on glass slides and covered with transparent cello tape/ cover slip. The slide was inserted into the foldscope in such a way that sample side was close to lens of foldcope. A LED light supplied with foldscope instruments was used a light source. The clear images under foldscope for each sample were photographed using cell phone camera (Samsung, Galaxy Tab A) by adjusting zoom and focusing of camera and foldscope.

Result and Discussion

Higher plants obtain nutrients from soil and synthesize the organic matter in leaves through photosynthesis. Fungi on the other hand grow in nature on all sorts of vegetable waste and they play an important part in maintaining the natural cycle. However, being heterotrophic, fungi mushroom draw their nutrition from substrate, known as compost [3]. Mushrooms are capable of breaking down organic matter that other microbes cannot break down. However, white button mushroom *Agaricusbisporus* has unusual substraterequirement compared to other commercially grown mushroom. Hence, preparing a suitable composted substrate for growing button mushroom is vital for successful crop [6].Composting is best approached through the system of ecsosyetm management. Starting with initial ingredients, composting requires the management of various chemical and physical factors, which select for succession of microbial population and substrate changes. The culmination of these successional environments is the development of dominate mushroom population. Composting is brought about by number of microbes like bacteria, fungi, actinomycetes). Among these thermophilic actinomycetes developed during the course of plant residues and form a selective substrate for development of *A.bisporus* [2]. These actinomycetes, therefore play a key role in process of composting and bring about selectivity of substitute. In this study, foldscope based analysis was carried out to detectactinomycetes which eventually developed during biological active phase of compost formation at various turnings(Fig 1)

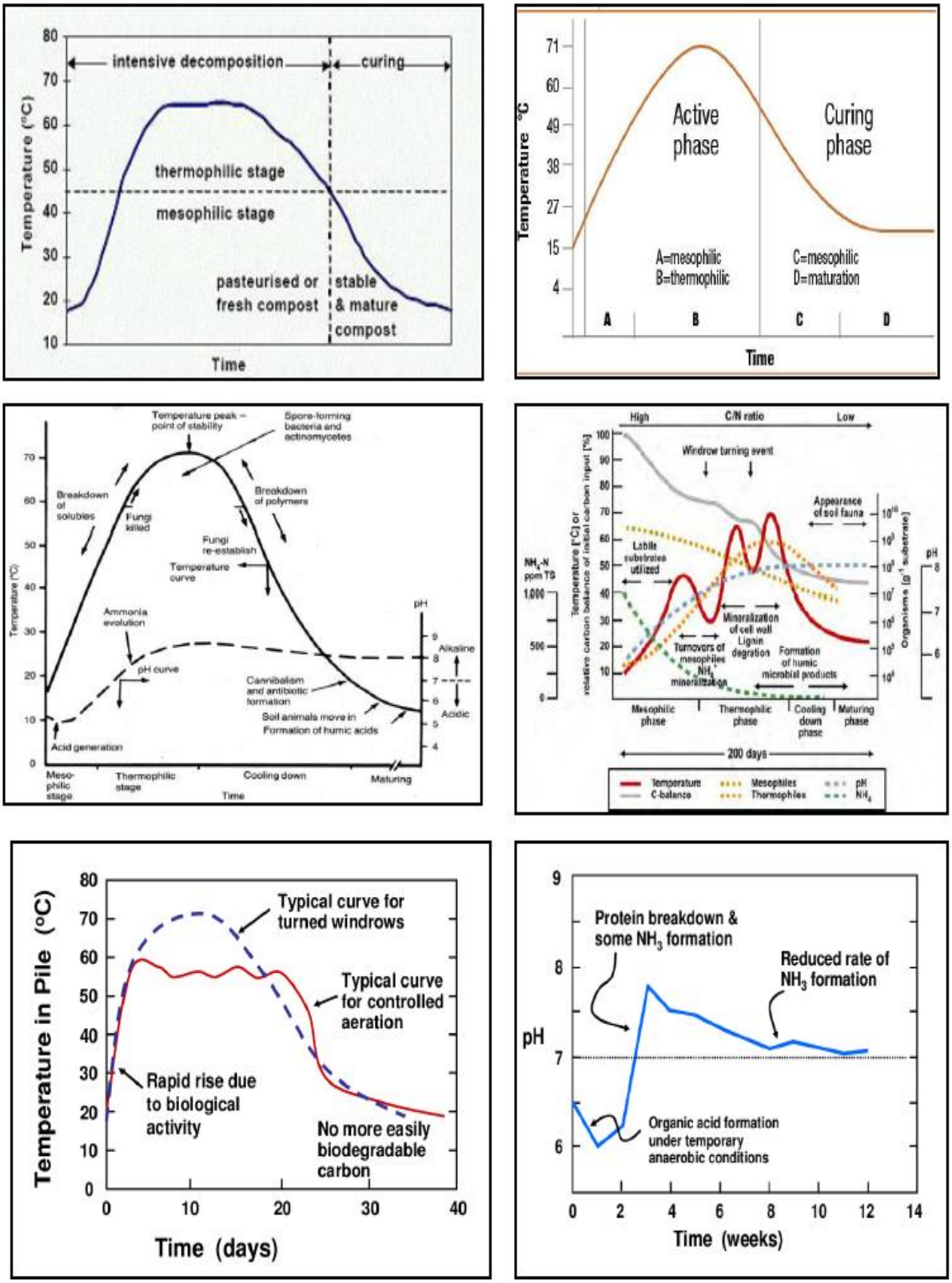

Fig 2: Dynamics of changes observed during composting

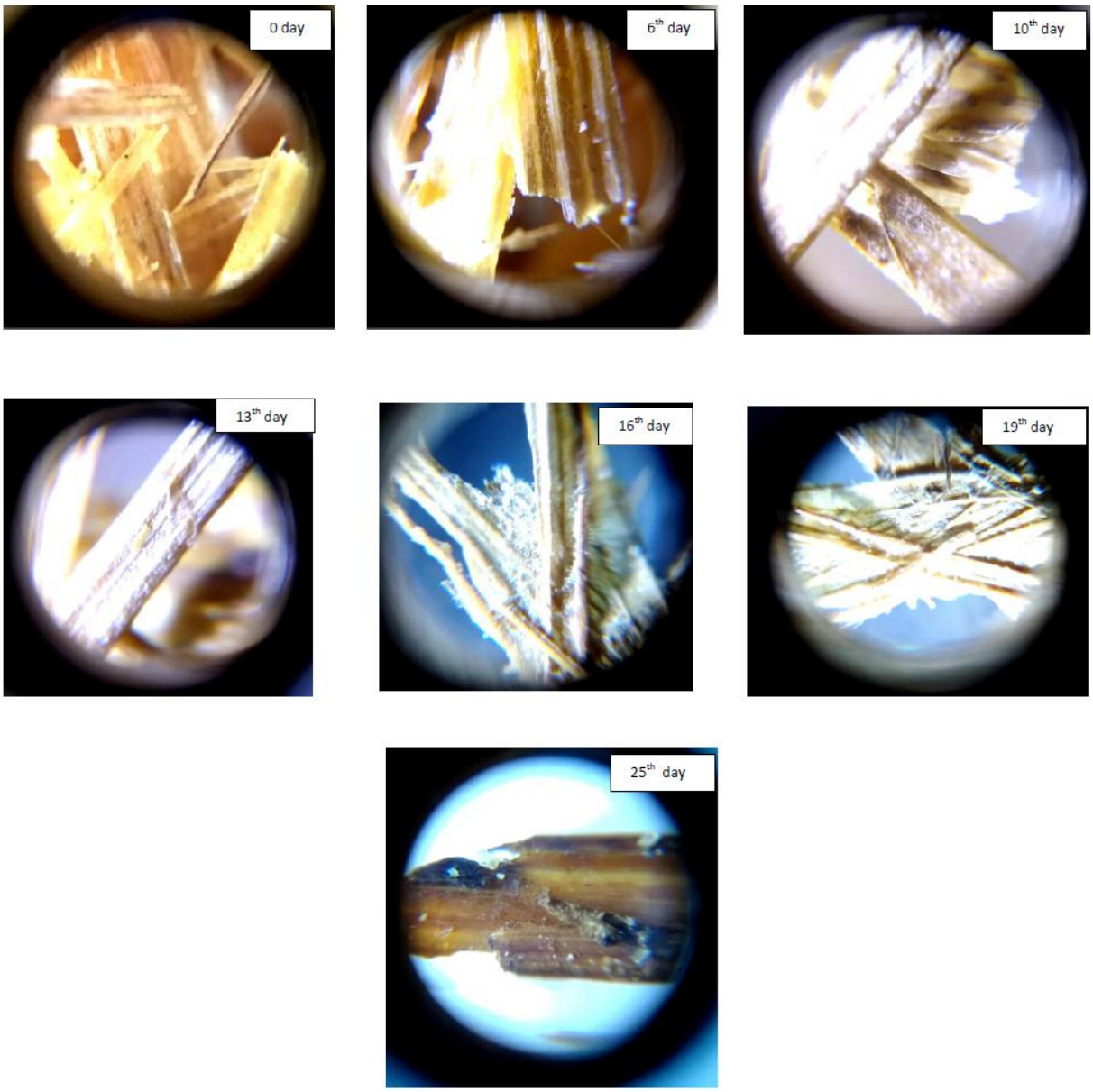

Fig:2 Foldscope view of compost at various turnings.

Since compost is an aerobic process, therefore, each portion should receive proper air and water supply. Hence, turnings at specific intervals are a key process for compost formation. It is coupled with steady rise in compost temperature, thermophilic microbial load, ammonia release and protein breakdown processes (Fig. 1). Other physical and chemical changes are illustrated in Fig 1. In addition, as shown in Fig. 2, at 0day of turning no actinomycetes were detected. However during the course of composting, actinomycetes started appearing. For instance: heavy growth of actinomycetes were detected from 13-19 days of turnings, which get disappeared at 25th day of turning, indicating the crucial role of this microbial community in composting process. It has been reported by previous studies that as the compost temperature, rises, a progressive reduction occurs in the number of mesophilic bacteria in favour of thermophilic spore formers like actinomycetes. Actinomycetes are abundant in compost and give rise to white grey "fire

fang", as indicated in our experiments (data not shown).

Based on these findings it was concluded that foldscope is an efficient tool to study microbial population during mushroom composting process. Further studies are required to identify specific strain of actinomycetes detected during compositing process.

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Conflict of interest

Authors declares no conflict of interest

Compliance with Ethical Standards

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors

Author contributions

ADS: designed the study and prepared manuscript

IK: designed the study and prepared manuscript

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