

Studies on the diversity of coprophilous microfungi from hybrid cow dung samples

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Abstract: Saprophytic and coprophilous fungal flora on dung samples of different hybrid cow in Lawspet area of puducherry, India was studied by moist chamber method during 2014-15. During the isolation and identification of fungi from hybrid cow dung samples, 16 species under 7 genera of hyphomycetous fungi were isolated with 44 isolates from the dung samples. Aspergilli and penicilli were dominated among all the isolates from the hybrid cow dung, but the populations of aspergilli were more in the dung samples in comparison to penicilli. *Aspergillus niger* was recorded with the maximum (20.45%) followed by *Aspergillus fumigatus* (11.36%) and *Aspergillus ochraceus* (9.09%). Other saprophytic fungi were recorded at minimal concentrations. *Absidia* sp., *Curvularia intermedia*, *Drechslera* sp., *Rhizopus stolonifer* and *Saccharomyces cerevisiae* were also observed from the dung samples during the study period. Relative occurrence of the isolated fungi from the dung samples showed the incidence of each fungus in the dung samples as well as its distribution in the dung. Aspergilli were recorded with seven species viz., *Aspergillus awamori*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. sydowii* and *A. terreus* and penicilli were recorded with four species viz., *Penicillium chrysogenum*, *P. digitatum*, *P. notatum* and *P. purpurogenum*.

Key words: Diversity, Coprophilous microfungi, Hybrid cow, Moist chamber method.

Introduction

Dung is a source of organic matter and also a potential home for saprotrophs. The fungi which germinate, grow and sporulate on dung are termed as 'coprophilous'¹. Herbivore dung suitably supports a wide variety of coprophilous fungi. The word coprophilous literally means "dung loving". Dung types of different herbivorous animals are partially digested highly complex, organic matter. They composed of the remains of ingested vegetation in the form of waste products, along with microbial population residing in the herbivore rumen. These dung types contain nitrogen which is as high as 4%, a three to four-fold increase over the ingested material². On dung, especially after voided off, the nitrogenous compounds influence the growth and fruiting of coprophilous fungi². Herbivore dung generally has a higher pH, usually above 6.5. The high pH has some desirable effect on the fungi to make them sporulate and grow over this dung. Further, coprophilous fungi posses a wide variety of characteristics that assist their survival and reproduction on nutrient rich dung substrate. Although fungi are reported from all types of dung, herbivore dung is considered as rich repository of coprophilous mycoflora³. Bacteria play an important role in the decomposition of carnivore and omnivorous dung⁴. Earlier works on dung mycoflora were done in few countries but this work is very rare in India. Moreover, no such work was done on hybrid cow dung samples which are mainly composed of cellulose fibers.

The present work deals with the isolation and identification of saprophytic as well as coprophilous fungi from different dung samples of hybrid cow in Pondicherry, India.

Materials and Methods

Isolation and identification of coprophilous fungi from different dung samples of a domesticated animal viz., hybrid cow in and around of Lawspet area of Puducherry, India was carried out in the Microbiology Laboratory, Department of Botany, K. M. Centre for Post Graduate Studies (Autonomous), Lawspet, Pondicherry - 605008, India during the year 2014-2015.

Isolation and identification of fungi

The dung samples were subjected to isolation and enumeration of saprophytic and coprophilous fungi by moist chamber method. Each dung sample of the domesticated animals was kept in moist chamber plates equidistantly with wide space for the growth of fungi. They were incubated for 5 – 15 days at $25 \pm 3^{\circ}\text{C}$ for fungal growth and sporulation. The moist chamber plates don't need any type of medium for the growth of saprophytic as well as coprophilous fungi from the dung samples. In this method, the fungi grow on its own on the host, getting the moisture produced from the wet condition prevailing inside the blotting paper coated petriplates. All the plates were incubated at $25 \pm 3^{\circ}\text{C}$ temperature in the incubation chamber. Incubation time was maintained differently since, 7-8 days is meant for the fungal growth of fungi in agar plate method, but in moist chamber method, 1 to 3 weeks are required for the growth of fungi. Every day watch of the petriplates and check the growth of fungi was almost necessary in our present study after 3rd day of incubation. A direct isolation method was carried out by using a fine sterile needle to pick up the fungal hyphae and spores from the dung surface under a stereomicroscope. A hyphal tip also was transferred to a slant of Sabouraud's dextrose agar (PDA) and kept as pure cultures in the Microbiology Laboratory, Department of Botany, K. M. Centre for Post Graduate Studies (Autonomous), Pondicherry, India. Identification was made based on morphological characteristics, such as colony growth pattern, colour, texture on different agar media and spore ornamentation which was examined by trinocular Olympus photographic light microscopy by the help of taxonomic expertise and available manuals^{5,6,7,8,9,10,11}.

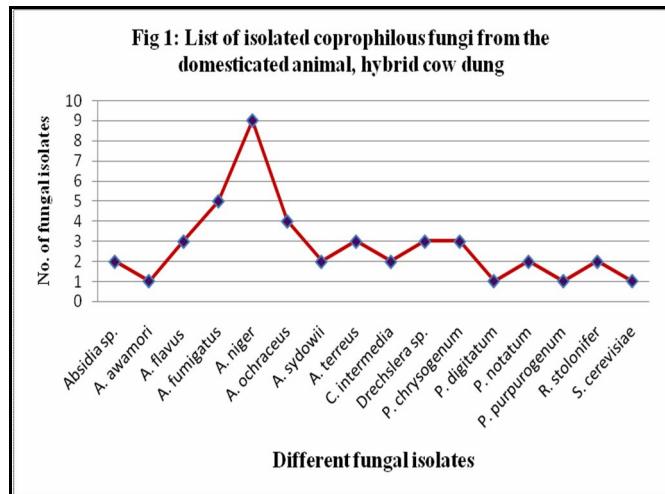
Results and Discussion

During the isolation and identification of saprophytic and coprophilous fungi from hybrid cow dung samples, 16 species under 7 genera of hyphomycetous fungi were isolated with 44 isolates from the dung samples (Table 1). Aspergilli and Penicilli were dominated among all the isolates from the hybrid cow dung, but the populations of aspergilli were more in the dung samples in comparison to penicilli. *Aspergillus niger* was recorded with the maximum (20.45%) followed by *Aspergillus fumigatus* (11.36%) and *Aspergillus ochraceus* (9.09%). Other saprophytic fungi were recorded at minimal concentrations. *Absidia* sp., *Curvularia intermedia*, *Drechslera* sp., *Rhizopus stolonifer* and *Saccharomyces cerevisiae* were also observed from the dung samples during the study period. Relative occurrence of the isolated fungi from the dung samples is given in Fig 1, which showed the incidence of each fungus in the dung samples as well as its distribution in the dung. Aspergilli were recorded with seven species viz., *Aspergillus awamori*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. sydowii* and *A. terreus* and penicilli were recorded with four species. Hyphomycetes group of fungi were commonly isolated in our present study, which is attributed that they may belong to coprophilous fungi but, Seifert et al¹² stated that hyphomycetes might be coprophilous fungi, but may be the contaminants of the air or soil, deposited over the dung after its release on the ground. They also stated that some hyphomycetes were known only from dung, such as *Arthrobotrys*, *Basidimbia* and *Oedocephalum*. Richardson¹³ observed from his study on coprophilous ascomycetes on different dung types and found 137 ascomycetes on the dung samples of sheep, horse, cow, roe deer, rabbit and hare. Fungi like, *Phomatospora coprophila* sp. nov., *Ascophanus microsporus*, *Podospora cumula*, *Coprobia granulata* and *Ascobolus immersus* were associated predominately with ruminant dung. *Podospora appendiculata*, *Thelebolus stercoreus* and *Sporormia intermedia* occurred more frequently on lagomorph dung, whilst *T. nanus*, *P. vesticola*, *A. albidus* and *Saccobolus versicolor* were frequent on all dung types¹³. Fungi are well known agents of decomposition of organic matter, in general, and of cellulosic substrates in particular^{2,13}. Works on isolation of coprophilous fungi from the dung environment has been done by Krug and Jeng¹⁴. Different people have done number of works on dung mycoflora of various herbivores all over the world. Manoch et al¹⁵ recorded two

isolates of *C. irregularis* on rabbit and toad dung and five isolates of *A. candidus* from deer, banteng, bird and chicken dung from Huay Khang Wild Life Sanctuary, Uthaithani province. Seifert et al¹² reported *Arthrinium phaeospermum* on millipede pellets, *Arthrobotrys oligospora* on sheep, frog, bird and horse dung, *Cephaliophora irregularis* on ass, mouse and monkey dung, *Cladosporium cladosporioides* on horse, *Memnoniella echinata* on cow and rabbit dung, *Nodulisporium* sp. on cow, *Oidiodendron* spp. Richardson¹⁶ opined that the vegetation generally possesses certain amount of other fungal spores from the vicinity, which in turn is taken up by the grazing herbivores along with the spores of coprophilous fungi. However, the harsh conditions in the alimentary canal, to which these spores are subjected, provide no prospect of survival for fungi other than coprophilous^{17,18}. Bell² mentioned three hyphomycetes from dung samples viz., *Athrobotrys oligospora*, *Penicillium claviforme* and *Sepedonium* sp. Subramanian¹⁹ stated that hyphomycetes were a normal component of the mycoflora of dung and animal excrements, including birds and mammals, both herbivores and carnivores.

Table 1: Relative occurrence of coprophilous fungi isolated from hybrid cow dung.

Sl. No.	Isolated fungi	Percentage Occurrence
1	<i>Absidia</i> sp.	4.54
2	<i>Aspergillus awamori</i>	2.27
3	<i>A. flavus</i>	6.81
4	<i>A. fumigatus</i>	11.36
5	<i>A. niger</i>	20.45
6	<i>A. ochraceus</i>	9.09
7	<i>A. sydowii</i>	4.54
8	<i>A. terreus</i>	6.81
9	<i>Curvularia intermedia</i>	4.54
10	<i>Drechslera</i> sp.	6.81
11	<i>Penicillium chrysogenum</i>	6.81
12	<i>P. digitatum</i>	4.54
13	<i>P. notatum</i>	2.27
14	<i>P. purpurogenum</i>	2.27
15	<i>Rhizopus stolonifer</i>	4.54
16	<i>Saccharomyces cerevisiae</i>	2.27



Conclusion

During our study, most of the isolated fungi belonged to saprophytes and considered as coprophilous based on their growth on hybrid cow dung in Pondicherry city. The moist chamber method was found to be suitable one to record coprophilous fungi from the dung samples. Saprophytic fungi like aspergilli and penicilli were more common in the dung samples followed by *Absidia* sp., *Curvularia intermedia*, *Drechslera* sp.,

Rhizopus stolonifer and *Saccharomyces cerevisiae* were isolated moderately from the hybrid cow dung and *Aspergillus awamori*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus* and *A. terreus* were the most common species and were found from all the dung samples.

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