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Coprophilous Fungi and Their Utilities

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ABSTRACT: Coprophilous fungi (dung-loving fungi)^[1] are a type of saprobic fungi that grow on animal dung. The hardy spores of coprophilous species are unwittingly consumed by herbivores from vegetation, and are excreted along with the plant matter. The fungi then flourish in the feces, before releasing their spores to the surrounding area. Coprophilous fungi release their spores to the surrounding vegetation, which is then eaten by herbivores. The spores then remain in the animal as the plants are digested, pass through the animal's intestines and are finally defecated. The fruiting bodies of the fungi then grow from the animal feces.^[2] It is essential that the spores of the species then reach new plant material; spores remaining in the feces will produce nothing. As such, some species have developed means of discharging spores a large distance.^[3] An example of this is the genus *Pilobolus*. Fruiting bodies of *Pilobolus* will suddenly rupture, sending the contents over 2 metres away.^[4]

Animal feces provide an environment rich in nitrogenous material as well as various enzymes from the animal's digestive system. The spores themselves survive digestion by being particularly thick-walled, allowing them to germinate in the dung with minimum competition from other organisms.^[2] This thick wall is often broken down during digestion, readying the spore for germination.^[1] The spores are so hardy that samples of dried dung can later be rehydrated, allowing the fungus to fruit weeks later.

KEYWORDS: coprophilous, fungi, dung, loving, herbivores, faeces

I.INTRODUCTION

Animal dung is a special substrate for fungi. Fungi growing thereon have been called coprophilous (or sometimes coprophilic). The term is derived from two Greek terms, viz. copros = dung; and philous = having a love for, preferring. (The word fimicolous, to denote the same habitat preference is derived from the Latin *fimicus* or *fimum* = dung, and *cola* – inhabiting, however coprophilous is the term most often used in the scientific literature, and this usage is also followed in this essay.) Some fungi occur exclusively on dung, whereas other species occupy broader niches, also occurring on certain forms of organic matter. Most coprophilous fungi are found on dung of herbivores, both wild herbivores and domesticated herbivores like cattle, horses and sheep. Rabbit dung is also rich in coprophilous fungi; [1,2,3] as it constitutes a tractable substrate for experimental studies, it has frequently been studied. Rabbit dung can be easily converted into so-called copromes, standardised dung pellets created through collecting, drying, powdering, sterilising and reconstituting these to pellets (Wood & Cooke, 1984). It needs to be assessed whether a similar technique will also be beneficial when studying fungi on dung of larger domesticated herbivores. While copromes have been mainly used in the study of fungal succession on dung, their use could also be beneficial for a range of other questions, e.g. the role that interference competition plays in the upregulation of the production of antimicrobial compounds (Bills et al., 2013), or the role that species mixtures play in enhancing or reducing dung degradation rates. Compared to the dung of herbivores, pig dung is not known to be rich in coprophilous fungi. Dung of carnivores and dung of birds is generally also (very) poor in these fungi. The most likely explanation is that dung of these organisms contains mostly easily degradable compounds and low amounts of lignin, as a consequence of which coprophilous fungi (and especially coprophilous Basidiomycota) are either outcompeted or do not have sufficient time to complete their life cycle before the dung pellet is degraded. Coprophilous fungi have generally been linked to dung of endothermic (warmblooded) animals. In its natural habitat animal dung is usually found as smaller to larger individual resources, but due to animal husbandry dung may be collected and, mixed with plant residues, be piled as manure heaps. Both classes of substrates (dung pellets, manure heaps) partly select for different fungi. Manure heaps are likely to heat during the composting process, resulting in a strong selection for a small number of thermophilic or thermotolerant fungi. Dung of different animal species usually harbours its own fungal community. Such differences arise from the different food items that the animals have been consuming (Kruys & Ericson, 2008), from differential selection during gut passage, and from the properties of the dung when excreted. Important recent publications dealing with coprophilous fungi are Krug et al. (2004) and Doveri (2004). Many coprophilous species germinate only after passage through the animal gut. Coprophilous fungi therefore have adaptations that maintain their viability in such hostile environments.[4,5,6] Many species have thick and dark walls, while the spores of some species are covered by a



gelatinous sheath. After gut passage and deposition of dung, coprophilous fungi develop and form spore-bearing organs, called fruitbodies. From these fruitbodies spores are actively discharged. They often land on nearby vegetation (and the gelatinous sheath likely facilitates attachment), which then increases the chances that the spores are consumed with the vegetation. However, not all species that occur on dung have a life cycle that is dependent on passage through the animal gut. Several grassland fungi also occur on dung and separating true coprophilous fungi from these subcoprophilous fungi (Griffith & Roderick, 2008) is not always easy. Coprophilous fungi are most common in grasslands (Griffiths & Roderick, 2008), however they can occur in every habitat where large and smaller herbivores defecate, including dunes, heathlands and forests. Several coprophilous fungi have also been reported to have the ability to live as plant endophytes (Herrera et al. 2011; Newcombe et al. 2016). Occurrence of coprophilous fungi from surface-sterilised plant tissue had been reported before, however it had remained unclear whether these fungi were incidental contaminants that were not killed by alcohol or bleach (which may even have enhanced spore germination, just like gut passage might achieve) or whether these fungi were true endophytes. Newcombe et al. (2016) provided evidence for an endophytic life style of *Sordaria fimicola* and also for negative fitness effects on the growth of the grass *Bromus tectorum*. However, Griffith et al. (2017) reported that the same fungus was more common on the same grass species under more drought-prone environments, suggesting a possible role of the endophyte in drought tolerance. It has also been reported that *Sordaria fimicola* can reduce symptoms of the cereal disease take-all in rye and wheat (Dewan et al., 1994). The endophytic life style, which likely causes higher fungal selectivity towards certain plant species, could also be an explanation for the positive correlation between the number of plant species foraged by herbivores and the number of coprophilous species found on the dung of these herbivores. Endophytic occurrence has also been reported for subcoprophilous fungi such as *Psilocybe semilanceata* (Keay & Brown, 1990). Dung is often characterised by its high amounts of nitrogen and also phosphorus; from a stoichiometric perspective dung has a low N:P ratio, much lower than is needed from the perspective of fungal demand (and plant demand as well). There are only few studies that have linked the occurrence of coprophilous fungi to dung C:N ratio. Richardson (2001) listed C:N ratios of dung of five mammal species (sheep, deer, cattle, rabbit and hare)[7,8,9] ranging between 20 and 30. It is likely that these dung samples came from animals that were fed with relatively nitrogen-poor and lignin-rich plant material. Cattle that is fed a more nitrogen-rich diet has lower C:N ratios, often ranging 10-15, whereas pig manure has even lower C:N ratios, up to 6. Even though dung is enriched in P, focus has been on N-content in dung rather than on P-content as an explanation for the fungal specificity or selectivity for dung. Because of its high content of mineral nutrients, often accompanied by easily degradable carbon compounds, dung is a habitat with intense competition between fungi and bacteria, and between different species of fungi. As a consequence of the saprotrophic life style the dung is degraded – although there do not seem many studies that have assessed the decomposition process of various types of dung (Nagy & Harrower, 1980) and the enzymes that are responsible for the degradation of cellulose and lignin, as some of these could have biotechnological application (see below). After dung has been deposited a succession of fungal species has been observed (Richardson, 2001; Richardson, 2002). Succession has most often been studied on the basis of the appearance of reproductive structures. The first fungi to appear are members of the Mucoromycota (*Pilaira*, *Pilobolus* – Fig. 1). Species of *Pilobolus* take somewhat more time than *Pilaira*, on average 6.5 compared to 3.5 days, before their fruitbodies are visible (Richardson, 2002).

II.DISCUSSION

Coprophilous fungi are a large group of fungi mostly found in herbivore dung and have an exclusive life cycle. This group of fungi produces many important metabolites which can be consumed in medicine or agriculture. The present study aimed to investigate the antibacterial effects of these fungi on bacterial mastitis. A total of 50 dung samples were collected from four herbivores (cows, buffalos, sheep, and camels) from different areas of Basra. The moist chamber method was used for each sample to establish a fungal fruiting body and detect the type of the fungi. The coprophilous fungi included *Aspergillus sp.* (*A. niger*, *A. fumigatus*, *A. flavus*, *A. terreus*), *Chaetomium sp.*, *Sordaria sp.*, and *Podospora sp.* which belong to the Ascomycetes class.[10,11,12] PCR test was performed using the ITS region for confirmatory detection of species. The highest and the lowest number of isolated species was associated with cow dung and camel dung, respectively. The antimicrobial property of three different partitioned extracts (petroleum ether [F1], ethanol [F2], and water [F3]) prepared from some fungal mycelia was evaluated in vitro. All fractions were tested to detect antimicrobial activity using the disc diffusion assay against five pathogenic bacteria *Staphylococcus aureus*, *Streptococcus Enterobacter*, *Proteus mirabilis*, and *E. coli*. which is isolated from bovine mastitis. Data revealed that all fractions could inhibit the tested bacteria. However, inhibitory activity was found to be dependent on (i) the used fungal strains; (ii) the extracted solvent; and (iii) the tested bacteria. In general, the petroleum ether extracts (F1) derived from all fungi displayed the highest inhibitory activity against the testing bacteria. The extracts prepared from the fungal mycelia contain bioactive compounds with antibacterial properties. This study was first conducted in Iraq and further studies are required to develop new treatments.



III.RESULTS

Malachite green in screening experiments and its degradation using varied species of coprophilous fungi obtained from dung samples of different herbivores viz. cow, goat, camel, elephant, horse found in Jaipur. For this purpose, the dye effluent from industries of Sanganer were collected and concentrations of Malachite Green dye were determined. These concentrations of MG were used for degradation using coprophilous fungal spp in vitro A total of 9 coprophilous fungal species were isolated from these dung samples. The cowdung sample showed maximum number of coprophilous fungi (8) followed by horse dung sample (6). Minimum number of coprophilous fungi were found in elephant dung sample (3). The coprophilous fungal spp found in herbivore dung samples were *Rhizopus stolonifer*, *Mucor racemosus*, *Oidiodendron grieseum*, *Geotrichum candidum*, *Phoma betae*, *Chaetomium globosum*, *Microascus cinereus*, *Chrysosporium tropicum* & *Scopulariopsis brevicaulis*. Some species were specifically found in particular dung sample only while some fungal spp were common to maximum herbivore dung samples. The present study lays down the best degradation by *Rhizopus stolonifer* at varied MG dye concentrations of 4ppm, 7ppm and 10 ppm. It can be inferred that varied concentration range from 1ppm to 10 ppm can be easily degraded by *R. stolonifer*. By experimental studies it has been concluded that low concentrations of MG (1ppm to 4ppm) can be easily degraded within 3 days.[13,14,15] The concentrations ranging from 5ppm to 7ppm can be degraded till 5 days and higher concentrations 8ppm to 10 ppm can be degraded within 9 days. This proves that *R. stolonifer* is the best decolorizer and degrader of MG utilized in textile dye industry in general concentrations of 1 to 10 ppm. Further investigations can be utilized in screening of varied concentrations of other textile dyes using *Rhizopus stolonifer*. The future scope of the study is the degradation of varied textile dyes using coprophilous spp of fungi which are easily available in herbivore dung samples. The different textile dyes at various concentrations can be screened and decolorized using coprophilous spp. Thus biodegradation using coprophilous fungi with cost effective and cheap methodology can prove a boon in decolorization experiments and treatment of textile wastewaters

Coprophilous fungi have been known to competitively interfere with other fungi, producing chemical agents that impair the ability of rival species to access resources.^[13] There is evidence to suggest that slower-growing fungi, such as *Poronia punctata*, employ antagonistic strategies more often in order to hamper the reproductive potential of quicker-growing fungi in dung.^[13] *Podospora appendiculata*, itself a slow-growing fungus, has likewise been shown to produce three molecules with antimicrobial properties: Appenolide A, Appenolide B, and Appenolide C.^[5] Each molecule is a 2(5H)-furanone.^[5]

Appenolides A, B, and C display microbicidal activity against a variety of fungi, with 150 micrograms of each compound enough to produce 12-14 millimeter zones of inhibition against *Candida albicans* in standard disc assays.^[5] Similar antifungal effects were noted with all Appenolides against the coprophilous fungi *Sordaria fimicola* and *Ascobolus furfuraceus*.^[5]

Furthermore, Appenolides B and C exhibited additional antibacterial properties in disc assays against *Bacillus subtilis*. Zones of inhibition of 8 millimeters were noted at concentrations of 150 micrograms per disk.^[5] The exact mechanisms of bacterial inhibition for Appenolides B and C remain unknown, but other 2(5H)-furanones appear to interfere with bacterial growth by blocking the activity of N-Acyl homoserine lactones and autoinducer 2 (AI-2), signalling molecules that help mediate quorum sensing.^{[14][15]}

Quorum sensing, a process which allows for differential gene expression in response to changes in cell density, can trigger bacterial biofilm formation when bacteria are present in sufficiently high concentrations.^[15] Biofilm formation in turn drives resistance to a range of environmental and biological stressors, including antibiotics and human immune responses, and the 2(5H)-furanone-mediated disruption of quorum sensing has been shown to negatively impact the growth rate of *Campylobacter jejuni*, a clinically significant food-borne pathogen.^[15] 2(5H)-furanone derivatives have also demonstrated in vitro bactericidal effects against *Mycobacterium tuberculosis* and methicillin-resistant *Staphylococcus aureus*, two species that have demonstrated increased resistance to more traditional antibiotics.

Coprophilous and litter-decomposing species (26 strains) of the genus *Coprinus* were screened for peroxidase activities by using selective agar plate tests and complex media based on soybean meal. Two species, *Coprinus radians* and *C. verticillatus*, were found to produce peroxidases, [16,17,18] which oxidized aryl alcohols to the corresponding aldehydes at pH 7 (a reaction that is typical for heme-thiolate haloperoxidases). The peroxidase of *Coprinus radians* was purified to homogeneity and characterized. Three fractions of the enzyme, CrP I, CrP II, and CrP III, with molecular masses of 43 to 45 kDa as well as isoelectric points between 3.8 and 4.2, were identified after purification by anion-exchange and size exclusion chromatography. The optimum pH of the major fraction (CrP II) for the oxidation of aryl alcohols was around 7, and an H₂O₂ concentration of 0.7 mM was most suitable regarding enzyme activity and stability. The apparent *K_m* values for ABTS [2,2'-azinobis(3-ethylbenzthiazolinesulfonic acid)], 2,6-dimethoxyphenol,




benzyl alcohol, veratryl alcohol, and H₂O₂ were 49, 342, 635, 88, and 1,201 μM, respectively. The N terminus of CrP II showed 29% and 19% sequence identity to *Agrocybe aegerita* peroxidase (AaP) and chloroperoxidase, respectively. The UV-visible spectrum of CrP II was highly similar to that of resting-state cytochrome P450 enzymes, with the Soret band at 422 nm and additional maxima at 359, 542, and 571 nm. The reduced carbon monoxide complex showed an absorption maximum at 446 nm, which is characteristic of heme-thiolate proteins. CrP brominated phenol to 2- and 4-bromophenols and selectively hydroxylated naphthalene to 1-naphthol. Hence, after AaP, CrP is the second extracellular haloperoxidase-peroxygenase described so far. The ability to extracellularly hydroxylate aromatic compounds seems to be the key catalytic property of CrP and may be of general significance for the biotransformation of poorly available aromatic substances, such as lignin, humus, and organopollutants in soil litter and dung environments. Furthermore, aromatic peroxygenation is a promising target of biotechnological studies.[19,20,21]

IV.CONCLUSION

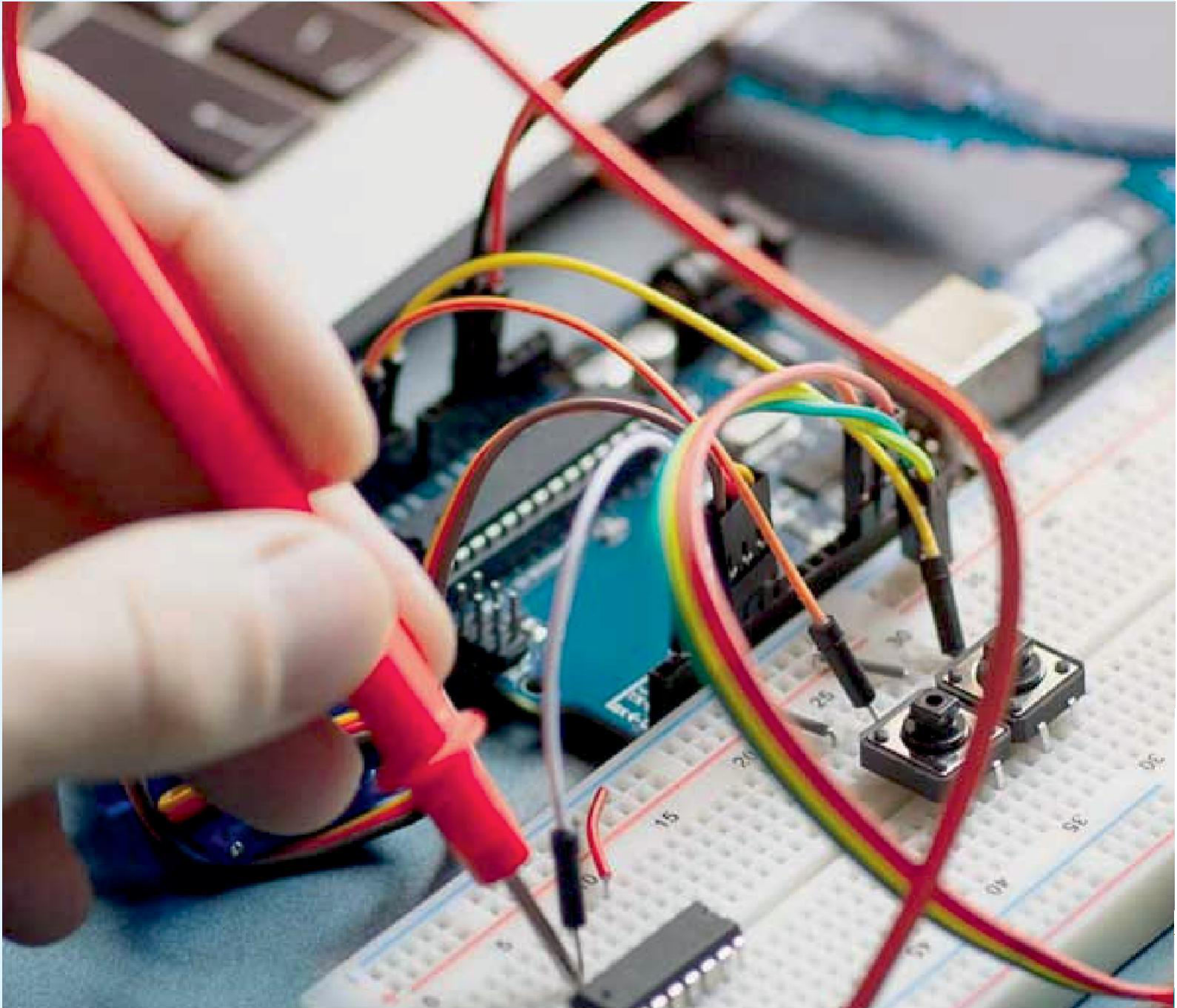
In nature manure is recycled by unique fungi (coprophilic fungi), which are capable of growth on substrates with high nitrogen contents. They bind a lot of the nutrients and in a delayed release they are making these nutrients available for plants, animals and insects, thereby closing nutrient cycles. This may provide opportunities for processing of manure. Within project KB-40-005-008; Closing the loop: improving circularity with manure-loving mushrooms), part of the Investment theme Connected circularity, we have been provided with the opportunity to work on a this topic.[22,23,24] A literature study was performed on the options that coprophilic fungi offer. It focused on the taxonomic and ecological knowledge of coprophilous mushrooms present in the Netherlands and on the threats of fungal diversity on dung. Next to this the literature study focusses on the options that coprophilous fungi offer as a source of secondary metabolites or enzymes. Furthermore it briefly focusses on an overview of genomes available of coprophilous fungi. The literature study is finalized with a brief outlook towards possibilities of using coprophilous mushrooms in a circular agriculture system. In the second part of the project we able to build a collection of coprophilic basidiomycete strains comprising of 38 strains distributed over at least 23 species. Limited tests of their ability to grow on a small range of types of manure demonstrated growth of 23 strains on chicken manure (ranging from limited growth to abundant growth). A total of 19 strains showed growth on cow manure (again ranging from limited growth to abundant growth). Pig manure was least favorite in our experiments, with only 4 strains showing growth with different abundances. [25,26,27]We believe that this project will provide a starting point for a study of applicability of coprophilic fungi in circular agriculture.[28]

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