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Isolation and Identification of Fungi Located in Horse Manure

KEYWORDS: Isolation fungi,horse manure

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ABSTRACT *The present study was included the isolation and identification of fungi located in horse manure were collected from horses located in the Equestrian Club in Jadriya – Baghdad during fourmonths (July .August,September. and October). Fifty samples of horse manure were collected belonging to sixteen genera were isolated using Plating technique. Aspergillus genus was the most frequent and occurrence (98%), followed byRhizopus (86%) and Mucor (78%).the less frequent and occurrence distribution between theMonillia (52%) .Absidia(18%)andSyncephylum(14%).*

Introduction

Manure commonly includes various fungi. Apart from microbial colonists of the Gastro Intestinal Tract (GIT), the fungi of manure include fungi accidentally consumed, and that are specifically adapted to manure as a resource. Manure is a very rich medium for fungal growth. It consists of the remains of plant material plus the microbiota associated with its digestion. Much of the material contains readily available carbohydrate in addition to cellulose and lignin. The material is complex, and includes fatty acids, vitamins and amino acids. In addition, initially, the waste is moist, and the pH is close to neutral. Manure is an ideal medium for microbial growth.However, most studies of the ecology of manure have noted that the fungi that fruit on the surface consist of a group of recognizable taxa commonly including (Zygomycota ,Ascomycota ,Basidiomycota)(Bell,2005).

Some observers have noted a true ecological succession on manure in that first the Zygomycetes, then the Ascomycetes, and finally the Basidiomycetes appear.

Manure fungi are important components ofecosystems, actively participating in the cycling of nutrients inanimal excrements (Richardson, 2008). These organisms often grow andreproduce under a narrow range of conditions, occurring atdifferent temperatures, pH and moisture levels, and are important biological control agents, source of enzymes andantibiotics(Dix and Webster,1995).

Recently Scientists have discovered that the fungi grows on manure plays a big role in breaking down the plant material — a process that, in the biofuel industry, is expensive. But taking a cue from the fungi could provide a cheaper way (Jesse,2013). There for this study aims to isolation and identification the fungi located in horse manure.

Methods

1- Samples collection:

Samples were takenfrom horse manure were collected from horses located in the Equestrian Club in Jadriya– Baghdad and investigated from (July to October). Itwerecollected one or two times per month. The samples are placed in sterile sealed containers and were transferred to laboratory.

2-Media Preparation

Potato dextrose agarused for isolation and Czapecdoxe agar used for identified were prepared according to the manufacturing company instructions. Autoclaved at 121°C and pressure 1.5 kg/cm 2 for 15 min, after cooling the media , a 500mg/L of cycloheximide and 50 mg/L of chloramphenicol were added . (Detandt and Nolard ,1995) .

3-Isolation by plating technique:

Treatment the sample of horse dropping as a sample of soil. One gm of collected horse manure sample was added to 99 ml of sterile distilled water in a 250 ml Erlenmeyer flask and kept in a mechanical shaker at 120 rpm for 15 min. Serial dilutions up to 106 was done. Potato dextrose agar (PDA) medium using for the isolation.Filter sterilized chloramphenicol (50 mg/L) was added and thoroughly mixed to avoid bacterial growth. One ml aliquots of 10-1 – 10-6 dilutions was pipette out into sterilized petridishes and about 15 ml of Potato dextrose agar medium was pour plated in duplicate. The dishes were then rotated clockwise and anti-clockwise for uniform distribution of the samples. The solidified plates were incubated at 30°C in an incubator from 5-10 days (Karthikeyanet al.,2012).

4-Single hyphal tip method of purification (Mundkur, 1959)

Czapek-Dox agar was prepared,and dispensed onto sterile petridishes. After solidification, peripheral mycelia from the slants were carefully lifted and stabbing on to CzA plates and incubated at 30°C for 5 days. After incubation, the colonies were observed for hyphal developments. The peripheral tip of the mycelial growth was taken from the plates, reinoculated onto CzA medium and incubated at 30°C for 5 days (Jaivel and Marimuthu ,2010).

5- Fungi identification

Isolation and identification of fungi were achieved by macroscopic colony characterization and microscopic examination. For characterization of the morphology of fungal isolates; slides prepared from cultures were stained with bromothymol blue reagent and examined with a compound microscope. Identification was based on morphological characteristics such as growth pattern, hyphae, colour of colony and medium, surface texture, margin char-

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acter, aerial mycelium.

6-Indicator of Fungal Occurrence and Frequency
1- Occurrence %= No. of the samples in which the genus occurs/No. of the total samples ×100.

2-Frequency %= No. of the isolates of each genus /No. of the isolates of all genera ×100 (Choi et al.1999).

Results
1- Isolation of fungi located in hors manure:
From July to October / 2013, fifty samples of horse manure were collected from horses located in the Equestrian Club in Jadriya - Baghdad. Different colonies of fungi were observed on (PDA) at 30°C for 5-10day and appeared with different morphological features such as (Colonies dense, Colonies dark green,Colonies compact white or yellowColonies at first white to pale yellowish, Creamish – yellow in daylight, more grayish in darkness,Light grayish ,Colony pale brownish-gray. Colony smooth radial furrows, whitish to cream-colored or grayish–brown, fig (1).

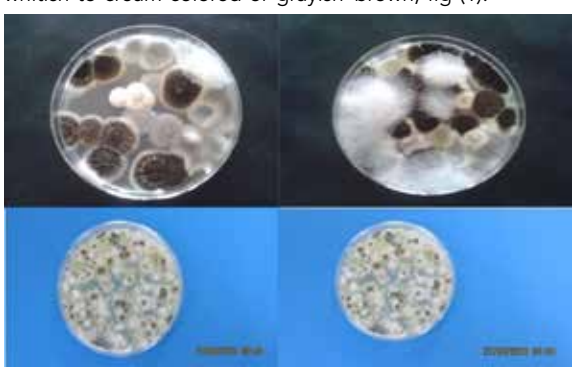
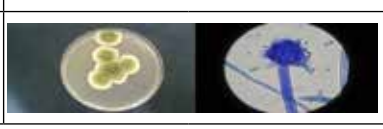


Fig (2): Different types of fungicolonies isolated from horse manure

2- Identification of fungispecies
The survey of horse manuresamples revealed that Sixteenspices fungiwere obtained, after culturing for 5 day at 30°C and identified by compound microscopeaccording the key in (John et al., 2010; Robert and Reenen, 1988).Eight speciesof fungi which belong to the genera; Aspegillus(A. niger ,A. parasiticus,A.flavus ,A.terreus, A.fumigatus, A. tamari, A.ustus, A. nidulaus)(Table 1).Three species of Rhizopusisolated (Rh. Oryza, Rh.oligosporus, Rh.stolniform .Genus Mucor including two species (M.hemalis, M.racemosum). (.Regarding by fungi observed with one species were isolated from horse manure, including (Absidiacerymbifera,Syncephalstrumracemosum) and(monillia).

Table (1): Showed the most important characteristics of the Aspergillus spices on the Cz.A media and under microscope.

No.	Strains	View on CzA at 30°C after 5 days of incubation.	Microscopic feature under(400x)
1	A. niger		
2	A. flavus.		
3	A. paracitcus.		
4	A.fumigatus.		
5	A. ustus .		
6	A. tamarii.		
7	A. terreaus.		
9	Emericellanidulaus		

3-Indicator of Fungal Occurrence and Frequency
The results of the survey for fungal genera indicate that different types of fungi among the more frequent isolated genera wereAspergillus(98%)Rhizopus (86 %) and Mucor(78%) . (Table 2)

Table (2) : Frequency and occurrence of Fungal genera isolated fromhorse manure

Genus	Occurrence%	Frequency%
1- Aspergillus niger	98%	62.5%
2-A.fumigatus	60%	31.25%
3-A.paraciticus	48%	25%
4-A.nidulaus	64%	31.5%
5-A.tamarii	40%	18.75%
6-A.flavus	34%	12.5%
7-A.terreaus	44%	18.75%
8-A.ustus	10%	6.25%
9-Rhizopusoryza	86%	43.75%
10-Rh.oligosporus	48%	31.25%
11-Rh. stolnifer	42%	31.25%
12-Mucorhemalis	78%	25%
13-M.racemosum	70%	18.75%
14-Absidia cerymbifera	18%	6.25%
15-Syncephalstrumracemosum	14%	6.25%
16-monellia	52%	25%

This result is based on the notion thatAscomyceteslike Aspergillus genus was the most frequent and occurrence (98%)that utilize more complex carbohydrate, followed byZygomycetes likeRhizopus (86%) and Mucor (78%) .whichcan only utilize readily available nutrients; they lack the necessary array of enzymes to digest complex carbohydrate.These results are corroboratedby researchers Francis (1988)They postulated that the Zygomycetes, or sugar fungi, appear first, because their spores germinate quickly and their mycelium grows rapidly, exploiting the fresh substrate; that is, they utilize the simple sugars and hemicel-

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luloses present in manure . The Zygomycetes are not capable of utilizing the cellulose and lignin found in manure. When the simpler carbon sources are metabolized, the Zygomycetes disappear and are replaced by the Ascomycetes, which can utilize cellulose. These are then replaced by the Basidiomycetes, which can utilize both the cellulose and the lignin.Also These results are corroborated by reports ofAndréet al.(2011): Delgado et al. (2005)andRichardson (2008), who observed the occurrence of different-species of Zygomycetes in manure of different animals, mainlyherbivores, in different countries.

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