University of Thi-Qar Journal Of Science (UTsci)
Website: http://jsci.utq.edu.iq Email: utjsci@utq.edu.iq
Volume 6, Number 3, December 2017

Isolation and identification of fungi from extreme environments in Nassiriyah city soils

Saad S. Hamim Mohammed H. Mashhad Maysam Kareem Hassan*

Biology department- Science collage- Thi-Qar university

*E. mail: maysamalkhafagi1992@gmail.com

Abstract:

The present study aimed to isolation and identification some mycoflora from 40 soil sample in 6 sites (Remnants of fat-born, parks, edges of the river, animal wastes, sewage and rubbish) during October 2015 to January 2016 in Nassiriyah city, Iraq. According to different environmental factors. The present study showed the isolated genera from different sits and that were include *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Cladosporium*, *Sepedonium*, *Alternaria*, *Bipolaris*, *Chrysosporium*, *Candida*, *Rhododendron*, *Humicola*, *Geotrichum*, *Fusarium* and *Acremonium.* They were isolated by dilution method, direct plate method, alcohol and heat treatment technique using the cultural media viz. PDA, SDA and PCA. *Aspergillus* represented the highest fungal isolate which represent 62 (37.12%) isolation followed by *Penicillium* with 47 (28.14%), *Mucor* 22 (13.16%), *Rhizopus* 15 (8.98%), *Cladosporium* 6 (3.59%), *Sepedonium and Alternaria* 3 (1.80%), *Bipolaris*, *Chrysosporium* and *Candida* 2 (1.20%), and finally *Rhododendron, Humicola and Geotrichum* recorded the lowest fungal isolation with one isolate (0.60%). The study results showed that soil dilution method gave a best fungal growth in comparison with direct plate method and alcohol and heat treatment technique in 25 ^{o}C and pH= 6. Potato Dextrose Agar appeared as an optimum in comparison with other media such as SDA and PCA.

Key words: Fungi, Dilution method, PDA, SDA,PCA.

Introduction:

Fungi are an important component of the soil microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions, the role of fungi in the soil is an extremely complex and is fundamental to the soil ecosystem. They perform ecological services that strongly impact the quality of human life and have enormous potential for providing economic benefits, e.g., the isolation and identification of the soil fungus *Penicillium* leading to a large pharmaceutical industry of antibiotics (Takahashi *et al.*, 2008*).* As fungi play a major role in soil ecosystems along with bacteria, protists, small invertebrates and plants, through complex trophic interactions. Most soil fungi are regarded as saprobes, decomposing organic matter and contributing to nutrient cycling, while several species form mycorrhizal associations with plants or are plant pathogens (Pfenning & Abreu, 2006). Also recognized as prolific secondary metabolite producers, fungi have provided several bioactive compounds and chemical models currently used as pharmaceuticals, and soils are traditionally the main source of fungal genetic resources for bioprospection programs (Adrio & Demain, 2003). Despite that, the biodiversity and biotechnological potential of the soil mycobiota in many tropical regions is still poorly studied. This environment is thought to select species with adapted metabolism and good potential for delivering new bioactive metabolites. The present study aimed to isolation and identification fungi from different sits in Nassiriyah city soils.

Materials and methods:

Isolation of soil Fungi:

Fungi were isolated from soil, which were collected from six sites of Nassiriyah city, such as Remnants of fat-born, parks, edges of the river, animal wastes, sewage and rubbish, In case of soil, the collection site of samples were cleaned of all the superficial deposit such as; stone, grass, litter. and for a pit of 5-15 cm. The soil was loosened inside the pit and collected in sterile bags, which were brought to the laboratory. Three methods were followed to isolate the fungi.

Dilution method:

One gram of soil were added to 9 ml of distilled water and were agitated on a shaker (memert, Germany) for 15 min. The samples were taken out of shaker and allowed to settle for 15 min. one ml of the supernatant liquid was used for the isolation of the fungi by applying the liquid on three media included Potato Dextrose Agar (PDA), Sabouraud Dextrose Agar (SDA) and Potato Carrot Agar (PCA) using a serial dilution plate technique (Ayse, 2003).

Direct Plate Method:

In this procedure a small amount of soil sample (0.015 g) was taken from the main sample by means of a sterile nichrome needle with a flattened tip and dropped into the bottom of a sterile plate. Agar medium was poured and particles were distributed throughout the medium by shaking and rotating the plate. After solidification plates were incubated at 25±1ºC, and observation were made as above. After this, isolation were made from the plates, different fungal species were picked up with the help of sterile needle and then streaked into the slant, containing Potato Dextrose Agar (PDA) medium (Warcup ,1950).

Alcohol and heat treatment techniques:

One gram of soil samples was soaked in 4.5 ml of 60% ethanol for 8 min. To remove ethanol, the samples were centrifuged at 3,000 rpm for 10 min (MSE, England). Thereafter, soil pellets were washed twice with sterile distilled water and incubated in a water bath at 80 °C for 10 min (Memert, Germany). Finally, samples were 10-fold serially diluted concentration and spreader on different media (PDA, SDA and PCA) plates, supplemented and incubated at room temperature in dark for 2-3 weeks to induce sporulation and recovered as single colonies (Seifert & Labeda, 1990).

Samples culture:

The samples were cultured on different media with 0.05 g/L chloramphenicol to reduce contamination with fast growth bacteria. Cultures were incubated at 25±1ºC for 7-21 days. Petri dishes were examined daily after 7 to14 days.

Identification of soil fungi:

The morphologies of the fungal isolates were identified through macroscopic and microscopic observations (De Hoog *et al*., 2002).

Macroscopic Examination:

The cultures were observed and physical characteristics were identified such as top and reverse color, growth behavior, mycelia mat, and changes of medium.

Microscopic Examination:

The slide culture of the fungal isolates was prepared. A small sample of fungus and agar was cut out from the fungal culture and were transferred onto microscope slide. The sample was covered with a cover slid supported by plasticins. Subsequently, the culture slide was placed into petri dish which was then sealed with parafilm. After 5 days of incubation at room temperature, examination of the slide culture was carried out by Binocular Compound Microscope. Microscopic characteristics such as mycelia end, branching, structure of hypha, and presence of spore were observed using light microscope.

Statistical analysis:

Statistical analysis was performed with (SPSS) 17. Descriptive statistics for categorical

data were expressed as frequency and percentage. Chi-square was used for the comparison of categorical data. P-value of ≤ 0.05 was considered as the level of significant.

Results:

Isolation of soil Fungi:

A total of (40) soil sample were collected from six sites in Nassiriyah City during October 2015 to January 2016. The results showed that dilution method gave the best growth of fungi in comparison with direct plate method ,alcohol and heat treatment techniques. The isolated fungi from the soil grown on Potato Dextrose Agar (PDA) with 0.05 g/l Chloramphenicol at pH= 6 and 25°C, which seem to be the best selective medium for growth and culturing of fungi, in comparison with (SDA and PCA) at pH (6.5 and 5.6) respectively) at the same temperature. The present study showed that the main sites of fungal isolates was remnants of fat-born with 38 isolates (22.75%) followed by edges of the river 36 (21.56%), parks 29 (17.37%), animal waste 26 (15.57%). Whereas sewage and rubbish recorded the lowest isolation sites with 20 (11.98%) and18 (10.77%) ($p \leq 0.05$). *Aspergillus* statistically, recorded the highest fungal species prevalence with 62 (37.12%), followed by *Penicilium* 47 (28.14%),*Rhizobus* 15 (8.98%),*Mucor* 22 (13.16%),*Cladosporium* 6 (3.60%). *Sepedoneum* and *Alternaria* 3 (1.80%), while *Bipolaris*, *Chrysosporium* and *candida* 2 (1.20%) And finally *Rhododendron*, *Humicola* and *Geotrichum* with one isolate (0.60%) ($p \leq 0.05$) (Table 1-1 and 2-2).

Genera	PDA	SDA	PCA
Aspergillus	+	+	+
Penicillium	+	+	+
Mucor	+	+	–
Rhizopus	+	+	–
Cladosporium	+	+	–
Alternaria	+	+	–
Sepedoneum	+	+	–
Bipolaris	+	–	–
Chrysoporium	+	–	–
Candida	+	–	–
Geotrichum	+	–	–
Humicola	+	–	–
Rhododendron	+	–	–
Acremonium	–	+	–
Fusarium	–	+	–

(+) = Presence of the genus. (-) = Absent of the genus. (PDA) = Potato Dextrose Agar. (SDA) = Sabouraud Dextrose Agar. (PCA) =Potato Carrot Agar

Table (1-1): Isolated genera from soil samples in six sites by dilution method

Table (2): Number and percentage of genera isolates isolated in the study sites on PDA.

Sites / Species	Remnants of fat-born No.%	Parks No.%	edges of the river No.%	Animal wastes No.%	Sewage No.%	Rubbish No.%	Total No.%
Aspergillus	15 8.98%	6 3.59%	13 7.78%	10 5.99%	10 5.99%	8 4.79%	62 37.12%
Penicillium	9 5.39%	10 5.99%	7 4.19%	9 5.39%	6 3.59%	6 3.59%	47 28.14%
Rhizopus	4 2.40%	5 2.99%	6 3.59%	-	-	-	15 8.98%
Mucor	5 2.99%	6 3.59%	5 2.99%	6 3.59%	-	-	22 13.16%
Cladosporium	4 2.40%	-	-	-	2 1.20%	-	6 3.59 %
Sepedonium	-	-	3 1.80%	-	-	-	3 1.80%
Alternaria	-	-	1 0.60%	1 0.60%	-	1 0.60%	3 1.80%
Bipolaris	1 0.60%	-	-	-	1 0.60%	-	2 1.20%
Chrysosporium	-	2 1.20%	-	-	-	-	2 1.20%
Candida	-	-	-	-	-	2 1.20%	2 1.20%
Rhodendendron	-	-	1 0.60%	-	-	-	1 0.60%
Humicola	-	-	-	-	1 0.60%	-	1 0.60%
Geotrichum	-	-	-	-	-	1 0.60%	1 0.60%
Total of colonies	38 22.75%	29 17.73%	36 21.56%	26 15.57%	20 11.98%	18 10.77%	167 100%

Test statistics

	Part	Fungal
Chi-Square	11.958	364.754
df	5	12

Tab. $\chi 2$: (df =5; α= 0.05)=11.070

Tab. $\chi 2$: (df =12; α= 0.05)=21.026

Discussion:

Isolation of soil Fungi:

Soil sustains an immerse diversity of microbes, which to a large extent, remains unexplored. Bacteria including actinomycetes and fungi are most preferably used as screening sources from various habitats. Fungi are well known as prolific producers of biologically active natural products (Hara Kishore *et al*., 2007).

Most of the naturally occurring antibiotics have been isolated from soil microorganisms. These substances play a significant role in their establishment on (rhizoplane) and around (rhizosphere) the roots of plants. Keeping in view this fact, searches are to be made for the isolation of novel compounds from the soil microorganisms to fight against pathogenic microorganisms involved in dental caries and periodontal diseases. Isolating microorganisms from the environment is the first step in screening for natural products such as secondary metabolites and enzymes (Hunter-Cevera *et al*., 1999). Through the results of the current study, dilution method and selective culture medium (PDA) at pH = 6 and 25°C was the best in comparison with other used methods. The possible explanation that this method may be offering greater opportunity for the growth of fungi, as well as their ability to isolate the fungus whether spore or hypha (Benson, 2002). This reliable to use dilution method without the other methods and these finding was in agreement with (AL-Bayati, 2005). The direct plating method may be used to detect fungi in soil in the form of thick colonies and combined unlike dilution method which used to give single colonies (Obire & Anyanwu 2009 ; Sharma *et al*., 2011). Alcohol and heat treatment techniques was used only to encourage the growth of the ascomycete fungi and induce sporulation and recovered as single colonies (Hong *et al*., 2006). Most fungi were able to grow in a wide range of pH (4-12). Decline of soil pH was positively correlated with the development of specific microbial community. Stated that, the majority of known fungi are mesophyll growing between 25-37C in cultural media (Harley &

Prescott,1996). By using dilution plate method, it was found that *Aspergillus* and *Penicillium* were the dominant genus in the soil (Ayse, 2003 & Philip, 2004). Other fungal genera were isolated in moderate and low occurrence, with the using of different media in this study, it appeared that the percentage of the presence of each genera were parallels to those recorded by (Pandey *et al*., 2001).

References:

Adrio, L. and Demain, L. (2003). Fungal biotechnology. International Microbiology, 6 (3): 191-199.

AL-Bayati, A. (2005). Isolation of some fungi from Salahaddin provinces soil and characterized the fungal extract of *p.brasilianum batista*. Ph .D ,thesis, Collage of education, University of Tkirit. 15-21.

Ayşe, D.A. (2003). Isolation and identification of soil-borne fungi in fields irrigated by GAP in Harran Plain using two isolation methods . Turkish Journal of Botany. 27: 83-93.

Benson, H. j. (2002). Microbiology application (Laboratory manual in general microbiology). gth ed. published by McGra-Hill, New York. U.S.A : 202-203.

De Hoog, G.; Guarro, J.; Gene, J. and Figueras, M. (2002). Atlas of clinical fungi. 2nd ed. Utrecht/Reus: Centra albureau voor Schimmel cultures.

Hara Kishore, K.; Misra, S.; Chandra, D.; Prakash, K. and Murty, U. (2007). Antimicrobial efficacy of secondary metabolites from *Glomerella cingulata.* Braz. J. Microbial, 38: 150-155.

Harley, J. and Prescott (1996). Laboratory exercises in microbiology. 3rd ed . Publisher Mcgrow- Hill, USA.244-253

Hong, S.; Cho, H.; Shin, H.; Jens, F. and Robert, A. (2006). *Neosartorya* species isolated from soil in Korea, Int J System Evol Microbial, 56:477–486.

Hunter-Cevera, J. and Belt, A. (1999). Isolation of cultures. In: Demain, A. and Davies, J. (Eds.). Manual of Industrial Microbiology and Biotechnology. 2nd ed. ASM Press. Washington, DC.

Obire, O. and Anyanwu, E. (2009). Impact of various concentrations of crude oil on fungal populations of soil. International Journal of Environmental Science and Technology, 6 (2) : 211-218.

Pandey, A.; Palni, L. and Bisht, D. (2001). Dominate fungi in the rhizosphere of established tea bush and their interaction with the dominate bacteria under in Situ conditions. India. Microbial, 156: (4) 377-382.

Pfenning, H. and Abreu, M. (2006). Diversity of micro fungi in tropical soils. In: Moreira, F.M ; Siqueira, J.O. and Brussard, L. eds. Soil Biodiversity in Amazonian and Other Brazilian Ecosystems. Wallingford, Oxford shire, UK, CABI Publishing, 1:184-205.

Philip, B.M. (2004). Training in mold isolation, identification, handling, and evolution of conditions leading to mycotoxin production. USA. Food and Drug Administration.

Seifert, A.K. and Labeda, D. P. (1990). Isolation of filamentous fungi, in Isolation Of Biotechnological Organisms from nature, New York, 21-51.

Sharma, K.; Luka and Deo, S. (2011). Fungal spore in soil of Lachung, Kavaka, 38 :67-68.

Takahashi, A.; Lucas, M. and Esther, F. (2008). Occurrence and structural diversity of fungal metabolites with antibiotic activity. Quimica Nova, 31(7): 1807-1813.

Warcup, J.H. (1950). On the origin of colonies of fungi developing on soil dilution plates. Transactions of the British Mycological Society, 38(3): 298-301.