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Original Research Article

Phenotypic and Molecular Diagnosis of *Penicillium limosum* Isolated from some Diabetics Patients in Al- Diwaniyah City

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Abstract: In general, plants are the primary host for the *Penicillium* fungus, which can cause a variety of diseases and losses. Additionally, some species are useful to us in a variety of sectors and the manufacture of antibiotics; nevertheless, in this study, the *Penicillium* fungus was isolated from sick cases of individuals suffering from diabetes, implying that it took advantage of immunity. A small percentage of these patients may experience isolation repeatedly. PCR testing is the most popular technique for finding fungal DNA in clinical materials. In this study, the genus *Penicillium* was identified through phenotypic analysis under a microscope. Genetic diagnosis confirmed this identification as *Penicillium limosum*. Products of the polymerase reaction were given in multiplexed DNA. The nitrogenous base sequence was discovered by sequencing isolated specimens from diabetic patients through the Korean Macro gene Company. The fungal isolates have been added to the Global GenBank.

Keywords: *Penicillium*, *Penicillium Limosum*, Diabetics Patients, Molecular Diagnosis.

INTRODUCTION

Patients with diabetes represent a very diverse group in terms of diabetes type, length of disease, glucose control quality, presence of complications, interference of comorbidities like obesity and hypertension, and kind of glucose-lowering medication, among other factors. Determining the separate roles of diabetes and obesity may be challenging, if not impossible, due to their close relationship. (Scheen *et al*., 2020; Kavanagh & McCowen 2010). Insufficient metabolic control persists despite notable advancements in diagnosis and treatment. Both patients' inability to manage their diabetes on their own and physicians' ineffective intervention tactics may be signs of poor glycemic control (Dailey, G., F *et al*., 2001; Nam, S *et al*., 2011). The Gender, race, and ethnicity all have different rates of diabetes prevalence, which rises with age (Skyler & Oddo 2002).

Patients with diabetes mellitus generally have higher rates of serious and/or frequent infectious illnesses, which may worsen their morbidity. The hyperglycemic environment that promotes immunological dysfunction, micro- and macro-angiopathies, neuropathy, a decline in urine's antibacterial activity, gastrointestinal and urinary dysmotility, and a higher need for medical interventions are the main causes of the higher frequency of infections in diabetic patients. Every organ and system is affected by the illnesses. Some of these conditions, like gangrenous cholecystitis, rhinocerebral mucormycosis, foot infections, and malignant external otitis, are more common in diabetics. Apart from the heightened morbidity, viral processes could be the initial indication of diabetes mellitus or the catalyst for comorbidities like hypoglycemia and diabetic ketoacidosis (Casqueiro *et al*., 2012; Vardakas, K. Z *et al*., 2007) and the diabetes patients have changes in several elements of immunity. Function of polymorphonuclear leukocytes is decreased, especially in the presence of acidosis. There could be an impact on phagocytosis, chemo taxis, and leukocyte adhesion (Nirmal, J. *et al*., 2004).

Mycotic infections, may raise the risk of diabetic foot syndrome in individuals with diabetes. On the other hand, not much information is known regarding

the frequency of fungal foot infections in diabetic individuals (Eckhard, *et al*., 2007).

Numerous infections, including respiratory system infections, urinary system infections, liver and intestinal tract infections, soft skin infections, and mucormycosis, have been reported in diabetic patients. These infections can be caused by fungi, bacteria, or viruses (Casqueiro *et al*., 2012; Nicolás *et al*., 2020).

One of the most prevalent and well-known fungi is *Penicillium.* Although the Penicillium fungus has numerous applications, such as being used in the food business and to make antibiotics that fight germs, it also creates Mycotoxins, more than 58 species of Penicillium fungi have been reported to produce mycotoxins; the majority of these toxins are hazardous to the kidneys, liver, or immune system (Frisvad & Samson 2004, Pitt & Hocking 2009, Samson *et al*., 2010) which can be dangerous for those with weakened immune systems, like diabetics. Secondary metabolites, mycotoxins appear to have no purpose in the regular metabolism of fungi. When the fungus achieves maturity, they are mostly, though not entirely, produced. Their configurations range from simple heterocyclic rings with molecular weights as low as 50 Da to groups containing 6–8 irregularly organized heterocylic rings with a total molecular weight greater than 500 Da. Interestingly, none of these compounds exhibit immunogenicity. (Vinokurova, *et al*., 2003; da Rocha *et al*., 2014).

Pitt in 1994, Schwab & Straus 2004; found that many types of Aspergillus and Penicillium, causing diseases, had been isolated from humans and animals, and most of these types were opportunistic types.

METHODS

a- Materials: Extract the DNA of the fungi under study using a specialized kit (Hipure A fungal DNA Purification), adhering to the manufacturer's steps and instructions.

b- Isolation: The specimens, which included specimens from patients who were recumbent (diabetics), were gathered at Al-Diwaniyah Teaching Hospital in Iraq. After being collected in a sterile manner and cultivated, the specimens were brought into the lab for testing and incubation.

c- The Diagnostic of Molecular: The CTAB approach was utilized to extract DNA from fungal mycelia after they had been collected.

The internal transcribed spacers (ITS) amplification was carried out utilizing primers P-ITS4 {revers} (5'-TCCTCCGCTTATTGATATGC-3') and P-ITS1{forward} (5'-TCCGTAGGTGAACCTGCGG-3') in accordance with. (White *et al*., 1990; Xie, *et al*., 2008; Visagie *et al*., 2014).

RESULTS & DISCUSSION

A: Isolation and Diagnosis of *Penicillium Limosum*

In Table One, the percentage of *Penicillium limosum* fungi isolated from the total number of specimens is 6.66 percent. If this indicates something, it indicates its actual.

Table 1: *Penicillium limosum* positive specimens from total specimens

Total samples	Positive samples	%
45	3	6.66

Table No. 2 shows us the location of sample collection. As shown in the table below, 18 sputum specimens were studied from 27 nasal swab specimens. The positive specimens for the *Penicillium limosum* fungus were two isolates from sputum samples and one isolate isolated from nasal swabs.

Table 2: Distribution of positive *Penicillium limosum* specimens according to the type of samples

Type of sample	Total samples	Positive samples	%
Sputum	18	2	11.11
nasal swabs	27	1	3.7
Total	45	3	6.66
Calculated X^2	0.952		
Calculated P	0.329*		

* No significant difference (P>0.05)

Table 3: Distribution of positive *Penicillium limosum* specimens according to the age of patients

Age interval	Total samples	Positive samples	%
46-61	15	1	6.66
62-77	16	2	12.5
78-96	14	0	0
Total	45	3	6.66
Calculated X^2	1.87		
Calculated P	0.392*		

* No significant difference (P>0.05)

In Table No. 3 above, the distribution of positive isolates according to age was studied, and there was no significance here, as we have one isolate for people aged between 46-61 and two isolates for people over sixty years of age. The reason may be due to the fact that they were three isolates out of 45 specimens. In Table No. 4, we studied other chronic diseases associated with people with diabetes. There were five people out of 45 people who had other chronic diseases in addition to diabetes, and among these five, one isolate was positive for the fungus *Penicillium limosum*, and two isolates were from people who did not have chronic diseases other than diabetes, and from this we find The main reason behind these infections or those carrying such fungi that may cause illness may be low immunity in diabetics.

Table 4: Distribution of positive *Penicillium limosum* specimens according to the presence of other disease

Status	Total samples	Positive samples	%
Yes	5	1	20
No	40	2	5
Total	45	3	6.66
Calculated X^2	1.60		
Calculated P	0.205*		

* No significant difference (P>0.05)

The Morphological Diagnosis

After phenotypic diagnosis of *Penicillium* according to the appearance of the growth in the dish and according to the appearance under the microscope and identifying the fungus, it is assigned to the genus Penicillium, as shown in Figure No. 1. (Visagie *et al*., 2014; Sciortino Jr, C. V. 2017).

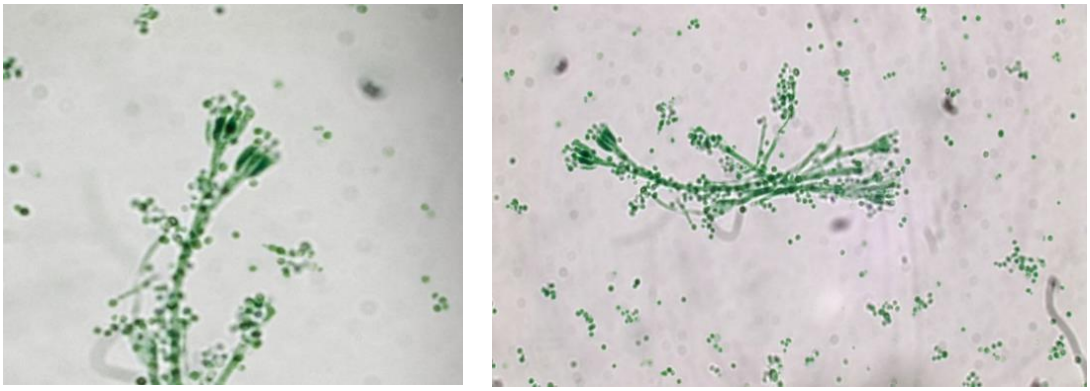

Fig. 1: (Penicillium genus under microscope 40 x)

The Molecular Diagnosis

The *Penicillium* was identified molecularly to confirm its genus. The result was the fungus *Penicillium limosum*. The isolate was registered in GenBank, as shown in the appendices. The genetic tree of the isolate was drawn for comparison between other similar isolates in the world, as in Figure 2.

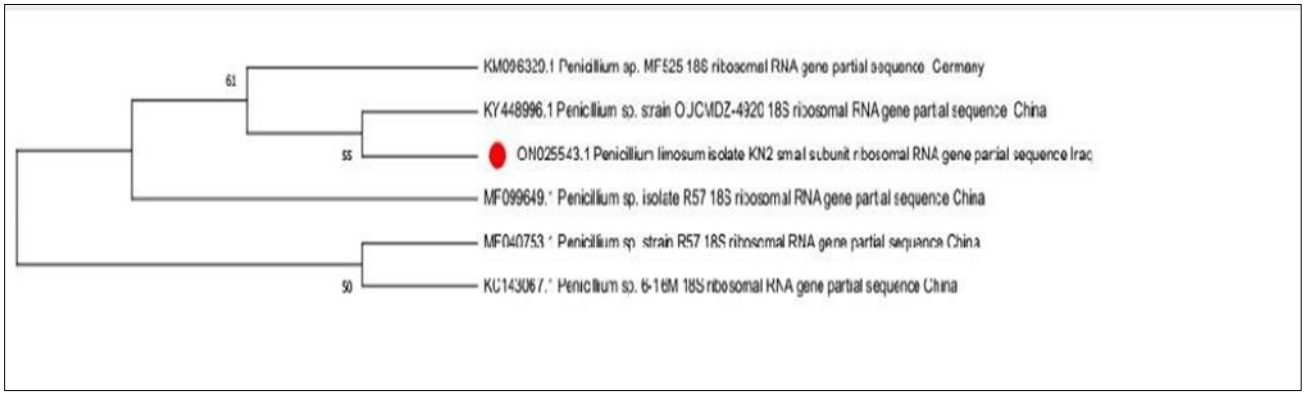

Fig. 2: The genetic relationships between the fungal isolates in this study and other isolates that have been previously registered with the National Center for Biotechnology Information (NCBI) are displayed in a neighbor-joining tree. (ONO25543 the study's isolate)

In the genetic tree below, there is a comparison between our isolate of the fungus *Penicillium limosum* and the isolates of the genus *Penicillium* registered in the GenBank, one from Germany, three from China, and our isolate with the genetic number ONO25543.1, The nitrogenous base sequence in Appendix 2 illustrates the similarities and differences.

CONCLUSION

Diabetes is a chronic disease whose disease develops with weakened immunity. Therefore, we searched for fungi that suffer from diabetes to be associated with some patients. It turns out from our research that one of the species belonging to the genus *Penicillium* was identified, which is in fact a saprophytic fungus that exploits the weakness of patients and infects them. The phenotypic diagnosis was made and the final diagnosis was assigned, which is the final diagnosis taken from the phenotypic diagnosis, which purifies the result at the level of the species *Penicillium limosum.*

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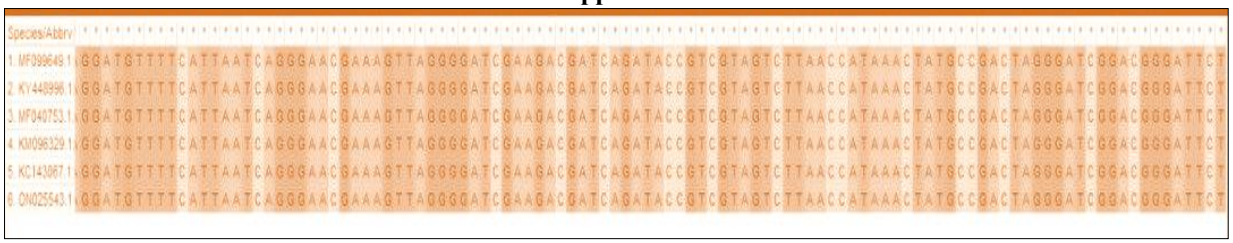
Appendix



REFERENCES

- Casqueiro, J., Casqueiro, J., & Alves, C. (2012). Infections in patients with diabetes mellitus: A review of pathogenesis. *Indian journal of endocrinology and metabolism*, *16*(Suppl1), S27.
- da Rocha, M. E. B., Freire, F. D. C. O., Maia, F. E. F., Guedes, M. I. F., & Rondina, D. (2014). Mycotoxins and their effects on human and animal health. *Food control*, *36*(1), 159-165.
- Dailey, G., Kim, M. S., & Lian, J. F. (2001). Patient compliance and persistence with antihyperglycemic drug regimens: evaluation of a medicaid patient population with type 2 diabetes mellitus. *Clinical therapeutics*, *23*(8), 1311-1320.
- Eckhard, M., Lengler, A., Liersch, J., Bretzel, R. G., & Mayser, P. (2007). Fungal foot infections in patients with diabetes mellitus–results of two independent investigations. *Mycoses*, *50*, 14-19.
- Frisvad, J. C., Smedsgaard, J., Larsen, T. O., & Samson, R. A. (2004). Mycotoxins, drugs and other extrolites produced by species in Penicillium subgenus Penicillium. *Stud. Mycol*, *49*, 201-241.
- Kavanagh, B. P., & McCowen, K. C. (2010). Glycemic control in the ICU. *New England Journal of Medicine*, *363*(26), 2540-2546.
- Nam, S., Chesla, C., Stotts, N. A., Kroon, L., & Janson, S. L. (2011). Barriers to diabetes management: patient and provider factors. *Diabetes research and clinical practice*, *93*(1), 1-9.
- Nicolás, F. E., Murcia, L., Navarro, E., Navarro-Mendoza, M. I., Pérez-Arques, C., & Garre, V. (2020). Mucorales species and macrophages. Journal of Fungi, 6(2), 94. oryzae adheres to, is phagocytosed by, and damages endothelial cells in
- Nirmal, J., Caputo, M., Weitekamp, M. R., & Karchemer, A. W. Infections in patients with diabetes mellitus The New England Journal of Medicine 4. *Hyperglycemic crises and lactic acidosis in diabetes mellitus (Postgrad Med J 2004*, *80*, 253-261.
- Pitt, J. I. (1994). The current role of Aspergillus and Penicillium in human and animal health. *Journal of medical and veterinary mycology*, *32*(Supplement_1), 17-32
- Pitt, John I., and Ailsa Diane Hocking. *Fungi and food spoilage*. Vol. 519. New York: Springer, 2009.
- Samson, R. A., Houbraken, J., Thrane, U., Frisvad, J. C., & Andersen, B. (2019). *Food and indoor fungi*. Westerdijk Fungal Biodiversity Institute.
- Scheen, A. J., Marre, M., & Thivolet, C. (2020). Prognostic factors in patients with diabetes hospitalized for COVID-19: Findings from the CORONADO study and other recent reports. *Diabetes & metabolism*, *46*(4), 265-271.
- Schwab, C. J., & Straus, D. C. (2004). The roles of Penicillium and Aspergillus in sick building syndrome. *Advances in Applied Microbiology*, *55*, 215-240.
- Sciortino Jr, C. V. (2017). *Atlas of clinically important fungi*. John Wiley & Sons.
- Skyler, J. S., & Oddo, C. (2002). Diabetes trends in the USA. *Diabetes/metabolism research and reviews*, *18*(S3), S21-S26.
- Vardakas, K. Z., Siempos, I. I., & Falagas, M. E. (2007). Diabetes mellitus as a risk factor for nosocomial pneumonia and associated mortality. *Diabetic Medicine*, *24*(10), 1168-1171.
- Vinokurova, N. G., Ozerskaya, S. M., Baskunov, B. P., & Arinbasarov, M. U. (2003). The Penicillium

commune Thom and Penicillium clavigerum Demelius fungi producing fumigaclavines A and B. *Microbiology*, *72*, 149-151.

- Visagie, C. M., Houbraken, J., Frisvad, J. C., Hong, S. B., Klaassen, C. H. W., Perrone, G., ... & Samson, R. A. (2014). Identification and nomenclature of the genus Penicillium. *Studies in mycology*, *78*(1), 343-371.
- White TJ, Bruns T, Lee S, Taylor JW: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. San Diego 1990
- Xie, J., Fu, Y., Jiang, D., Li, G., Huang, J., Li, B., ... & Peng, Y. (2008). Intergeneric transfer of ribosomal genes between two fungi. *BMC Evolutionary Biology*, *8*(1), 1-7.