Isolation and Identification of Aspergillus Niger Associated with Rice Grain in Babylon Local Markets

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Abstract
The current research involved the identification and diagnosis of contaminated fungi found in rice grains collected from various local markets in Hilla city, located in the Babylon Governorate of Iraq. A total of 121 fungal isolates were obtained from 90 rice grain samples and identified based on their cultural and morphological characteristics. Among the isolates, 31 were identified as Aspergillus niger, accounting for 25.61% of the total isolates and appearing in 34.44% of the samples. Aspergillus flavus comprised 12 isolates, representing 9.91% of the total isolates and appearing in 13.33% of the samples. A total of 7 isolates of A.terrus were identified, making up 5.78% of the isolates, while 11 isolates of Alternaria spp. accounted for 9.09% of the isolates.
Keywords: Rice, Fungi, Aspergillus

1. Introduction

Rice, Oryza sativa L., is an important summer grain crop in the world It is the third after wheat and barley and is grown in the countries of Asia, America, Australia, Africa and Europe, as it belongs to the Poaceae family and is the main food for more than half of the world's population. Its seeds contain starch, protein, fat, iron, calcium, phosphorus and vitamins (1).
Rice is the seed of the grass species Oryza sativa, it is a monocotyledonous angiosperm. The genus Oryza contains more than twenty species, only two of which are referred to as cultivated rice: O. sativa, cultivated in South-east Asian countries and Japan, and O. glaberrima cultivated in West Africa (2). The rice is the agricultural commodity with the third-highest worldwide production in 2016 (741.5 million tones), after sugarcane (1.9 billion tones) and maize (1.0 billion tones). In order to meet the requirements of a growing world population, worldwide production and yield of cereals has been increased for the last 50 years (3).
The rice crop faces many problems during harvesting, storage and after harvest, and the most prominent of these problems are fungal infections, as the Aspergillus fungus is at the forefront of fungi that attack rice grains due to its ability to grow and produce spores when the crop is exposed to moisture and heat (4).
Rice is typically cultivated in favorable climatic conditions that promote fungal infection 5)
In Babylon Province corn seeds were found to be associated with various fungi such as Penicillium spp., Aspergillus spp., Rhizopus spp., Alternaria spp., Fusarium spp., Rhizoctonia spp., and Mucor spp. Among these fungi, Aspergillus spp. had the highest occurrence (55%) according to a study by (6).
The current research involved the identification and diagnosis of contaminated fungi found in rice grains collected from various local markets in Hilla city, located in the Babylon Governorate of Iraq.

2. Materials and Methods

Collection of samples

Ninty of rice samples of several kinds and trade marks were collected randomly (approximately 1kg for each sample) in the period from Oct to Dec 2022, from the local markets (Iraqi rice samples and imported rice samples).

Isolation fungal contamination

To isolate the Aspergillus spp. (stored medicinal plants seeds) seed were taken randomly from each of the collected samples. It was sterilized using 2% sodium chloride for 2 minutes and then washed with sterile distilled water twice to remove traces of sterile material and dried with sterile filter paper. The seeds were transferred with sterile forceps to 9 cm petri dishes containing 20 ml of pre-prepared PDA (chloramphincol) (250 mg / l) to prevent bacterial growth (7) by 5 tablets per dish and three replicates per sample and then incubated in 25 c for 5-7 days as they appear. The fungi associated with the seeds were then purified by secondary forms for identification (7).
Fungal isolates were kept in clean, sterile glass containers containing the center of PDA and incubated at 25 °C for 7 days and then placed in the refrigerator at 4°C until it was used isolated fungi were then diagnosed based on the taxonomic keys of both Nelson et al.(1983)(8) and Seifert(1996) (9).
Identification and characterization of fungi:
1- Morphological examination including colony morphology, colony appearance was studied in terms of shape, color, texture and their margin on PDA(10) .
2- Microscopic examination was carried out to identify the hypha, septale, conidiophores, spores, basidia and cystidia as described by Odhiambo et al., (2013) (11).

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3- Molecular detection of A.niger by using Genomic DNA extraction kit (Favrogen) from Aspergillus spp and the set of primer used for moleculer detection were ITS5: GGAAGTAAAAGTCGTAACAAGG ITS4: TCCTCCGCTTATTGATATGC at 600bp White et al.,1990

In this study, sequencing analysis was conducted on PCR products of 40 Aspergillus isolates. For each isolate, the 20 µl PCR product generated from AFU5S primer was directly sequenced by Macrogen Laboratory in Korea. The resulting sequencing data for each isolate were compared to the NCBI Blast nucleotide database to identify the fungal species.

The 40 isolates of Aspergillus species in this study were analyzed to construct a phylogenetic tree using Mega 6 software program, based on the sequences data amplified by AFU5S primer. The unweighted pair group method with arithmetic mean (UPGMA) tree type was used for the analysis.

3. Results and discussion

In current study the culture of rice samples showed many types of fungi were 31 isolates of Aspergillus niger species, which accounts for 25.61% of the total isolates and has an appearance frequency of 34.44%.

There were 12 isolates of Aspergillus flavus species, representing 9.91% of the total isolates with an appearance frequency of 13.33%.

The results in table (4-2). Show there was 1 Aspergillus fumigatus isolate as well as 1 isolate Bipolarus accounting for 0.82% of the total isolates with an appearance frequency of 1.11%. There were 7 isolates of Aspergillus terrus, representing 5.78% of the total isolates with an appearance frequency of 7.77%. Alternaria species 11 isolates of, accounting for 9.09% of the total isolates with an appearance frequency of 12.22%. There are 23 isolates of Cladosporium species, accounting for 19.01% of the total isolates with an appearance frequency of 25.55% and 2 isolates of Curvularia species, representing 1.65% of the total isolates with an appearance frequency of 2.22 while White sterile Mycelium were 10 isolates, accounting for 8.26% Penicillium spp. 12 isolates which representing 9.91%3 isolates of Rhizopus, accounting for 2.47% of the total isolates with an appearance frequency of 3.33%.

There was 1 isolate of Scopulariopsis, representing 0.82% of the total isolates with an appearance frequency of 1.11% and 7 isolates of yeast species, accounting for 5.78% of the total isolates with an appearance frequency of 7.77%

Table (1) show the distribution of fungi isolated from different types of rice in Babylon market

Type of rice	origin	Location of collect	production date	type of fungi
Abu Dahab	Indian	Hilla Center market	2022	Curvularia ,Cladosporium Whitesterile mycelium, penicillium,yeast
Abu Araba - gold	Indian	Hilla Centermarket	2022	A.niger, Alternaria, White sterile mycelium,A.flavus, A.terrus, Rhizopus,yeast
golden hawk	Indian	Hilla Centermarket	2022	Alternaria A.niger
Falcon (Rose Sila)	Indian	Hilla Cente rmarket	2022	A.terrus, Alternaria, penicillium A.niger,A.flavusCladosporium, Rhizopus,Penicillium,Yeast, White sterile mycelium
Thai basmati rice		Hilla Cente rmarket	2022	A.niger, Penicillium ,A.flavus, Rhizopus, Curvularia,A.terrus, Cladosporium , Alternaria, White sterile mycelium, Yeast
Amber Jasmine	Iraqi	Jewel grinder	2022	A.niger, Alternaria, White sterile mycelium, Cladosporium,
Amber Jasmine	Iraqi	Honest promise grinder	2022	Alternaria,Cladosporium, yeast
Amber Jasmine	Iraqi	Maryam's grinder	2022	Alternaria, Scopulariopsis, A.niger,A.flavus
Amber Jasmine	Iraqi	Al-Hassan grinder	2022	A.niger, Cladosporium, yeast,A.flavus, penicillium,A.niger Penicillium,Alternaria
Amber Jasmine	Iraqi	Kawthar grinder	2022	A.niger
Amber Jasmine	Iraqi	Jewel grinder	2022	A.niger
Amber Jasmine	Iraqi	Duha grinder	2022	Alternaria,A.terrus, Cladosporium
green amber	Iraqi	Honest promise grinder	2022	A.niger,
green amber	Iraqi	Al-Hassan grinder	2022	Alternaria , Penicillium
green amber	Iraqi	fulfillment grinder	2022	A.flavus , white sterile mycelium
green amber	Iraqi	King of amber grinder	2022	A.niger, White sterile mycelium ,penicillium
green amber	Iraqi	The apparent grinder	2022	Bipolarus , Cladosporium
green amber	Iraqi	Jewel grinder	2022	Penicillium

Table (2): percentage of frequency and appearance of fungal species isolates from different type of rice in Babylon province				
No.	*Fungi*	No. of isolates	Percentage of frequency	Percentage of appearance
1	*A. niger spp.*	31	25.61 %	34.44
2	*A.flavus spp.*	12	9.91 %	13.33
3	*A.fumigatus*	1	0.82 %	1.11
4	*A.terrus*	7	5.78 %	7.77
5	*Alternaria spp.*	11	9.09 %	12.22
6	*Bipolarus*	1	0.82 %	1.11
7	*Cladosporium spp.*	23	19.01 %	25.55
8	*Curvularia spp.*	2	1.65 %	2.22
9	*White sterile mycelium*	10	8.26 %	11.11
10	*Penicillium spp.*	12	9.91 %	13.33
11	*Rhizopus*	3	2.47 %	3.33
12	*Scopulariopsis*	1	0.82 %	1.11
13	*Yeast spp.*	7	5.78 %	7.77
Total		121	100 %	

The results show the fungus Aspergillus spp came as a first contaminat of rice grains, followed by the. Cladosporium spp and the Penicillium spp. came at the third rank as showed in table (2).

With many studies These results coincide in terms of the fact that the fungal species that are included in this study are in the fungi of typical stores that have been monitored in different countries of the world (12). The samples yielded 19 isolates in 9 species: A. tubingensis, A. fumigatus, A. niger, T. radicus, T. purpureogenum, T. pinophilus, T. islandicus, P. citrinum, and P. .chermesinum, the genus Aspergillus (80%) was the most common in this refrenses (13) .

(14) indicated that regions between latitudes 26°-35° north and south support the thriving of Aspergillus spp., which includes Iraq known for its hot and warm climate. The growth of Aspergillus spp. is favored by cereals rich .

In Babylon Province corn seeds were found to be associated with various fungi such as Penicillium spp., Aspergillus spp., Rhizopus spp., Alternaria spp., Fusarium spp., Rhizoctonia spp., and Mucor spp. Among these fungi, Aspergillus spp. had the highest occurrence (55%) according to a study by (6). (15) revealed that Aspergillus and Penicillium were the most common fungi found in cereal grain samples (maize and wheat) and peanuts collected from central Delta province in Egypt.

Rice is not as frequently reported to have mycotoxin contamination compared to another cerealicrops (16). In the study conducted, the main genera of fungi found polluting rice be present Trichoderma, Curvularia, Fusarium, Penicillium, Rhizopus, Cladosporium, Aspergillus, Mucor, Helminthosporium, and Alternaria. Other fungal species identified included Arthrinium, Geotrichum Syncephalastrum,, , Rhodotorula Bipolaris, Cryptococcus, Gilocladium and Nocardia. The most common fungi species contaminating rice in Niger State were A. niger, Alternaria spp., Penicillium spp., Rhizopus spp., A. parasiticus, Mucor spp., and A. flavus, fungi Aspergillus spp, and Penicillium. Remain linked to rice grains and what helps its growth and developmentis the availability of appropriate conditions for its growth like availability of storage temperature, moisture and the rate of exposure to insects before and during storage (17). Reported by (18), the most frequently identified fungal genera in cereal fields were Aspergillus and Penicillium. These fungi, along with Rhizopus, were found to thrive. Colleagues and Trung (2001) reported that Aspergillus sp. accounted for 43.8%, Penicllium sp. for 10.9%, Fusarium sp. for 21.9%, and other fungi for 23.4%. During periods of drought and insect harm, these events can occur when situations are stored in fields found in sub-tropical and tropical regions (19).

Some references indicate an increasingly widespread presence of fungi that have the ability to produce mycotoxins in hot and dry areas (20).The research pinpointed a variety of mycotoxins, such as ochratoxins, aflatoxins, along with over twelve other toxins generated by species of Aspergillus (21). Rice samples collected from local and non-local markets in Iraq as well as other countries were compared regarding fungal isolation. It was found that rice samples from Iraqi markets contained a higher level of fungi compared to non-local samples. Under specific conditions, fungi can grow on rice grains, and some strains are capable of secreting mycotoxins. This poses a high risk to the population due to the associated health effects, including liver cancer (6).

The growth of these fungi can be influenced by factors such as the product's moisture content (22), temperature, duration of storage, and the level of fungal contamination before storage. Insect and mite activity can assist in spreading the fungi (23).

Diagnosis of Aspergillus Spp

1- Morphological Characterizes of Aspergillus niger isolates

Of the 51 Aspergillus spp. isolates, 31 were identified as A. niger, which typically exhibit a cottony appearance, initially white to yellow, and eventually turning black. Their structure is made up of felt-like conidiophores, and the reverse is white to yellow. In microscopy, the conidial heads are radiate with biseriate conidiogenous cells.

Upon microscopic observation of A. niger, it was

noted that the fungus possesses smooth-colored conidiophores and conidia. The conidiophores are projections arising from hyaline hyphae. The conidial heads exhibit a radial arrangement and are arranged in columns (biseriate). The conidiophore vesicle produces sterile cells called metulae, which support the phialides on the conidiophores. The phialides, in turn, produce conidia with a rough texture. Are dark brown colored .

Numerous taxonomists have investigated the taxonomy of Aspergillus section Nigri. and was recently reviewed by (23).

In recent years, there have been a reported the contamination of rice with mycotoxins such as AFs and fumonisins, especially with AFs in certain areas of China, Filamentous fungi (often described as molds in this context) and mycotoxins have become the most frequently measured food and feed contaminants at the global level, with the prevalence of fungal invasion still increasing as a reflection of global climate change. Of particular concern is the fungal and mycotoxin contamination of staple foods, such as grains including rice, which is the major dietary product in the Asia-Pacific region. Hence, the present study focused of fungal contamination and the presence of mycotoxins in commercial samples of rice taken at random from supermarkets in Bangkok, Thailand. The results showed that Aspergillus, Talaromyces, and Penicillium were the three dominant fungal genera found in the commercial rice samples (25).

In study done by Seavchou et al. (2023)(26) the marketed rice, samples showed a high prevalence of Aspergillus, Penicillium, and Talaromyces, but no occurrences of Cladosporium and Alternaria. A. niger (eight isolates) was the most prominent species and our results supported other findings (27). Some studies have number of reports from various countries on the occurrence of fungal contamination in rice with high levels of aflatoxin)28).

2- Molecular Identification of Aspergillus Niger Group.

A 31 pure cultures of Aspergillus niger group were obtained by isolating 49 different strains. The initial identification was performed using traditional taxonomy, relying on the morphological features. However, this method has limitations as it provides a restricted number of distinguishing characteristics, making the identification of closely related species difficult. Fortunately, advancements in molecular biology techniques have significantly enhanced taxonomy by enabling highly sensitive and specific genetic differentiation of species. To identify the fungal strains, 24 isolates from the Aspergillus Niger group underwent DNA extraction and the ITS region of rDNA was selected as the barcoding region.

Identification of Aspergillus spp. group based on PCR Methods

The results successfully identified (24) isolates of Aspergillus niger group based on amplification amplicone included ITS1-5.8S-ITS2 with flanking primer region by primer pair: ITS5: and ITS4: All isolates shown monomorphic Figure (1). These results were consonant with results of Skladny (26).

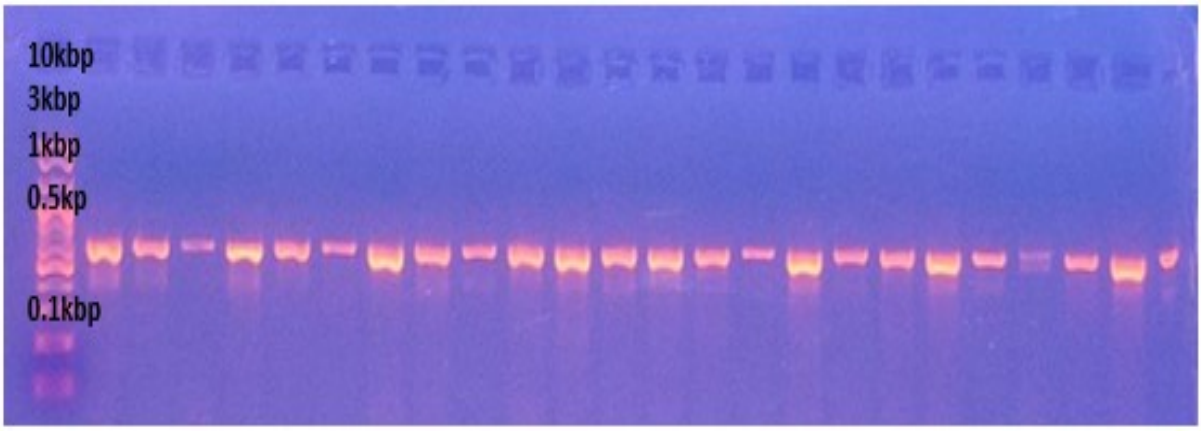

Figure 1: Profile PCR product for amplification IST region with flanking primers, all strain (1-24) of Aspergillus niger group shown 610 bp. M=molecular marker 100-3000bp.1.5% agarose TBE buffer, 100 volt 45 min.

The PCR products were shown clear bands of ITS1-5.8S-ITS2 and flanking regions of primer pair was illustrated on the agarose gel. These products shown bands were sent for sequencing. This sequence results were identified based on NCBI search and shown 24 different fungal species, most of them 18 species were A.niger, others four species were A.brasillensis and tubigensis, two for each.

Identification of Aspergillus Niger group based on sequence method

A 24 isolates of Aspergillus Niger group were identified, distributed among 20 isolates were A. niger, 2 isolates were A.brasillensis and A.tubigensis for each.

1. Multiple alignment sequence of 24 isolates of A.niger

In order to comprehensive the identity and mutation sites among all strains sequence charts under interest, a multiple alignments of 24 isolates based on Edit software was performed. The results shown variation among A.niger strains and between other species in figure (2).

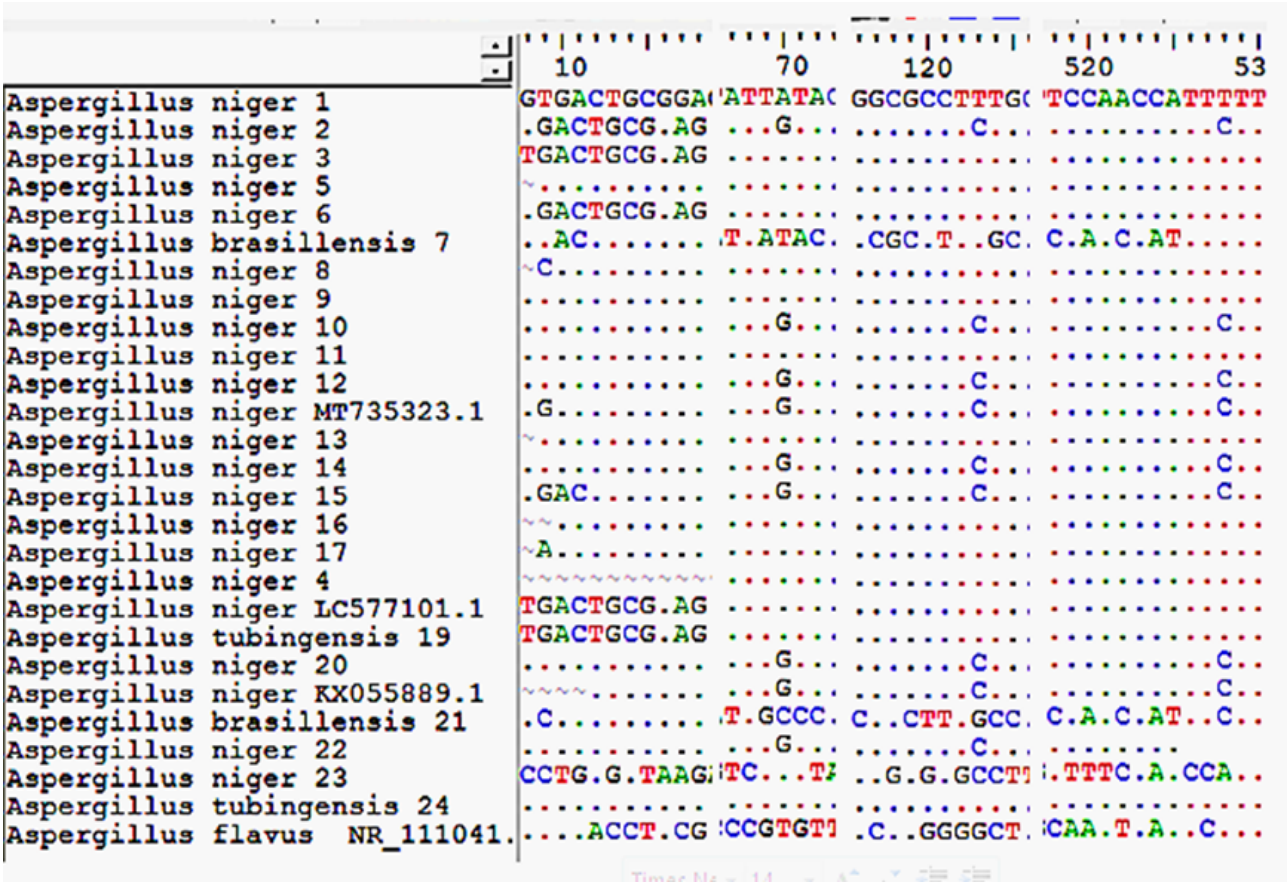

Figure 2: Multiple alignment of partial sequence of ITS region for 24 isolates of Aspergillus niger and closed related species with reference strains with accession numbers.

The common mutations sites were oriented not randomly, for example: the Adenine base was subtitled by Guanine base at site 70. In addition, Cytosin in site 123 and 558 respectively Figure (2) substituted Thymine.

The isolates of A.niger:3-6, 8-9, 11, 13, 16-17, were shown highly identity in their sequences. Also the two isolates of A.brasillensis (7&21) were shown afar evolution from others A.niger isolates. On the other hand, the two isolates of A.tubingenesis were shown trend similar to the A.niger isolate, this may explained the closes relationship and microevolution from A.niger isolates figure (2)

A mutation in the ITS region may have caused the microevolution, which could be credited to the divergent evolution such as the isolates 2,10 and 12 , these results consistent with (29).On the other hand, the sequences of ITS region other isolates of A.niger showed a high percentage of similarity (>98%).This attributed to convergent evolution, this consistent with (30).

In this study, the DNA sequence of the ITS region was utilized as a barcoding region to identify isolated Aspergillus Niger groups. This region is characterized by its high level of conservation, frequent repetition, and variation among interspecific and intraspecific species. Often utilized for DNA-centric mycological research at subgeneric stages and species recognition, the nuclear ribosomal repeat unit's internal transcribed spacer (ITS) region is highly esteemed. The sequence of words has been shuffled, ensuring that unchanged terms are located at least four words apart, and punctuation and referencing have been accurately maintained (31)(32).

According to the findings of this research (Figure 2), it is evident that the ITS region displays uneven variability among different A.niger species. Moreover, there seems to be no clear correlation between this variation and the organized connection or nutritionary way of these speciesi (33)..

2. Phylogeny tree

Many clades and sub clades of A.niger (A-E) , A.tubingensis (F) and A.brasillensis (G) with reference strains were sprouted from construction phylogeny tree for twenty-four sequences of local isolates of A.niger group based on MEGA X software with closes related A.niger , A.tubingensis and A.brasillensis reference strains figure (3)

The isolates of A.niger with numbers:22,12,10,14,2,15 were clustered with reference strains KXO55889.1 in separated clade A, but the isolates 22 and 12 clustered in sub clade A1 while isolates 10 and 14 clustered in sub clade A2 and isolates 2 and 15 away from them figure (3).

On the same way, the isolates 3, 4, 11, 13 were clustered in closes clade B, and clade C. figure (3).

The two isolates of A.tubingensis (clade F) and A.brasillensis (clade G) were situating in separated clade for each Figure (3). Many isolates of A.niger were fare away from these clusters in separated lines.

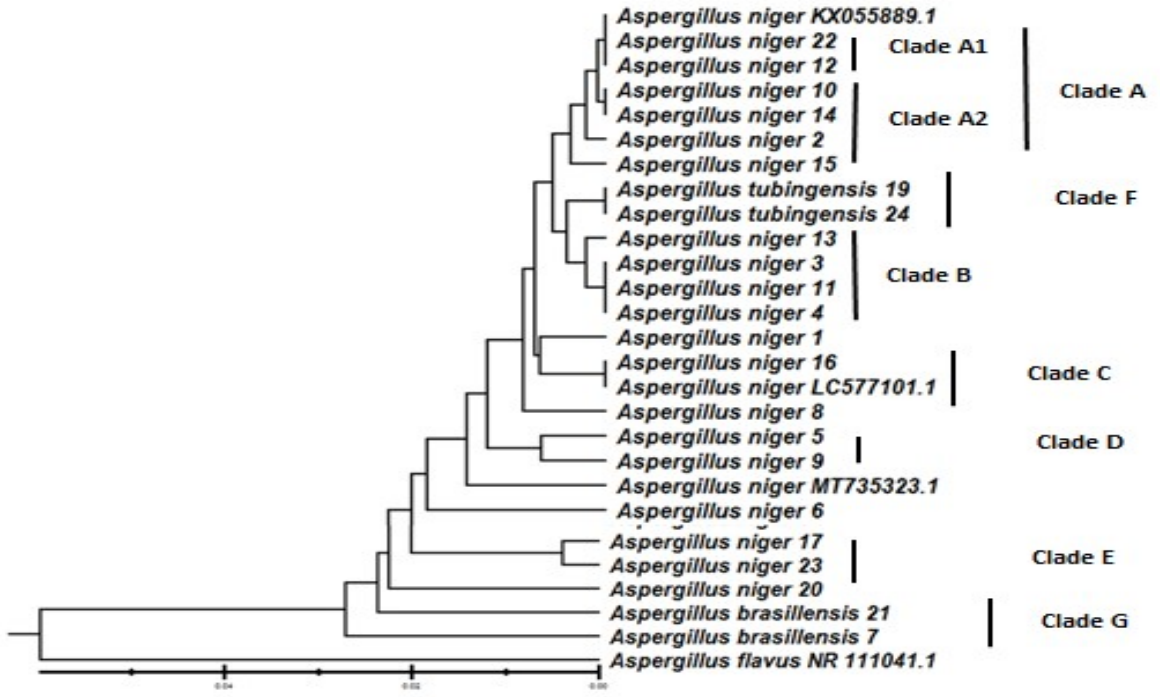

Figure (3): Phylogeny tree for 24 isolates of Aspergillus niger and closed related species.

The closes isolates in each clades and sub clades of Aspergillus Niger group were explained the highly or low sequence identity (34)(35). That means highly identity 98-100% situating in one clade while far away due to low identity 96-97%. The deference in clustering due to micro-evolutionary in some isolates. These results were consistent with results of (36). In addition, the mutation in ITS region may cause this evolution (30).

In general, the evolutionary power are mutations, recombination, gen flow, gen draft and hybridization (37). Mentioned to the evolution acts on mutations that naturally arise within the genome and are shaped both by intrinsic genomic features and by the cellular environment and he pointed to the role of microevolution of C. albicans. In many cases the environmental condition such as sun ray may cause mutations this explanation was agree with , when they pointed to the role of ecological interactions in micro evolutionary(38).

4. References

1- Motlagh, M. R. S. (2011). Evaluation of Curvularia lunata as an biological control agent in major weeds of rice paddies. Life science journal, 8(2), 81-91.

2. Watanabe, Y. (1997). Genomic constitution of Genus Oryza. (Tokyo: Food and Agriculture Policy Research Center). 3: 29-69

3. Park, H.; Oh, N.; Jang, J.; Yoon, H. R. and Cho, M. (2016). Study on Importance-Performance Analysis Regarding Selection Attributes of Rice-Convenience Foods. Journal of the Korean Society of Food Science and Nutrition. 45: 593-601.

4- Makun, H. A., Gbodi, T. A., Akanya, O. H., Salako, E. A., & Ogbadu, G. H. (2007). Fungi and some mycotoxins contaminating rice (Oryza sativa) in Niger state, Nigeria. African journal of Biotechnology, 6(2).

5- Sales, A. C., & Yoshizawa, T. (2005). Updated profile of aflatoxin and Aspergillus section Flavi contamination in rice and its byproducts from the Philippines. Food additives and contaminants, 22(5), 429-436.

6- Ali, A. M., AL-Thwani, A. N., & Yousif, S. A. (2018). Comparing the fungi contamination of rice samples collected from local and non-local markets. Iraqi journal of biotechnology, 17(3).

7- Pitt, J., & Hocking, A. (1997). Fungi and food spoilage (2nd edn). England, UK. In: Cambridge University Press. https://doi. org/10.1007/978-1-4615-6391-4.

8- Nelson, P.E.; Toussoun, T.A.and Marasas, W.F.O., (1983).Fusarium Species. An Illustrated Manual for Identification. Pennsylvania State University Press, University Park, United States of America.

9- Seifert , K. (1996).Fus. key (Fusarium interactive key) Agriculture and AgriFood Canada Cat. 42-66 .

10- Atlas, R.M.; Parks, L.C. and Brown, A.E. (1995). Laboratory manual of experimental microbiology. 1st ed. Mosby-Year Book. Inc. U.S.A.

11- Odhiambo, B.O. and Murage, H.; Wagara, (2013). Isolation and characterisation of aflatoxigenic Aspergillus species from maize and soil samples from selected counties of Kenya. Afr. Journal Microbiol. 7: 4379–4388.

12- Proctor, R. H., Hohn, T. M., & McCormick, S. P. (1995). Reduced virulence of Gibberella zeae caused by disruption of a trichothecene toxin biosynthetic gene.

13- Laut, S., Poapolathep, S., Piasai, O., Sommai, S., Boonyuen, N., Giorgi, M., . . . Poapolathep, A. (2023). Storage Fungi and Mycotoxins Associated with Rice Samples Commercialized in Thailand. Foods, 12(3), 487.

14- Sinaga, S. (1986). Controlled atmosphere storage to reduce microflora [Aspergillus sp.] and aflatoxin production in maize.

15- El-Shanshoury, A. E.-R. R. (2014). Occurrence of

moulds, toxicogenic capability of Aspergillus flavus and levels of aflatoxins in maize, wheat, rice and. Int. J. Curr. Microbiol. Appl. Sci, 3, 852-865.
16- Tanaka, K., Sago, Y., Zheng, Y., Nakagawa, H., & Kushiro, M. (2007). Mycotoxins in rice. International Journal of Food Microbiology, 119(1-2), 59-66.
17- Haque, A., & Russell, N. J. (2005). Phenotypic and genotypic characterisation of Bacillus cereus isolates from Bangladeshi rice. International Journal of Food Microbiology, 98(1), 23-34.
18- Al-Obaidy, S. R. (2015). DETECTION OF FUSARIUM SPECIES THAT P.
19- Ominski, K., Marquardt, R., Sinha, R., & Abramson, D. (1994). Ecological aspects of growth and mycotoxin production by storage fungi. Mycotoxins in grains. Compounds other than aflatoxin. Eagen Press, USA. p, 287-305.
20- Cantalejo, M. J., Carrasco, J., & Hernandez, E. (1998). Incidence and distribution of Fusarium species associated with feeds and seeds from Spain. Revista iberoamericana de micología, 15(1), 36-39.
21- De Scott, B. (1965). Toxigenic fungi isolated from cereal and legume products. Mycopathologia et mycologia applicata, 25(3-4), 213-222.
22- Giorni, P., Battilani, P., & Magan, N. (2008). Effect of solute and matric potential on in vitro growth and sporulation of strains from a new population of Aspergillus flavus isolated in Italy. Fungal ecology, 1(2-3), 102-106.
23- Suleiman, M.N. and Omafe, O.M. (2013). Activity of three medicinal plants on fungi isolated from stored maize seeds (Zea mays (L.). Global Journal of Medicinal Plant Research, 1(1): 77-81.
24-Abarca, M., Bragulat, M., Castella, G., & Cabanes, F. (1994). Ochratoxin A production by strains of Aspergillus niger var. niger. Applied and environmental microbiology, 60(7), 2650-2652.
25-Olagunju, O., Mchunu, N., Venter, S., Guibert, B., Durand, N., Metayer, I., . . . Ijabadeniyi, O. (2018). Fungal contamination of food commodities in Durban, South Africa. Journal of Food Safety, 38(6), e12515.
26-Laut, S., Poapolathep, S., Piasai, O., Sommai, S., Boonyuen, N., Giorgi, M., . . . Poapolathep, A. (2023). Storage Fungi and Mycotoxins Associated with Rice Samples Commercialized in Thailand. Foods, 12(3), 487.
27-Reddy, K., Reddy, C., Abbas, H., Abel, C., & Muralidharan, K. (2008). Mycotoxigenic fungi, mycotoxins, and management of rice grains. Toxin reviews, 27(3-4), 287-317.
28-Sun, X. D., Su, P., & Shan, H. (2017). Mycotoxin contamination of rice in China. Journal of food science, 82(3), 573-584.
29- Skladny, H., Buchheidt, D., Baust, C., Krieg-Schneider, F., Seifarth, W., Leib-Mösch, C., & Hehlmann, R. d. (1999). Specific detection of Aspergillus species in blood and bronchoalveolar lavage samples of immunocompromised patients by two-step PCR. Journal of clinical microbiology, 37(12), 3865-3871.
30-Nosil, P., & Feder, J. L. (2012). Genomic divergence during speciation: causes and consequences. In (Vol. 367, pp. 332-342): The Royal Society.
31-Leander, B. S. (2008). A Hierarchical View of Convergent Evolution in Microbial Eukaryotes 1. Journal of eukaryotic microbiology, 55(2), 59-68.
32-Horton, T. R., & Bruns, T. D. (2001). The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. Molecular ecology, 10(8), 1855-1871.
33-Bridge, P., Spooner, B., & Roberts, P. (2005). The impact of molecular data in fungal systematics. In Advances in botanical research (Vol. 42, pp. 33-67). Elsevier.
34-Nilsson, R. H., Kristiansson, E., Ryberg, M., Hallenberg, N., & Larsson, K.-H. (2008). Intraspecific ITS variability in the kingdom Fungi as expressed in the international sequence databases and its implications for molecular species identification. Evolutionary bioinformatics, 4, EBO. S653.
35-Leander, B. S. (2008). A Hierarchical View of Convergent Evolution in Microbial Eukaryotes 1. Journal of eukaryotic microbiology, 55(2), 59-68.
36-Xie, X., Guan, J., & Zhou, S. (2015). Similarity evaluation of DNA sequences based on frequent patterns and entropy. Bmc Genomics,
37-Baho, S. (2022). Evolutionary Relationship and the Sequence Similarities among Different Fungal Species Infecting Birds Captured from Different Areas in Denmark. Archives of Razi Institute, 77(1), 491.
38-Ene, I. V., Farrer, R. A., Hirakawa, M. P., Agwamba, K., Cuomo, C. A., & Bennett, R. J. (2018). Global analysis of mutations driving microevolution of a heterozygous diploid fungal pathogen. Proceedings of the National Academy of Sciences, 115(37), E8688-E8697.
39-Drott, M. T., Rush, T. A., Satterlee, T. R., Giannone, R. J., Abraham, P. E., Greco, C., . . . Labbé, J. L. (2021). Microevolution in the pansecondary metabolome of Aspergillus flavus and its potential macroevolutionary implications for filamentous fungi. Proceedings of the National Academy of Sciences, 118(21), e2021683118.