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Molecular study to the fungus *Neosetophomasamarorum* was isolated from Al Chabaish marsh, South of Iraq

دراسة جزيئية للفطر *Neosetophomasamarorum* المعزول من هور الجبايش جنوب العراق

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ABSTRACT

*Neosetophomasamarorum*is one of the phoma fungi put up with Ascomycota isolated from sea phoma. In this study,*N. samarorum*was isolated fromAl Chabaish marsh, south of Iraq.This research aimed to the fungus *Neosetophomasamarorum's* molecular characterization using ITS gene and phylogenetic structuring tree.From35 samples of water collected from a different marsh area, eight samples were positive to *Neosetophomasamarorum* when cultured on PDA medium at 28C°, and 27samples were negative fungus. Genetic diagnosisresults for the fungus used specific primers for the ITS gene; thisdesign, especially for this project,showed that 8 (22.8%) were positive. The fungus was diagnosis depending onthe cultural feature and microscopic examination, and then PCR technology was used to ensurethe diagnosis of this fungus. Primers (ITS) and phylogenetic structuring treeanalysis were done by sequences and confirmation ofmicroorganism's homogenous data using the database (NCBI) after amplifying Fungi's ribosomal RNA. The resultshowed the genetic affinity percentage of *N. samarorum* between Germany and Canada is 99%, then among Poland, Canada and Germany are 99%, while between Netherland and Netherland is 96%, between Iran and China is 98%, and between Iran and China and Netherland are 96%.

Keywords

Neosetophomasamarorum, Molecular study, DNA isolation, ITS, Al ChabaishMarsh, fungi

Introduction

Al Chabaish marsh is one ofthe Iraqi marshes that ranges about 20,000 Km^2 of the sedimentary plain; ita Biotic ecosystem because they are good an environment for fish and invertebrates, and itshabitat for the number of water birds, added to that itcontainsdifferent types of organisms one of themfungi, itwidespread in this aquatic environment(Bedair*et al*., 2006;APHA, 2012;UNESCO, 2018). The study by Al-Maiah*et al*.(2006) in Thi-Qar governorate marsh water (Al-sayh, Mukadum Al-affar, Alchebaish, Al-fuhud, Al-hmmar) was isolated and diagnosis Twentyspecies of fungi that found on the remaining plants submerged. The result of AI-Jawhary and Hakeem (2014) study were isolated 19mold fungi from sediments collected fromSuqalshuyokh marshes of Governorate of Thi- Qar. So Emran*et al*.(2018), in his research on Al Chabaish marsh water- was isolated six species of fungi from marsh water, one of this fungi *Neosetophomasamarorum*in the rate of 43.3% from the total samples of the marsh water in his study (the fungus*Neosetophomasamarorum* was isolated in Emran*et al*. (2018) research for the first time in Iraq).*Neosetophoma*was: Fungi,Dikarya, Ascomycota, Pezizomycotina, Dothideomycetes, Pleosporomycetidae,

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Pleosporales, Phaeosphaeriaceae, *Neosetophoma*. The majorityof species in this collection of fungi aresoil-borne organismsand are often found a link with multi typesof Gramineae. Some of these species are *N. Gruyter*, *N. Mycosphere*, *N. iranianum*, *N. lunariae*, and *N. samarorum* (De. Gruyter *et al*., 2010).A pycnidialof *N. samarorum*is dimorph with big stagonosporoid conidia contain 1-3septate, reach 25mm in lengthand3.5mm in wide, and has been characterized as (Stagonosporopsisfraxini (Allesch.) Died. Specimens examined: FRANCE, Fraxinus excelsior, 1828, Desmazie`res, Pl. cryptog. N. France [ed. 1] Fasc. 7, No. 349 P (HOLOTYPE); THE NETHERLANDS, Bladel. On Phlox paniculata, 1982, G.H. Boerema (EPITYPE designated here CBS H-20319, culture ex-epitype CBS 138.96). Paraphoma Morgan-Jones & J.F. White, Mycotaxon 18:58. 1983.; Phoma sect. Paraphoma (Morgan-Jones & J.F. White) Boerema, van der Aa*et al*., Stud. Mycol. 32:7. 1990).The*Neosetophomasamarorum*combnovisinterredand represents speciesconcerningParaphoma; therearemorphological and molecular phylogeny differences (De. Gruyter *et al*., 2010).*Neosetophomasamarorum*has antimicrobial activity against G+ bacteria such as (MRSA and VRE) and G- Bacteria such as *Proteusvulgaris*, added to that yeast *Candida albicans.*

This study's goal to detect the molecular characteristic of fungus*Neosetophomasamarorum* was isolated from AlChabaish marsh, South of Iraq,by PCR using ITS gene and phylogenetic structuring tree.

Materials and Methods

Culture medium

In this study,Potato dextrose agar (PDA) (Himedia/ India) was used as a fungal medium. The medium howwas used prepared to dependon the instructions of the Manufacture firm. To inhibit the growth of bacteria, Ampilox(500) solution at a rate of 2 mg/ liter (1g dissolved in 5ml D.W.) was used for this purpose by addingit to the agar (Mohammed and Ali, 2020).

Collection of samples

The samples were isolated from the Al Chabaish marsh water according to the previous study byEmran *et al* (2018); 35samples of water were combined from a different area of the marshat periods from 20/2/2018 to 25/3/2018. Samples were collected from Surface strataof water (5-10)cm in depth randomly by used sterilizecontainers and tubes (50ml to each containertube).Samples were stored in a Refrigerator at 4°C until uses (Overya, *et al*., 2014).

Identification of *Neosetophomasamarorum*

All water samples were cultured on PDA medium and incubated at 28°C for 5-10 days to isolate the fungi, especially*Neosetophomasamarorum*, to get pure cultures subculturing was applied then the isolated funguswas identified according to themorphology and color of the colony on culturemedium added to thatthe feature under microscopic (Al-Temimay and Hasan, 2016).

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Fungus identification by PCR usinginternal transcribed spacer(ITS) gene

After identifying*Neosetophomasamarorum* from classic methods (morphology and microscopic examination),PCR extractionwas used to confirm thediagnosis. From the fungus growth, genomic DNA was isolated according to manufactures protocol; the DNA was isolated by ZR Fungal and Bacterial DNA MiniPrep™ by the following steps: Gel disintegration, DNA Binding, wash, and DNA Elution(Al-Temimay and Aswad, 2018).

The components of the ZR Fungal/Bacterial DNA MiniPrep™ kit was used in DNA extraction was shown in the following:

1. ZR BashingBead™ Lysis Tubes (0.1 and 0.5 mm)1: 50
2. Lysis - Solution: 40 ml
3. Fungal-Bacterial DNA Binding Buffer2: 100 ml
4. DNA Pre / Wash Buffer3: 15 ml
5. Fungal/Bacterial DNA Wash Buffer: 50 ml
6. DNA Elution Buffer: 10 ml
7. Zymo / Spin™ IV Spin Filters (Orange Tops): 50
8. Zymo / Spin™ IIC Columns: 50
9. Collection Tubes: 150
10. Instruction Manual: 1

Identification of fungus by PCR using ITS gene

The ITS gene was amplified using the primer ITS4**according to** (White *et al*. 1990;Karunarathna*et al.*2017).

Condition of PCR

PCR amplification was completed with a total volume (25 µl) consist of (1.5 µl)DNA, (5µl) Taq Pol PCR PreMix, (1 µl) of each primer (10pmol) then D.W. It was supplemented to the tube with a whole volume of (25 µl) (Muhammad and Ali, 2020).

The Program of PCR

Conditions of thermal cycling were carried out as the following:

1- At 94°C, PCR was denaturation for three mints.

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2- Follow up by thirty-five94°C for 45 seconds, 52°C for one mint, and 72°C for one mint. 3- A thermal cycler (Gene Amp, PCR system 9700: Applied Biosystems) was used for the final elongation steps at 72°C for 7 minutes)(Divya*et al*., 2013).

DNA agarose gel electrophoresis

UV exposure was used to visualize PCR items separated by 1.5 percent Agarose Gel Electrophoresis. (302 nm) after red dye staining (Carvalho-Pereira *et al*. 2020).

Samples preparation

The processor loading buffer (3µl) was blended with DNA (5µl)supposedto be electrocautery (loading dye), and after the combinationoperation, After placing the gel in the pool, this was exposed to a UV source with a wavelength of 336 nm. It contained 30 µl of safe DNA red staining solution and 500 ml of D.W(Bulat and Mironenko, 1996).

Sequencing and Sequence Alignment

The separated products PCR were done on at a 2% agarose Gel Electrophoresis and vision by expositionto U. V. light 302nmafter ethidium bromide.The sequencing ofthe gene was complete by the national instrumentation center for environmental management (nicem) online at (http://nicem.snu.ac.kr/main/?en_skin=index.html), biotechnology lab, the machine is DNA sequencer 3730XL, Applied Biosystem), the search of Homology was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and BioEdit program(Carvalho-Pereira *et al*. 2020).

Result andDiscussion

Isolation and Identification of *Neosetophomasamarorum*from AlChabaish marsh water

This study showed that 35 samples of water were composed of different Al Chabaishmarsh water areas,8 (22.8%) samples were positive to ***Neosetophomasamarorum***when cultured on PDA medium 28C°,and 27 samples were negative. The fungus diagnosis was made according to cultural and microscopic examination. The colony of *Neosetophomasamarorum* appears in a filamentous form at the beginning of their growth in white color. Then the colonies become dark brown to black as the colony gets older; when cultured on PDA medium, the color of the colony on the back of the plate is dark brown (figure;1). The fungus under the microscope showed in figure (2) filamentous (hypha) is Nonseptate, conidia septate with 1-2 septa.

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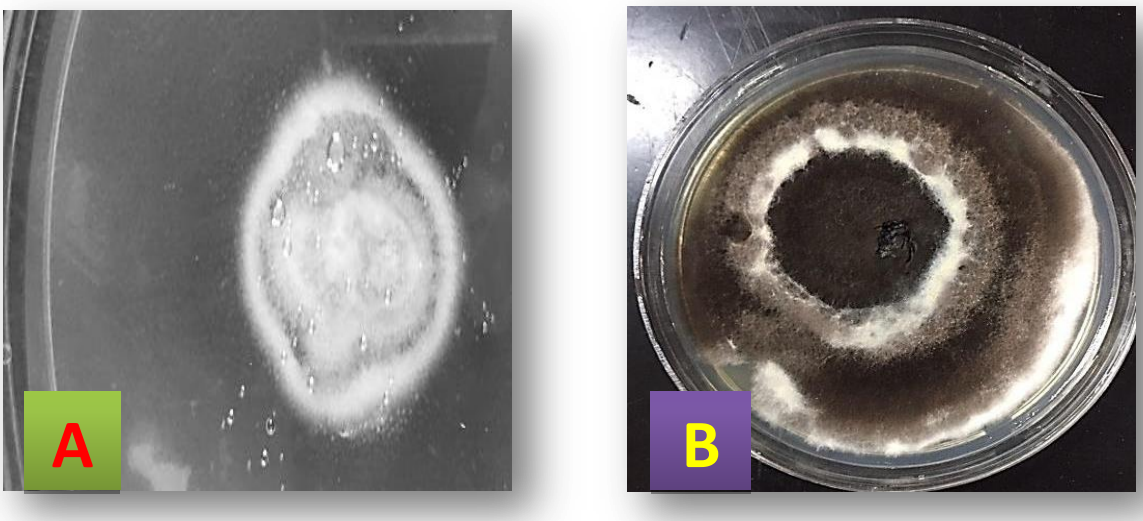

Figure (1): *Neosetophomasamarorum*appearance on PDA medium(5-10 days)incubation at 28C°(A) New colonywith white color filamentous appears (B) Older colonydark brown to black with white margins.

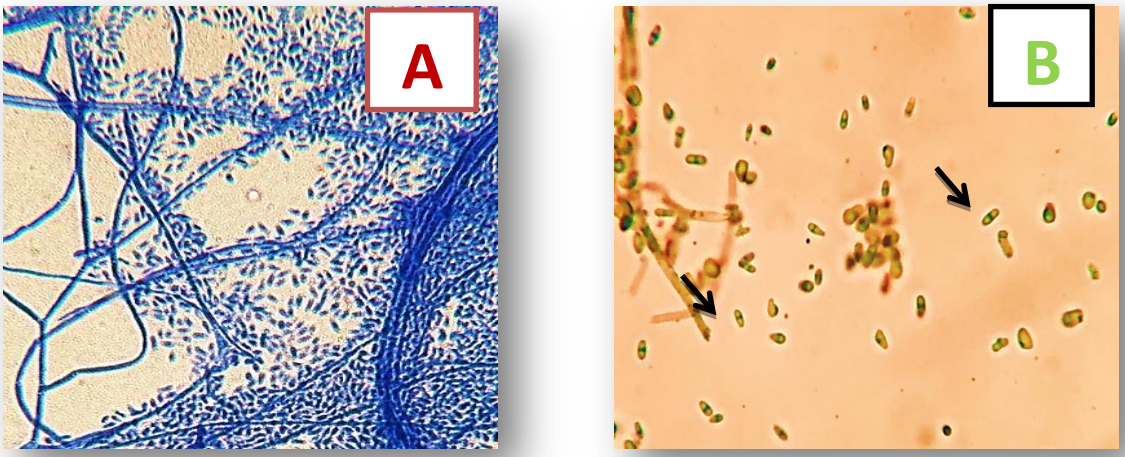

Figure (2) *Neosetophoma samarorum* under the microscope (A) Under 10X, Hypha and Conidia. (B) Under 40X Conidia with one septa.

Sequencing and Alignment of NCBI

The results are shown in table (1),referredto produce of a single band of the necessaryconsequence ofITS gene's regionsof *Neosetophomasamarorum*was acquired from 8 samples. The samplessent for sequencing regarding molecular weight 562bp. Eightsamples of PCR product sendfor sequence analysis; of isolated and extraction from*Neosetophomasamarorum* and 25 µl (10 pmol) from the introductory primer. The samples were processed using

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an Applied Biosystems AB13730XL machine at the National Instrumentation Centre for Environmental Management (NICM/USA) (http://nicem.snu.ac.kr/main/?en_ skin=index.html). The sequence analysis results were analyzed using blast in the National Centre Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov) and BioEd.to detect polymorphism and mutation in *ITS regions*. The isolates of*Neosetophomasamarorum* isolate showed 100% compatibility,as shown in table (1).

Table (1) Represent type of polymorphism*Neosetophomasamarorum*

No. Of sample	Type of substitution	Location	Nucleotide	Range of nucleotide	Sequence ID	Score	Expect	Identities	SOURCE
1	----------			190 to 398	ID: KY950236.1	209	8e-104	100%	Neosetophomasamarorum
	----------			**199 to 407**	ID: KX067820.1	209	8e-104	100%	Neosetophomasamarorum
	----------			**147 to 355**	ID: KJ812297.1	209	8e-104	100%	Neosetophomasamarorum
	----------			**186 to 394**	ID: JX981490.1	209	8e-104	100%	Neosetophomasamarorum
	----------			**214 to 422**	ID: KF251162.1	209	8e-104	100%	Neosetophomasamarorum
	----------			**214 to 422**	ID: KF251161.1	209	8e-104	100%	Neosetophomasamarorum
	----------			**190 to 398**	ID: FJ427062.1	209	8e-104	100%	Neosetophomasamarorum

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	----------	**190 to 398**	ID: KJ173536.1	209	8e-104	100%	Neosetophomasamarorum

1

Neosetophomasamarorum isolate 88SA1 internal transcript spacer1, fractionalsequence; 5.8S rRNA gene and internal transcriptspacer 2, whole sequenceand 28S rRNA gene, partial
Sequence ID: KY950236.1Length: 562Number of Matches:1
Related Information
Range 1: 190 to 398GenBankGraphicsNext MatchPrevious Match

Score	**Expect**	**Identities**	**Gaps**	**Strand**
387 bits(209)	8e-104	209/209(100%)	0/209(0%)	Plus/Plus

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Query  1    TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  190  TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  249

Query  61   AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  250  AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  309

Query  121  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  310  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  369

Query  181  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  209
            |||||||||||||||||||||||||||||
Sbjct  370  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  398
```

2

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Neosetophomasamarorum isolate RP411_3 internal transcribed spacer one, partial sequence; 5.8S rRNA gene and internal transcript spacer 2, whole sequence; and 28S rRNA gene, partial sequence
The ID of sequences: KX067820.1Length: 743Number of Matches: 1
Related Information
Range 1: 199 to 407GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
387 bits(209)	8e-104	209/209(100%)	0/209(0%)	Plus/Plus

```
Query  1    TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  199  TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  258

Query  61   AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  259  AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  318

Query  121  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  319  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  378

Query  181  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  209
            |||||||||||||||||||||||||||||
Sbjct  379  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  407
```

3

Neosetophomasamarorum isolate RKDO834 internal transcript spacer 1, fractional sequence; 5.8S rRNA gene and internal transcript spacer 2, whole sequence; and 28S rRNA gene, fractional sequence
The ID ofSequence: KJ812297.1Length: 519Number of Matches: 1
Related Information
Range 1: 147 to 355GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
387 bits(209)	8e-104	209/209(100%)	0/209(0%)	Plus/Plus

```
Query  1    TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  147  TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  206
```

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```
Query  61   AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  207  AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  266

Query  121  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  267  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  326

Query  181  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  209
            |||||||||||||||||||||||||||||
Sbjct  327  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  355
```

4

Neosetophomasamarorum strain WA0000019130 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

The ID of sequence: JX981490.1Length: 566Number of Matches: 1

Related Information

Range 1: 186 to 394GenBankGraphicsNextMatchPrevious Match

Score	Expect	Identities	Gaps	Strand
387 bits(209)	8e-104	209/209(100%)	0/209(0%)	Plus/Plus

```
Query  1    TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  186  TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  245

Query  61   AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  246  AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  305

Query  121  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  306  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  365

Query  181  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  209
```

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            ||||||||||||||||||||||||||||
Sbjct  366  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  394
```

5

Neosetophomasamarorum strain CBS 568.94 18S rRNA gene, fractional sequence; internal transcript spacer 1, 5.8S rRNA gene, and internal transcript spacer 2, whole sequence; and 28S rRNA gene, fractional sequence
The ID of sequence: KF251162.1Length: 570Number of Matches: 1
Related Information
Range 1: 214 to 422GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
387 bits(209)	8e-104	209/209(100%)	0/209(0%)	Plus/Plus

```
Query  1    TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  214  TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  273

Query  61   AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  274  AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  333

Query  121  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  334  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  393

Query  181  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  209
            ||||||||||||||||||||||||||||
Sbjct  394  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  422
```

6

Neosetophomasamarorum strain CBS 139.96 18S rRNA gene, fractional sequence; internal transcript spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, whole sequence; and 28S rRNA gene, partial sequence
Sequence ID: KF251161.1Length: 572Number of Matches: 1
Related Information
Range 1: 214 to 422GenBankGraphicsNext MatchPrevious Match

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Score	Expect	Identities	Gaps	Strand
387 bits(209)	8e-104	209/209(100%)	0/209(0%)	Plus/Plus

```
Query  1    TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  60
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Sbjct  214  TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  273

Query  61   AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  274  AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  333

Query  121  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  334  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  393

Query  181  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  209
            |||||||||||||||||||||||||||||
Sbjct  394  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  422
```

7

Neosetophomasamarorum strain CBS 139.96 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: FJ427062.1Length: 538Number of Matches: 1

Related Information

Range 1: 190 to 398GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
387 bits(209)	8e-104	209/209(100%)	0/209(0%)	Plus/Plus

```
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Sbjct  190  TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  249

Query  61   AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  250  AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  309
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Query  121  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  310  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  369

Query  181  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  209
            |||||||||||||||||||||||||||||
Sbjct  370  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  398
```

8

Neosetophomasamarorum strain EIODSF013 internal transcript spacer one and partial sequence;5.8SrRNA gene and internal transcript spacer 2, whole sequence; and 28S rRNA gene, partial sequence
Sequence ID: KJ173536.1Length: 571Number of Matches: 1
Related Information
Range 1: 190 to 398GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
387 bits(209)	8e-104	209/209(100%)	0/209(0%)	Plus/Plus

```
Query  1    TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  190  TAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCG  249

Query  61   AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  250  AAATGCGATAAGTAGTGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA  309

Query  121  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  310  TTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTCGAGCGTCATTTGTACCCTCAAGCT  369

Query  181  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  209
            |||||||||||||||||||||||||||||
Sbjct  370  CTGCTTGGTGTTGGGTGTTTGTCCACTCC  398
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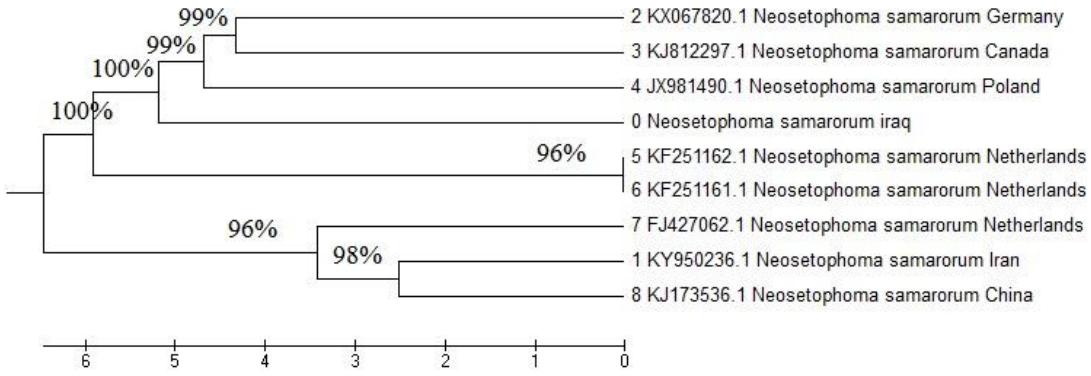
Figure (3): Sequencing of the sense flanking the partial *gliP*gene in compared *Neosetophomasamarorum*with standard *gliP*, obtained the query represents the sample, and the database of the National Centre Biotechnology Information is represented by the topic (NCBI).

3Phylogenetic tree: the phylogenetic tree of *Neosetophomasamarorum*as shown in Figure (4)



The genetic affinity percentage of *Neosetophomasamarorum* between Germany and Canada is 99%.

The genetic affinity percentageof *Neosetophomasamarorum* among Poland, Canada, and Germany is99%.

The genetic affinity percentage of *Neosetophomasamarorum* between the Netherland and the Netherland is 96%.

The genetic affinity percentageof *Neosetophomasamarorum* between Iran and China is 98%.

The geneticaffinity percentageof *Neosetophomasamarorum* between Iran and China, and the Netherland is 96%.

The geneticaffinity percentageof *Neosetophomasamarorum* among China, Iran, the Netherland, Iraq, and Poland, is 96%.

Dissuasion

Despite the genus PhomaSacc.EmendBoeremaandBollen is big distributed and omnipresent. It is still a poorly known group of mitosporic Ascomycetes, and it is widely regarded as a taxonomically challenging group of mitosporic Ascomycetes.Phoma species are known to be associated with not only land plants but also with marine plants. The development of single-celled, hyaline conidia in monophialidic, doliiform to flask-shapedconidiogenous cells in thin-walledpycnidiaare characterized by Phoma (Boerema and Bollen 1975;Aveskampand Verkle, 2009;

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Zhang *et al.*, 2014).*N. samarorum* is one of the phoma fungi isolated from the sea- phom,*N. samarorum* is characterizedby Mycelium on 1)MEA medium, 2) 14% strength Czapek's dox agar medium, and MEA medium developed floccose white sterile mycelia 3) Yeast Extract Peptone Agar.While *Neosetophomsamarorum* produces white fruitlessMycelium when grown on MEA mediumWhen it has grown on yeast extract malt agar and oatmeal agar, it produces olive- green orange- yellow colonies. (Overy*et al.*, 2018).A direct comparison of culture morphology or representative rDNA gene sequences is needed to prove or disprove that OS-F69284 is a strain of *N. samarorum*(Pan *et al.* 2004a, 2004b). This study is the first to report in *N. samarorum* molecular study in Iraq.

The study of Zhang *et al.*(2014),suggested that the deep-sea sediments from the East India Ocean can serve as habitats for new fungal communities compared with other deep-sea environments. In addition, different fungal community could be detected when using targeted environmental sequencing compared with traditional cultivation in this study, which suggests that a combination of targeted environmental sequencing and traditional cultivation will generate a more diverse fungal community in deep-sea environments than using either targeted environmental sequencing or traditional cultivation alone. Theenvironmental gene libraries were created after amplifying sediment DNA with an ITS rRNA gene primer set to find the most fungal species in the fivedeep-sea sediments collected from the East Indian Ocean and discovering new sequences for phylogenetic studies. Furthermore, the cultural fungi were isolated and classified in these sediments by amplifying and sequencing the ITS gene.

Based on a 99 percent similarity match (492/495 bases with no gaps) with *N. samarorum*strain CBS139.96, a Blastn search of the rDNA gene from RKDO834 in GenBank resulted in a provisional taxonomic classification of the strain as *N. samarorum.*According to Overy*et al*. (2014). A similarity match of 98 was found when ITS rDNA was compared to that of thisCBS138.96 is the species' type strain. A morphological comparison of strain RKDO834 to the type strain and other strains (CBS139.96, CBS568.94) confirmed RKDO834 as *N. samarorum*. Furthermore, secondary metabolite formation by RKDO834 and CBS strains was consistent when LC-HRMS profiles were compared. The origin of the *N. samarorum* strain is uncertain, but it is believed to have come from the sea. Due to assay screening results, they were interested in RKDO834 secondary metabolite growth, so fermentation extracts from this fungus were prioritized for further chemical investigations to establish and characterize the active constituents (Because previous taxon isolation was limited to terrestrial substrates).

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