

Myceliophthora verrucosa

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ITS

Myceliophthora verrucosa DNA
DNA
% 100
Myceliophthora verrucosa :

Molecular Identification of Fungi *Myceliophthora verrucosa* which Producing Laccase Enzyme

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ABSTRACT

Polymerase chain reaction (PCR) of ITS region was used to identify the fungus isolated from plastic garbage which can produce laccase enzyme. DNA bands were purified from agarose gel and then sequenced and after entering these nucleotides to the data base it was found that the DNA band belongs to the genus *Myceliophthora verrucosa* in 100%

matching. This method is a very sensitive method for the identification of species and strains of fungi as well as its simplicity in comparing between fungi species.

Keywords: Molecular Identification, *Myceliophora verrucosa*, Laccase.

(PCR) Polymerase Chain Reaction

White 1990

rDNA

Amplification

Variable region

Conserved region

DNA-PCR

DNA Polymerase

DNA

Reverse Forward

Oligoneucleotides

DNA

(dNTPs)

.PCR

Manganese Peroxidase

Lignocellulose

(Bridge *et al.*, Xylanase Cellulase Cellobiohydrolase Laccase Lignin Peroxidase
.1998)

rDNA

rDNA

(28S-like) rRNA

5.8S rRNA

(18S-like) rRNA

: rRNA

Internal Transcribed spacer

Intergenic

rDNA

(ITS) (ITS2 ITS1)

PCR

.spacer (IGS)

rDNA

ITS

1990

White

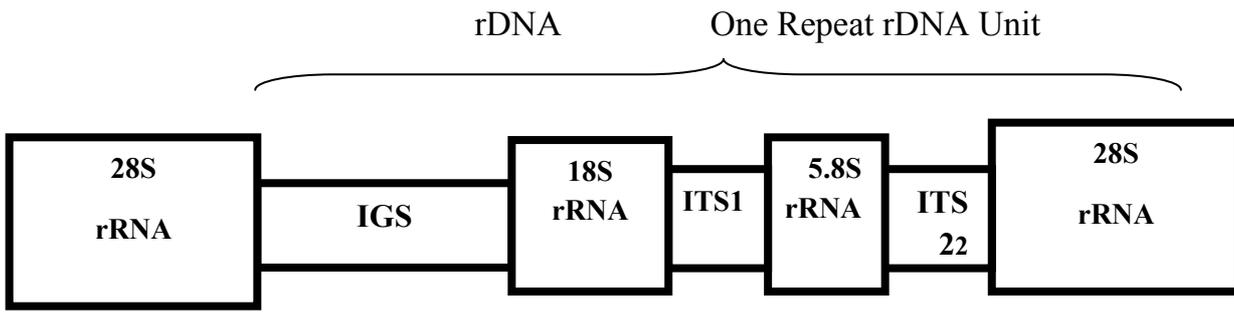
ITS

Phylogenetic

ITS

.(1)

(Bridge *et al.* ,1998)



الشكل 1: تركيب الـ ribosomal DNA

Lignocellulose

.CO₂

Lignocellulose

PCR

.(Bridge *et al.*,1998)

:

%70

. ° 28

(PDA)

DNA

:

PDA

% 0.01

20 -

Salt Solution

C- Free Broth

Auto Vortex Mixer

26

26

6.5 pH 20

40

50 Trace elements

76

	800		1000		400	
% 20	1		10	600	8	(Atlas, 2010)
		48	° 30			
					Towel Paper	
			(Brink and Samson, 2012)		° 80-	
		° 80-			DNA	
					150	
				Sand		
DNA			Eppendorf			
			DNA	DNeasy® Plant Mini Kit		
:			DNA		QIAGEN	
			4 (AP1 Buffer)		400	.1
Auto					100 RNase	
						Vortex Mixer
	-		° 65		10	.2
5			(AP2 Buffer)			.3
			(QIA shredder Mini spin column)			.4
			/ 14000		(Collection Tube)	
				Eppendorf		.5
			(AP3 Buffer)		1.5	.6
			(DNeasy Mini spin column)		650	.7
			/ 8000	1		
				(7)		.8

71

.....*Myceliophthora verrucosa*

500 (DNeasy Mini spin column) .9

/ 8000 AW Buffer

/ 14 AW buffer 500 .10

100 Eppendroff (DNeasy Mini spin column) .11

5 AE buffer

/ 14000

.(11) .12

:DNA

DNA

Gel loading Dye Blue)

DNA Ladder 100 bp

.New england BioLabs ((6X)

:

DNA

100 1 .% 2 PCR %1

1 20 Tris 40) 1X TAE (EDTA

1X TAE

1 DNA 5

Loading Buffer

/ 2 Electrophoresis

/ 0.5 Ethidium Bromid

BioDoc-It™ 210 UV Transilluminator

.Imaging Systems, with) FluorCam 210 , USA)

:

(Primers)

(2013)

5'TGA ATC ATC GAC TCT TTG AAC GC3' ITS86M Forward

.(Soeta *et al.*, 2009) 5'TTT CTT TTC CTC CGC TTA TTG ATA T3' ITS4M

DNA

DNA

DNA

NEW ENGLAND

50

.(1)

0.5

BioLabs INC

DNA

:1

()	
10	PCR Reaction Buffer 10 X
1	PCR Grade Nucleotide Mix dATP,dCTP,dGTP and dTTP
2.5	Primer UP
2.5	Primer Down
1	DNA Template
0.5	Taq DNA Polymerase
32.5	MiliQH ₂ O

10

0.5

The Eppendorf Master cycler [®]

:

° 94

15

° 94

15

° 50

° 10 = G

30

° 72

29

2

- 72
- 20

		:	DNA	
MiniElute®	DNA			
DNA		QIAGEN	Gel Extraction Kit	
	Benchtop UV Trans illuminators			
			:	
QG				.1
			.Buffer	
		10	◦ 50	.2
			. 3-2	
		Isopropanol		.3
Collection tube		QI Aquik spin column		.4
		. / 13000		
				.5
13000		QG Buffer	0.5	.6
			. /	
		PE	0.75	.7
		. / 13000		.8
	30	QI Aquik spin column		.9
	. / 13000	EB Buffer		

DNA :

DNA

12 DNA

.(2)

:2

()	
2	DNA Gel Extraction
1	1/10 Forward Primer
9	MiliQ H2O

Australian Genome Research Facility Ltd (agrif)

Sequence Scanner

NCBI

.DNA

5

DNA

DNA ladder 100 bp

DNA

(2)



. %2 :2
DNA .2 DNA ladder 100 bp .1

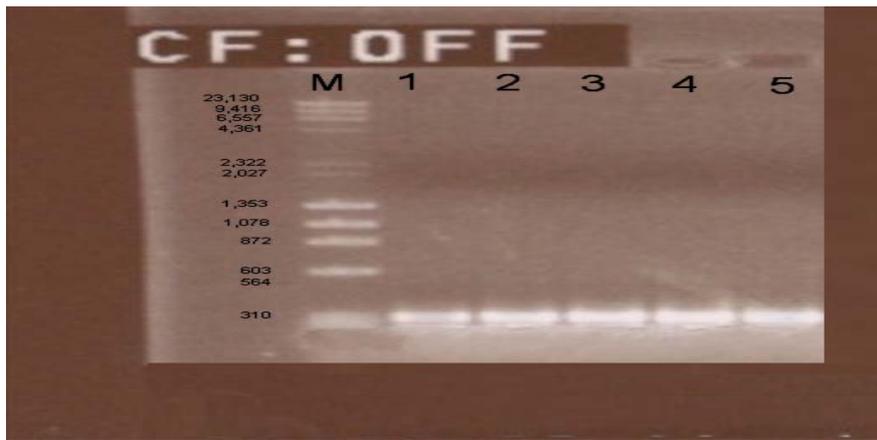
: DNA

DNA

310

.(3)

.....*Myceliophthora verrucosa*



(5-1)

:3

: M

agrI

CCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCATCAAGCCCCGGCTTGT
 GTTGGGGACCTGCGGCTGTCCGCAGGCCCTGAAAACCAGTGGCGGGCTCGCTA
 GTCACACCGAGCGTAGTAGCATAACATCTCGCTCAGGGCGTGCTGCGGGTTCCGG
 CCGTTAAACGACCTTCATAACCCAAGGTTGACCTCGGATCAGGTAGGAAGACCC
 GCTGAACTTAAGCATATCAATAAG.

.(4)

NCBI

Myceliophthora verrucosa

(5)

%100

Accession Description

[Max](#) [Total](#) [Query](#) [E](#) [Max](#) [Links](#)
[score](#) [score](#) [coverage](#) [value](#) [ident](#)

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
JQ246353.1	Myceliophthora verrucosa isolate MMI00058 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	442	442	100%	6e-121	100%	

Alignments

>gb|JQ246353.1| Myceliophthora verrucosa isolate MMI00058 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
 Length=566

Score = 442 bits (239), Expect = 6e-121
 Identities = 239/239 (100%), Gaps = 0/239 (0%)
 Strand=Plus/Plus

```

Query 1 CCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCATCAAGCCCCGGCTTGTGTTGGG 60
      |||
Sbjct 320 CCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCATCAAGCCCCGGCTTGTGTTGGG 379

Query 61 GACCTGCGGCTGTCCGCAGGCCCTGAAAACCAAGTGGCGGGCTCGCTAGTCACACCGAGCG 120
      |||
Sbjct 380 GACCTGCGGCTGTCCGCAGGCCCTGAAAACCAAGTGGCGGGCTCGCTAGTCACACCGAGCG 439

Query 121 TAGTAGCATAACATCTCGCTCAGGGCGTGTGCGGGTTCCGGCCGTTAAACGACCTTCATA 180
      |||
Sbjct 440 TAGTAGCATAACATCTCGCTCAGGGCGTGTGCGGGTTCCGGCCGTTAAACGACCTTCATA 499

Query 181 ACCCAAGGTTGACCTCGGATCAGGTAGGAAGACCCGCTGAACTTAAGCATATCAATAAG 239
      |||
Sbjct 500 ACCCAAGGTTGACCTCGGATCAGGTAGGAAGACCCGCTGAACTTAAGCATATCAATAAG 558
  
```

* Query = Sbjct =

.NCBI

:4



Khalil_rDNA_19_A01.ab1

KB 1.2 KB.bcp

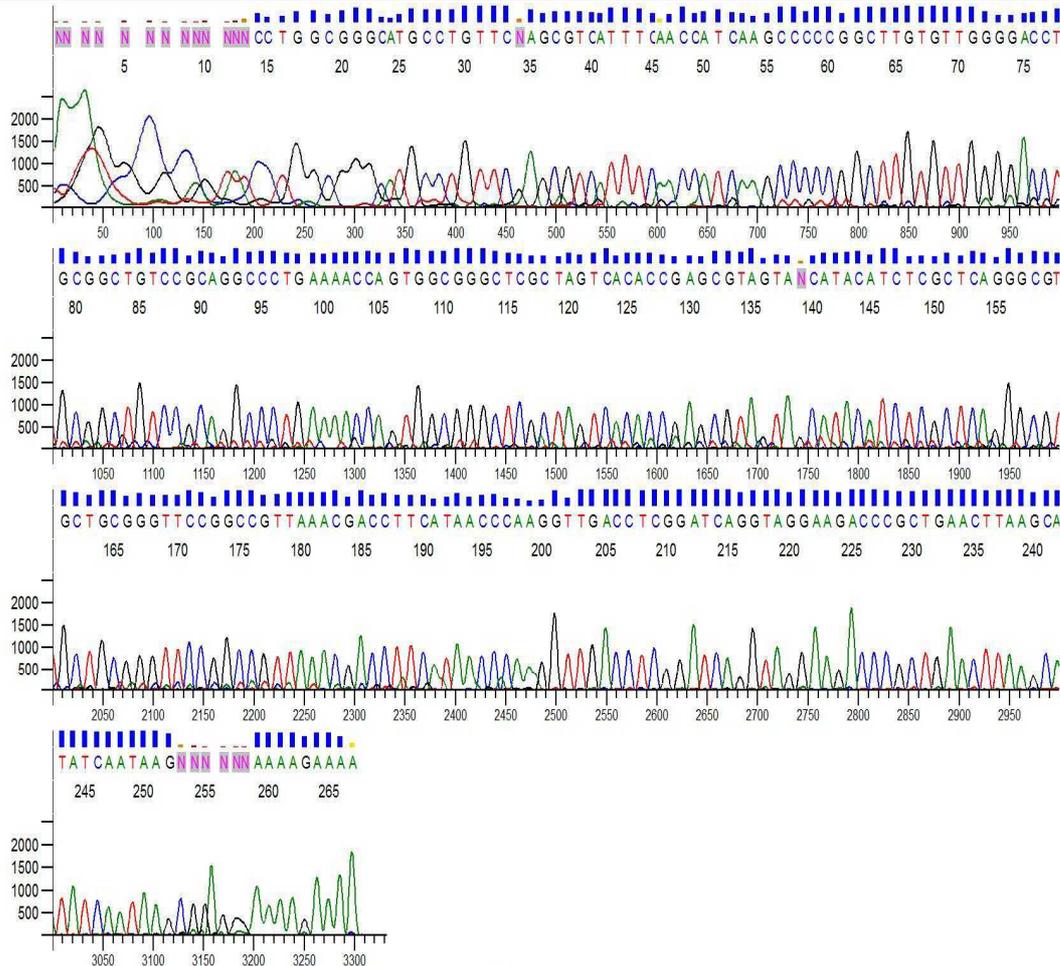
Khalil_rDNA_19_A01

KB_3730_POP7_BDTV3.mob

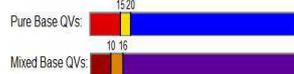
Signal: G:4207 A:4786 T:5587 C:5542 AvgSig: 5025

C#:15 W:A1 Plate Name:20120914PD4

TS:45 CRL:245 QV20+:244



Inst Model/Name:3730xl/Rich-16111-028



Printed on: ١٠/١٩/٢٥ ٢٧:٢٠:١٢ GMT

Sequence Scanner v1.0

Electropherogram Data Page 1 of 1

:5

DNA

ITS

.(Buchan *et al.*, 2002)

.(Luo and Mitchell, 2002)

.(2013)

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