

ITS

Rhizopus

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DNA D1-D2

MOLECULAR AND PHYLOGENETIC ANALYSIS OF *Rhizopus* ISOLATES BASED ON IT'S REGION AND D1-D2 REGIONS OF rDNA

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Rhizopus

	DNA	D1-D2	5.8S	ITS		
<i>MspI</i>	<i>HinfI</i>	<i>RsaI</i>	<i>HaeIII</i>	PCR		ITS1/NL4

Rhizopus

R. lycococcus *R. oryzae* *R. stolonifer* var. *stolonifer* *R. lycococcus* :

R. stolonifer var. *stolonifer*

	ITS	DNA	LSU	D1	D2
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DNA :

mohammadi_par@yahoo.com :

Zygomycota

Absidiaceae

Rhizopus

Mucorales

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- Rhizopus nigricans* (Alexopoulos *et al.* 1996)
- R. stolonifer* (Schipper 1984) (Vágvölgyi *et al.* 2004)
- Rhizopus* () *Rhizopus stolonifer*
- (Abdalla *et al.* 2008)
- °C
- (Schwartz & Gent 2005)
- Rhizopus* (Schipper 1984) *R. sexualis* *R. lyococcus* *R. oryzae* *R. stolonifer*
- (Dennis 1979)
- (Ben-Arie *et al.* 1991) (Martin 1964)
- (Wade & Morris 1982)
- (Maclean *et al.* 1993) *Rhizopus* (Jennessen *et al.* 2005)
- (Vágvölgyi *et al.* 2004) RAPD (Schipper *et al.* 1996, Voigt)
- (Park *et al.* 2003) PCR-RFLP (et al. 1999, Zycha *et al.* 1969)
- (Liou *et al.* 2007, Abe *et al.* 2006, 2007, Saito *et al.* 2004) (Mucormycosis)
- (Liou *et al.* 2007) ()
- (Hesseltine & Ellis 1973)
- (Ershad 2009) *Rhizopus*
- Mucor stolonifer*

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°C

PDA

R. stolonifer *R. oryzae*

(Ershad 2009)

°C

(Malt Agar) MA

°C

°C

°C

°C

°C

°C

°C

°C

°C

(Schipper 1984)

Rhizopus

DNA

PCR-RFLPs

(Potato Dextrose Broth) PDB

(Malt Yeast Extract) MY

°C

°C

)

(

()

°C

DNA

WA

DNA

(Potato Dextrose Agar) PDA (Water Agar)

°C

Table 1. Isolates of *Rhizopus* obtained from Tehran, West Azarbaijan provinces, Yazd and Kordestan Provinces

peach	Pakdasht	P1, P3, P5, P11, P12, P13, P14, P15, P16
peach	Urmia- Emamzade	P2
peach	Urmia	P4, P6
peach	Urmia-Nazloo	P7, P8, P9, P10
strawberry	Urmia	S1, S2, S3
strawberry	Urmia- Emamzade	S4
strawberry	Urmia-Nazloo	S5
strawberry	Urmia-Lashenloo	S6
strawberry	Sanandaj	S7
sunflower	Pakdasht	SUN1
sunflower	Urmia-Arnesa	SUN2
sunflower	Urmia- Sheikhsarmast	SUN3
apricot	Pakdasht	A1, A2
tomato	Pakdasht	T1, T2, T3
melon	Pakdasht	M1
melon	Yazd-Roknabad	M2
nectarine	Pakdasht	N1, N2, N3
persimmon	Pakdasht	KH

/ (10 mM) / (Dellaporta 1983)
 DNA (50 ng) (5 U/μl) *Taq* DNA
 / /
 °C PCR
 °C °C °C
 °C (rDNA
 .(Purkayastha *et al.* 2006) ITS1: 5'-TCC- rDNA
 White *et al.*) GTA-GGT-GAA-CCT-GCG-<G>-3'
 NL4: 5'- GGT-CCG-TGT-TTC- (*al.* 1990
 (O'Donnell 1992) AAG-ACG-<G>-3'
*Hae*III PCR DNA
 . *Msp*I *Rsa*I *Hin*fI
 PCR
 () () / (PCR buffer 10X) PCR
 Excel dNTPs (2.5 mM) / (50 mM) MgCl₂ (10X)

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D1-D2 ITS		RFLPs	SIMQUAL (Jaccard's coefficient)	(version 2.02) NTSYS-pc	
			SAHN		
	<i>R. stolonifer</i>				UPGMA
		<i>R. lyococcus</i>			
		<i>R. oryzae</i>			
					PCR (Sequencing)
			Eurofins MWG Operon		
			GeneDoc		
	<i>Rhizopus</i>				(Nicholas and Nicholas 1997)
°C	<i>R. stolonifer</i>		D1-D2	5.8S	ITS
°C	<i>R. lyococcus</i>				
<i>R. oryzae</i>			Clustal		
		°C	CLUSTAL		
		(Schipper 1984)			(Thompson <i>et al.</i> 1997) X (ver. 1.81)
°C	°C <i>R. stolonifer</i>				
	°C <i>R. oryzae</i>		(Neighbor-Joining) NJ		
	(Liou <i>et al.</i> 2007)			Bootstrap	
<i>R. lyococcus</i>	°C <i>R. stolonifer</i>			Maximum Parsimony	
°C <i>R. oryzae</i>	°C			Bootstrap	
			PAUP ver.		
					(Swofford 2003) 4.0b10
rDNA					
ITS	NL4	ITS1			
DNA		D1-D2		5.8S	

()

% . % UPGMA

(C) *R. lyococcus* ()

%

(B A) *R. oryzae* *R. stolonifer*

PCR

NL4 ITS1

RFLPs *HaeIII* *HinfI*

(S5 S4 M1 SUN2 P4 P2 P1) *RsaI* *MspI*

/

GenBank

R. oryzae

GenBank NL4 ITS1

R. lyococcus *R. stolonifer* *HinfI* PCR

R. oryzae *HaeIII* *R. oryzae*

ITS *R. stolonifer* *RsaI* *R. lyococcus*

(Liou *et al.* 2007) *MspI* *R. lyococcus*

R. lyococcus *R. oryzae*

ITS D1-D2 PCR

ITS

HaeIII *R. stolonifer*

RsaI *R. lyococcus*

D1-D2 *R. stolonifer*

GenBank *MspI*

Mucor indicus

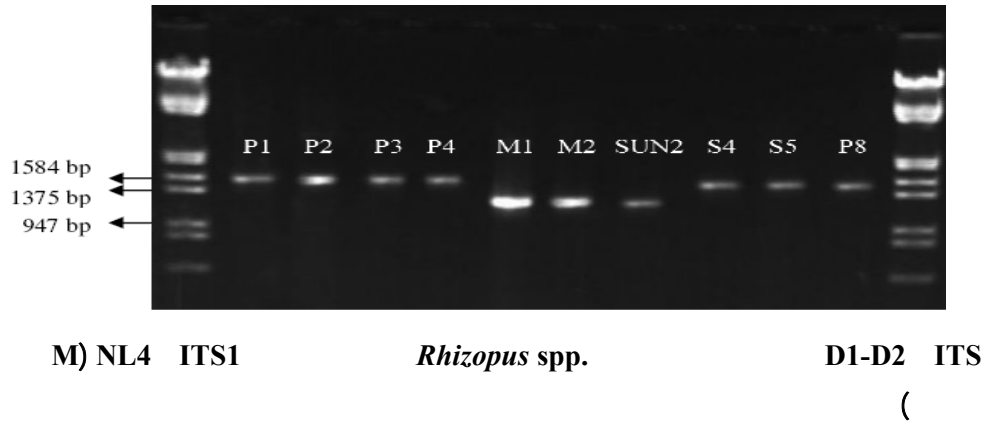


Fig. 1. ITS and D1-D2 regions amplified using ITS1 and NL4 primers (M, size marker with 1000 bp molecular weight)

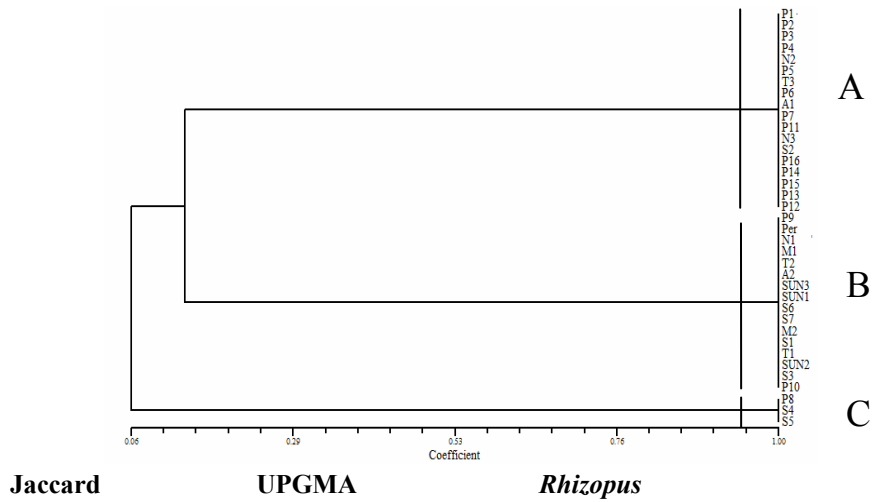


Fig. 2. Dendrogram of 37 isolates of *Rhizopus* genus created by UPGMA method with Jaccard coefficient

PAUP ver. 4.0b10 Maximum Parsimony NJ

(% %) GenBank

R. lyococcus *R. stolonifer* (C B A)

R. oryzae Bootstrap % NJ

R. stolonifer Bootstrap % Maximum Parsimony

A *R. lyococcus*

(NL4 ITS1)
Rhizopus spp.

Table 2. Restriction fragments obtained after digestion of PCR products with various enzymes

<i>Hinf</i> I	<i>Hae</i> III	<i>Rsa</i> I	<i>Msp</i> I	
490,430,250	750,430,220,80	700,450,430	1580	P1
490,430,250	750,430,220,80	700,450,430	1580	P2
490,430,250	750,430,220,80	700,450,430	1580	P3
490,430,250	750,430,220,80	700,450,430	1580	P4
490,430,250	750,430,220,80	700,450,430	1580	P5
490,430,250	750,430,220,80	700,450,430	1580	P6
490,430,250	750,430,220,80	700,450,430	1580	P7
500,410,90	700,320,240,100,100	580,420,310,150	860,350,250	P8
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	P9
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	P10
490,430,250	750,430,220,80	700,450,430	1580	P11
490,430,250	750,430,220,80	700,450,430	1580	P12
490,430,250	750,430,220,80	700,450,430	1580	P13
490,430,250	750,430,220,80	700,450,430	1580	P14
490,430,250	750,430,220,80	700,450,430	1580	P15
490,430,250	750,430,220,80	700,450,430	1580	P16
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	S1
490,430,250	750,430,220,80	700,450,430	1580	S2
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	S3
500,410,90	700,320,240,100,100	580,420,310,150	860,350,250	S4
500,410,90	700,320,240,100,100	580,420,310,150	860,350,250	S5
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	S6
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	S7
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	SUN1
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	SUN2
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	SUN3
490,430,250	750,430,220,80	700,450,430	1580	A1
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	A2
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	T1
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	T2
490,430,250	750,430,220,80	700,450,430	1580	T3
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	M1
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	M2
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	N1
490,430,250	750,430,220,80	700,450,430	1580	N2
490,430,250	750,430,220,80	700,450,430	1580	N3
490,430,210,110,90	700,350,200,80	400,300,200,130	730,600	KH

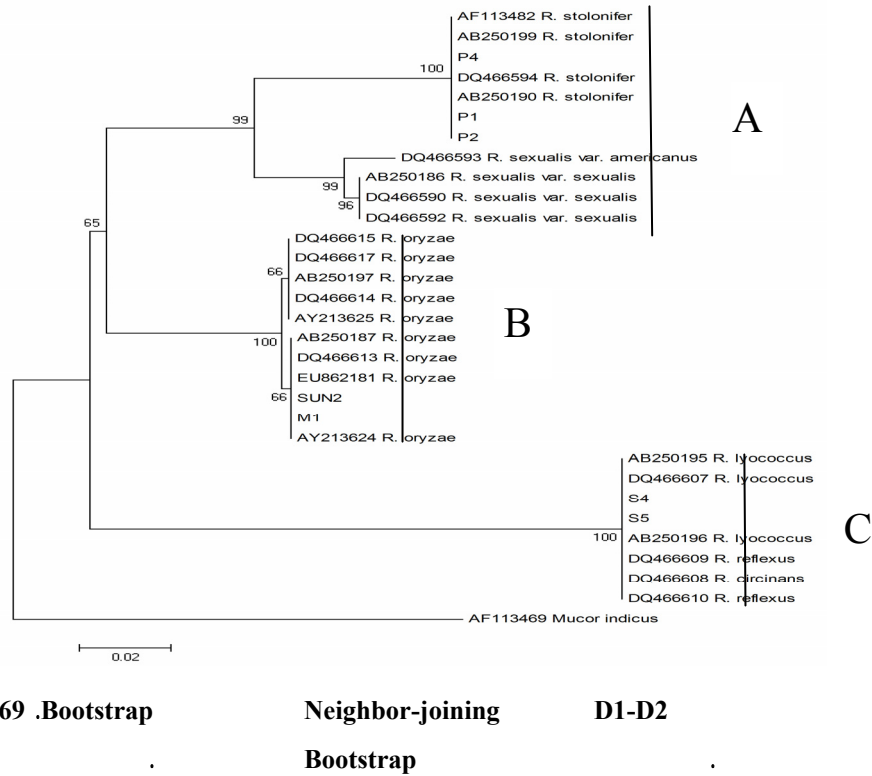


Fig. 3. Cladogram based on D1-D2 sequences with Neighbor-joining method and 1000 bootstrap repeats. AF113469 used as an outgroup.

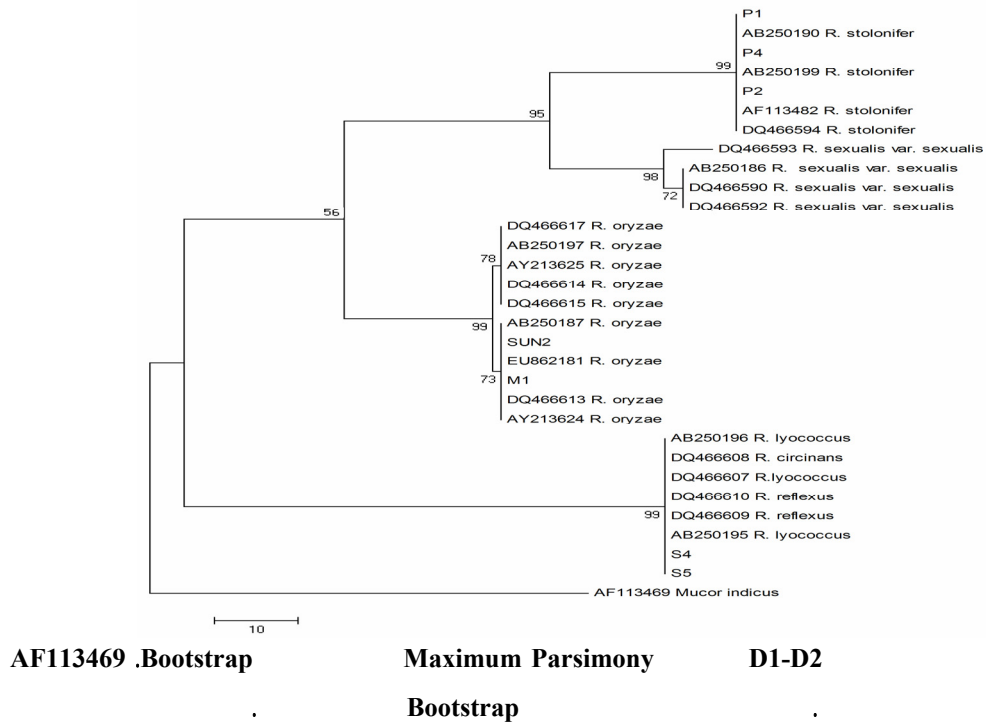


Fig. 4. Cladogram based on D1-D2 sequences with Maximum Parsimony method and 2000 bootstrap repeats. AF113469 used as an outgroup.

<i>R. stolonifer</i> var. <i>lyococcus</i>				<i>R. stolonifer</i>
<i>R. stolonifer</i> var. <i>stolonifer</i>	%		P4 P2 P1	
				Bootstrap
				<i>R. sexualis</i>
<i>R. lyococcus</i>			Bootstrap	%
	D1-D2		<i>R. oryzae</i>	B
% <i>R. lyococcus</i>		M1	SUN2	
Maximum Parsimony NJ		Bootstrap	%	
<i>R. lyococcus</i>			C	
D1-D2 (Abe <i>et al.</i> 2006)				<i>R. lyococcus</i>
<i>R. lyococcus</i>	18S		<i>R. lyococcus</i>	S5 S4
<i>R. stolonifer</i> var. <i>stolonifer</i>			Bootstrap	%
				D1-D2
D1-D2				
<i>R. lyococcus</i>				D1-D2
<i>R. stolonifer</i>				<i>R. stolonifer</i>
Abe <i>et al.</i> 2006, Vágvölgyi <i>et al.</i> 2004,)			% <i>R. stolonifer</i>	
(Liou <i>et al.</i> 2001, Schipper, 1984			Maximum Parsimony NJ	
				<i>R. stolonifer</i>
<i>R. oryzae</i>				(Liou <i>et al.</i> 2007)
				<i>R. stolonifer</i> (Ehrenb.: Fr.) Vuill. var. <i>stolonifer</i>
				<i>Rhizopus</i>
(Schipper 1984)		ITS 18S		(Abe <i>et al.</i> 2006)
	D1-D2			DNA 28S D1/D2
% <i>R. oryzae</i>				C B A
Maximum Parsimony NJ				(Schipper 1984; Schipper & Stalpers 1984)
<i>R. oryzae</i>				(<i>R.oryzae stolonifer</i> -group <i>microsporus</i> -group)
(Liou <i>et al.</i> 2007)				

stolonifer

R. stolonifer var. *stolonifer* .*R. oryzae* :

R. lyococcus

.R. lyococcus

R. stolonifer var.

(55-57)