

Fungal Identification

Objective

To identify to the species level a single fungal isolate by sequencing fungal D2 expansion segment region of the nuclear large-subunit (LSU) rDNA gene

Starting Materials

Fungal isolation on agar plate

Methods

1. Picking of a single colony from the agar plate
2. Genomic DNA extraction with PrepMan® Ultra Sample Preparation Reagent
3. PCR of the D2 LSU rDNA gene using MicroSeq® D2 LSU rDNA Fungal Identification PCR Kit
4. Double strand sequencing with MicroSeq® D2 LSU rDNA Fungal Identification Sequencing Kit
5. Alignment of the consensus sequence against GenBank public database

Results

D2 LSU rDNA Consensus sequence (322 bp):

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CCTCTTGGTCCGTGTTTCAAGACGGGCGGATTACGACCATTACGCCAGCA
TCCTAGCCGAAGCGCGGACCTCGCCCGGCGCTCGCTGCATCACAAGAGGC
TATAACACTCCCGAGGGAGCTACATTCCTCAAGTCTTATCCAGCGGCACC
AGACGATGCTGGCCTGCAACGCGGCGAGTAGACCGGTGAGAAGACCGGCC
GAACACCGCGAGCAAGTCTGGTTGCAATCCCTTCCCTTTTAAACAATTTCA
CGTGCTTTTTAACTCTCTTTCCAAAGTGCTTTTCATCTTTTCGATCACTCT
ACTTGTTTCGCTATCGGTCTAGG
```

First 10 Blast Hits

Distribution of 100 Blast Hits on the Query Sequence

Sequences producing significant alignments:	Score (Bits)	E Value
gi 37723106 gb AY152617.1 Mycosphaerella tassiana strain CBS...	626	9e-177
gi 29691392 gb AY234940.1 Cladosporium sp. SW073 large subun...	626	9e-177
gi 39547961 gb AY464845.1 Uncultured fungus clone FF10 26S r...	618	2e-174
gi 15808410 gb AY016367.1 Raciborskiomyces longisetosum 28S ...	618	2e-174
gi 83699212 dbj AB161065.1 Scolecobasidium terreum gene for ...	615	4e-173
gi 37786655 gb AY213694.1 Cladosporium cladosporioides strai...	613	1e-172
gi 51465753 dbj AB100658.1 Cladosporium coralloides gene for...	613	1e-172
gi 51465745 dbj AB100650.1 Cladosporium cladosporioides gene...	613	1e-172
gi 29691393 gb AY234941.1 Cladosporium sp. SW197 large subun...	611	6e-172
gi 37786656 gb AY213695.1 Cladosporium cladosporioides strai...	605	3e-170



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Identification results:

>gi|37723106|gb|AY152617.1| Mycosphaerella tassiana strain CBS289.49 28S ribosomal RNA
gene,
partial sequence
Length=316

Score = 626 bits (316), Expect = 9e-177
Identities = 316/316 (100%), Gaps = 0/316 (0%)
Strand=Plus/Minus

```
Query 4 CTTGGTCCGTGTTTCAAGACGGGCGGATTACGACCATTACGCCAGCATCCTAGCCGAAGC 63
      |||
Sbjct 316 CTTGGTCCGTGTTTCAAGACGGGCGGATTACGACCATTACGCCAGCATCCTAGCCGAAGC 257

Query 64 GCGGACCTCGCCCGGCGCTCGCTGCATCACAAGAGGCTATAAACTCCCGAGGGAGCTAC 123
      |||
Sbjct 256 GCGGACCTCGCCCGGCGCTCGCTGCATCACAAGAGGCTATAAACTCCCGAGGGAGCTAC 197

Query 124 ATTCTCAAGTCTTATCCAGCGGCACCAGACGATGCTGGCCTGCAACGCGGCGAGTAGAC 183
      |||
Sbjct 196 ATTCTCAAGTCTTATCCAGCGGCACCAGACGATGCTGGCCTGCAACGCGGCGAGTAGAC 137

Query 184 CGGTGAGAAGACCGGCGGAACACCGCGAGCAAGTCTGGTTGCAATCCCTTCCCTTTTAAC 243
      |||
Sbjct 136 CGGTGAGAAGACCGGCGGAACACCGCGAGCAAGTCTGGTTGCAATCCCTTCCCTTTTAAC 77

Query 244 AATTCACGTGCTTTTAACTCTCTTTCCAAAGTGCTTTTCATCTTTCGATCACTCTACT 303
      |||
Sbjct 76 AATTCACGTGCTTTTAACTCTCTTTCCAAAGTGCTTTTCATCTTTCGATCACTCTACT 17

Query 304 TGTTGCTATCGGTCT 319
      |||
Sbjct 16 TGTTGCTATCGGTCT 1
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Conclusions

Sequence data obtained from analyzed sample have 100% identity with *Mycosphaerella tassiana* (*Cladosporium herbarum*), GenBank Reference #AY152617. Based on this complete identity, the sample is positively identified as *Mycosphaerella tassiana* (*Cladosporium herbarum*).