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Abstract: Rhizoctonia sp. has an important role as a biocontrol agent. Its association with Phalaenopsis amabilis was molecularly identified through rDNA-ITS sequence analysis, conducted as one of wild orchid conservation efforts in Indonesia. A group of Rhizoctonia sp. were isolated from the root of orchid plant in Yogyakarta. Verification analysis of these isolates resulted in 600-750 bp DNA products located on the ITS1-5.8S-ITS4 region. The sequenced products showed insertion and substitution occurances, which may result in strain diversity and possible variation in severity. Reconstruction of phylogenetic trees using Maximum Parsimony and Bootstrap-1000 approach showed that Indonesian isolates have undergone speciation and have been positioned in the cluster, which are already far apart from the other isolates. Results of this study were expected to become the basic information in an effort of wild orchid cultivation and protection against infectious diseases in Indonesia. The study was the first to report regarding Rhizoctonia sp. isolated from wild tropical orchids in Indonesia.

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January 16th 2018

Dear Sir/Madam
Editor of Fungal Ecology,

I wish to submit a new manuscript entitled **Molecular Phylogeny of Mycorrhizal *Rhizoctonia* Isolated from Nature Tropical Orchids in Indonesia** for consideration by the Fungal Ecology. I confirm that this work is original and has not been published elsewhere nor is it currently under consideration for publication elsewhere. I also confirm that this submission for publication has been approved by all of the authors (other than myself), namely Budi Setiadi Daryono, Ari Indrianto, and Susamto Somawiyarjo.

In this paper, we report on phylogenetic analysis of mycorrhizal *Rhizoctonia* which was isolated from wild *Phalaenopsis amabilis* L. (Blume) in Indonesia. This is significant because it is the first taxonomic data related to mychorrizal *Rhizoctonia* from Indonesia. The *Rhizoctonia* was separated from other isolates on another countries. The data could be add to the global data of mychorrizal Rhizoctonia. The paper should be of interest to readers in the areas of mycology, plant pathology, and conservation because the mycorrizhal Rhizoctonia has a number of role in environment, one of which is to prevent *Odontoglossum ringspot virus* and *Cymbidium mosaic virus* replication.

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Please address all correspondence concerning this manuscript to me at bs_daryono@yahoo.com. Thank you for your consideration of this manuscript.

Sincerely,

Mahfut

MOLECULAR PHYLOGENY OF MYCORRHIZAL *RHIZOCTONIA* ISOLATED FROM WILD TROPICAL ORCHIDS IN INDONESIA

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ABSTRACT

Rhizoctonia sp. has an important role as a biocontrol agent. Its association with *Phalaenopsis amabilis* was molecularly identified through rDNA-ITS sequence analysis, conducted as one of wild orchid conservation efforts in Indonesia. A group of *Rhizoctonia* sp. were isolated from the root of orchid plant in Yogyakarta. Verification analysis of these isolates resulted in 600-750 bp DNA products located on the ITS1-5.8S-ITS4 region. The sequenced products showed insertion and substitution occurances, which may result in strain diversity and possible variation in severity. Reconstruction of phylogenetic trees using *Maximum Parsimony* and *Bootstrap*-1000 approach showed that Indonesian isolates have undergone speciation and have been positioned in the cluster, which are already far apart from the other isolates. Results of this study were expected to become the basic information in an effort of wild orchid cultivation and protection against infectious diseases in Indonesia. The study was the first to report regarding *Rhizoctonia sp*. isolated from wild tropical orchids in Indonesia.

Keywords: *Rhizoctonia* sp.; rDNA-ITS; *Phalaenopsis* sp.; Orchid Mycorrhizal Fungi.

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INTRODUCTION

Phalaenopsis is a genus of orchids, which some of its members have important role as parent crosses (Sarwono, 2002). Approximately 30 of the total 62 species are spread throughout Indonesia. The presence of this genus in its native habitat (nature) has been reported to have greatly diminished, even some of the members have been recorded to the IUCN red list version 2013.2 (IUCN, 2013) due to excessive exploration and forest degradation (Johanis et al., 2001). Thus, it is very necessary to conserve the existence of wild *Phalaenopsis* orchids in Indonesia through the efforts of preservation and protection of plants.

Infections by bacteria, fungi, and viruses are still major obstacles in conducting cultivation and development of wild orchids in Indonesia (Aruni *et al*., 2011). Cultivating and protecting *Phalaenopsis* sp. against diseases in Indonesia could be done through the induction of endophytic microorganisms (Tanawy, 2009), which includes the Orchid Mycorrhizal Fungi (OMF). Endophytic microorganisms are biological agents that can prevent and reduce the severity of disease caused by infectious pathogens by producing enzymes and secondary metabolites that are antagonistic (Harish et al., 2008), which in addition can also help the growth and development of plants. One type of OMF which have been isolated and identified is *Rhizoctonia* sp. (Suryantini, 2015).

Rhizoctonia solani could induce the resistance of *Phalaenopsis* sp. against infections by *Erwinia chrysanthemi* causing soft root disease (Wu et al., 2010), fungal infection by *Fusarium* sp. causing rotten stems, leaves, and shoots, as well as inhibit the replication *Odontoglossum ringspot virus* (ORSV) and *Cymbidium mosaic virus* (CymMV) (Tong, 2014). In addition for playing a role as biological control agents in crop protection (Otero et al., 2013), in general OMF has a role in stimulating the germination of orchid seeds (Andersen and Rasmussen, 1996), in supporting the

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provision of nutrients for growth and development of plantlets (Moreno, et al., 2000; Wu et al., 2011), and in helping the establishment of more buds and flowers (Moreno, et al., 2000). In this research, the identification of *Rhizoctonia solani* was molecularly conducted through analysis of rDNA-ITS sequence isolated from *Phalaenopsis amabilis* grown in Indonesia. This research was expected to become the basic information on the development of cultivation and protection of nature orchids and where possible, on the prevention of the occurence of the mentioned diseases in Indonesia.

MATERIALS AND METHODS

Plants Materials

The sampling of healthy roots of *Phalaenopsis amabilis* was conducted at four different locations: orchid garden in Condong Catur and Parakan (Yogyakarta), Balikpapan Botanical Garden (East Kalimantan), and Sultan Adam Forest (South Kalimantan). On the separated study, isolation results showed positive samples of mycorrhizal endophyte *Rhizoctonia* sp. based on morphological and microscopical analysis.

Molecular Analysis

Genomic DNA isolation was performed using techniques modified from *cetyltrimethylammonium bromide* (CTAB) method (Doyle and Doyle, 1987) on samples of pure cultures of isolated mycorrhizal endophyte *Rhizoctonia solani*. Genomic DNA was PCR amplified according to the manual instructions of *GoTaq® Green PCR mix (Promega)*. Predenaturation reaction and amplification was carried out using methods by Nadarajah et al. (2014), with a pair of universal primers, rDNA-ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and rDNA-ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). PCR products were visualized using electrophoresis on 2% agarose gel stained with ethidium bromide and 100 bp *Vivantis DNA ladder* was used as marker. The

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visualized DNA bands indicated the length of the targeted base pairs of rDNA-ITS, which were subjected to sequencing.

Phylogenetic analysis

Sequencing results were analyzed using *Sequence Scanner* software; the nucleotide sequences were combined using *EditSeq* and *SeqMan* of the *Software Suite for Sequence Analysis DNASTAR Lasergene DM Version 3.0.25*. *BLAST* software was used to determine and to compare the sequence homology with the data contained in the DDBJ database. Comparation between sequences of isolates was carried out using *Algorithm Multiple Alignment Parameters DNA* with *Kimura-2 Parameters*, relationship and phylogenetic analysis using the *Neighbour Joining* of *MEGA 5 Beta* program. Statistical analysis on internal branch was done using *the bootstrap* value with 1000 replication.

RESULTS

Sample Collection

A total of 12 samples of healthy roots of *Phalaenopsis amabilis* were isolated from 4 different locations, such as orchid garden in Condong Catur and Parakan (Yogyakarta), Balikpapan Botanical Garden (East Kalimantan), and Sultan Adam Forest (South Kalimantan). Positive result of *Rhizoctonia* sp. was found from orchid garden in Parakan (Figure 1), which had colony characteristic traits i.e. yellowish to white color, colony appearance like a cotton, 90^{o} branching hyphae shape, binucleate, with colony growth rate of 0.72 mm/hour, referring to Currah and Zelmer (1992) and Shan et al. (2002).

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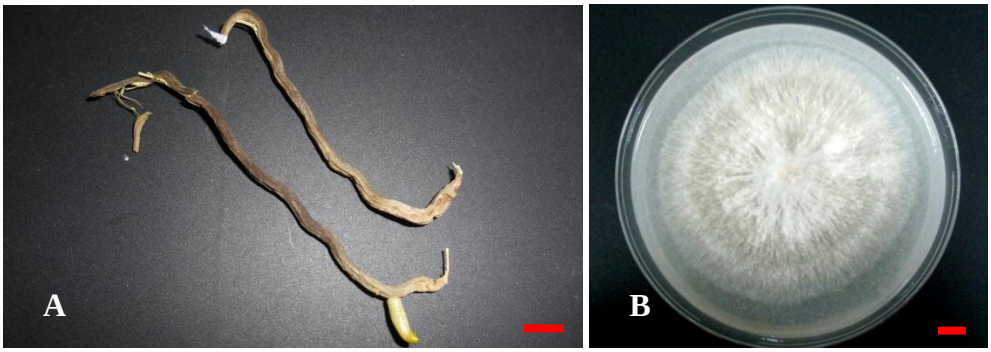

Figure 1. Sample collections of root of *Phalaenopsis amabilis* orchid (A) and *Rhizoctonia* isolate (B) from Parakan (Yogyakarta). Bar size 1 cm.

Molecular Analysis

ITS rDNA amplification results showed a specific band with a size between 600 and 750 bp (Figure 2) similar to that reported by Johansson et al. (1998), Nadarajah et al. (2014), and Pannecoucque et al. (2008).

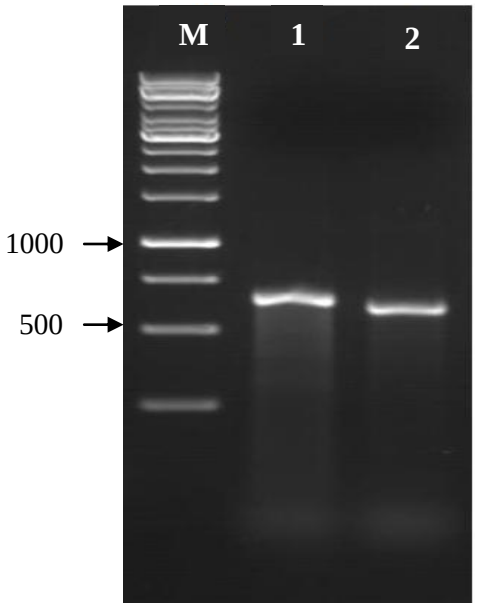

Figure 2. Results of ITS rDNA amplification on endophyte mycorrhizae *Rhizoctonia solani*; M = Marker (1 kb), 1 and 2 = Mychorrizae Parakan (MP)

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Internal transcribed spacer (ITS) is an area of the nuclear ribosomal DNA (nrDNA), which has the role of providing important information on the reconstruction of phylogenetic trees at different taxonomic levels (Bayer et al., 1996), as well as similarity at the level of intrageneric (Lee et al., 2010).

Sequencing results were combined and analyzed using *DNASTAR Lasergene DM Version 3.0.25*. Total number of nucleotide of MP isolates which succesfully scanned was 661 with 41.4% GC content. Analysis results were obtained by incorporating sequences to http://blast.ncbi.nlm.nih.gov/ site and confirmed that MP isolates were *Rhizoctonia* sp., which belong to the *Ceratobasidium*. Sneh et al. (1991) and Sharon et al. (2008) explained that genus *Rhizoctonia* in the anamorphic classification is divided into 3 main category: multinucleate *Rhizoctonia* (teleomorphs: *Thanatephorus* and *Waitea*), binucleate *Rhizoctonia* (teleomorphs: *Ceratobasidium* and *Tulasnella*), and uninucleate *Rhizoctonia* (teleomorph: *Ceratobasidium*). Based on microscopic observation, MP isolates were found to be binucleate (data not shown). Furthermore, search analysis of homologous sequences carried out using BLAST on NCBI site resulted in 47 sequences indicating a close relationship with high level of similarity of 99% (Table 1). This result showed that the MP sequences are representative of *Rhizoctonia* sp isolates, a non-pathogenic (endophyte) associating with the root of orchid (OMF).

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Table 1. Blast hits of *Rhizoctonia sp* (MP isolate) with *Ceratobasidium* rDNA-ITS sequence as query.

ID/accession	Organisms	Country	Totally bases	Max score
gi|70906627|gb|DQ102402.1|	Ceratobasidium sp. AG-G isolate Str14	USA	679 bp	1186
gi|70906626|gb|DQ102401.1|	Ceratobasidium sp. AG-G isolate Str16	Israel	680 bp	1186
gi|70906624|gb|DQ102399.1|	Ceratobasidium sp. AG-G isolate Str31	Israel	680 bp	1186
gi|27527695|emb|AJ318420.1|	Rhizoctonia sp. VJ15	Singapore	655 bp	1184
gi|762006709|gb|KP053814.1|	Uncultured Ceratobasidiaceae clone GX2-1	China	725 bp	1182
gi|630156424|gb|KJ495964.1|	Ceratobasidium sp. ANOF2	Taiwan	656 bp	1181
gi|528466578|gb|KC825348.1|	Rhizoctonia sp. AG-G isolate VRU-R3	Iran	694 bp	1181
gi|27527702|emb|AJ318427.1|	Rhizoctonia sp. M2ao1	Singapore	656 bp	1179
gi|440494654|gb|JX545228.1|	Uncultured Ceratobasidiaceae clone DOf-YC26	China	725 bp	1177
gi|70906625|gb|DQ102400.1|	Ceratobasidium sp. AG-G isolate Str35	Israel	681 bp	1177
gi|639127333|gb|KJ573103.1|	Ceratobasidium sp. ZeuS1-1	Taiwan	657 bp	1175
gi|70906622|gb|DQ102397.1|	Ceratobasidium sp. AG-G isolate Gm2	USA	679 bp	1175
gi|630156432|gb|KJ495972.1|	Uncultured Ceratobasidiaceae clone TANOF2	Taiwan	652 bp	1173
gi|27527708|emb|AJ318433.1|	Rhizoctonia solani	Singapore	655 bp	1171
gi|440494653|gb|JX545227.1|	Uncultured Ceratobasidiaceae clone DOf-YC11	China	725 bp	1168
gi|730911079|gb|KM488566.1|	Rhizoctonia sp. RM	Argentina	656 bp	1164
gi|27527704|emb|AJ318429.1|	Rhizoctonia sp. Abn1-2	Singapore	655 bp	1155

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Table 1. Continued

ID/accession	Organisms	Country	Totally bases	Max score
·gi|342349580|gb|JF519837.1|	Rhizoctonia sp. AG-G culture-collection CRA-COLMIA:Rh202	Italy	677 bp	1155
·gi|342349578|gb|JF519835.1|	Rhizoctonia sp. AG-G culture-collection CRA-COLMIA:Rh190	Italy	674 bp	1147
gi|154082130|gb|EU002954.1|	Uncultured Ceratobasidium clone 3b	USA	638 bp	1147
gi|154082121|gb|EU002945.1|	Uncultured Ceratobasidium clone 1g	USA	639 bp	1146
gi|312271011|gb|HM623627.1|	Ceratobasidium sp. AG-G isolate G-1B	China	632 bp	1142
gi|154082129|gb|EU002953.1|	Uncultured Ceratobasidium clone 3a	USA	637 bp	1133
·gi|71842199|gb|DQ097889.1|	Ceratobasidium sp. AG-G internal transcribed spacer 1	Japan	1500 bp	1122
gi|146186352|gb|EF536969.1|	Ceratobasidium sp. FPUB	India	617 bp	1118
gi|532733808|gb|KF171076.1|	Ceratorhiza sp. R63	Cuba	657 bp	1112
gi|532733724|gb|KF171071.1|	Ceratorhiza sp. 61	Cuba	663 bp	1112
·gi|62861810|gb|AY927319.1|	Rhizoctonia sp. AG-G isolate R11	Italy	617 bp	1112
gi|303305918|gb|HM625909.1|	Ceratobasidium sp. AG-G isolate RH169	Italy	635 bp	1107
gi|303305917|gb|HM625908.1|	Ceratobasidium sp. AG-G isolate RH168	Italy	636 bp	1105
gi|303305916|gb|HM625907.1|	Ceratobasidium sp. AG-G isolate RH167	Italy	637 bp	1105
·gi|62861820|gb|AY927329.1|	Rhizoctonia sp. AG-G isolate R25	Italy	617 bp	1105
·gi|62861814|gb|AY927323.1|	Rhizoctonia sp. AG-G isolate R16	Italy	617 bp	1105
·gi|62861818|gb|AY927327.1|	Rhizoctonia sp. AG-G isolate R22	Italy	618 bp	1103
·gi|62861811|gb|AY927320.1|	Rhizoctonia sp. AG-G isolate R13	Italy	616 bp	1103

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Table 1. Continued

ID/accession	Organisms	Country	Totally bases	Max score
gi|61612749|gb|AY738627.1|	Rhizoctonia sp. AG-G isolate R1	Italy	616 bp	1103
gi|62861839|gb|AY927348.1|	Rhizoctonia sp. AG-G isolate R56	Italy	618 bp	1101
gi|18181854|emb|AJ242897.1|	Rhizoctonia sp. C-653	Spain	659 bp	1098
gi|300303968|gb|HM597133.1|	Ceratobasidium sp. AG-G	USA	618 bp	1096
gi|224830295|gb|FJ752627.1|	Fungal sp. JIA3-1-1	China	614 bp	1085
gi|560940585|gb|KF688126.1|	Fungal sp. RTB2	India	602 bp	1083
gi|407741913|gb|JX514383.1|	Ceratobasidium sp. Ano_formo2	Taiwan	596 bp	1077
gi|83272293|gb|DQ279043.1|	Ceratobasidium sp. YA18	Netherlands	599 bp	1075
gi|62861815|gb|AY927324.1|	Rhizoctonia sp. AG-G isolate R17	Italy	592 bp	1062
gi|184186845|gb|EU605732.1|	Ceratobasidium sp. RR-2008	India	594 bp	1059
gi|67763786|dbj|AB196646.1|	Ceratobasidium sp. AG-G	Japan	560 bp	1059
gi|67763787|dbj|AB196647.1|	Ceratobasidium sp. AG-G	Japan	560 bp	1005

The whole isolates originated from various countries in Asia (Israel, China, Taiwan, Japan, Iran, Singapore, and India), America (USA, Cuba, and Argentina), and Europe (Italy, Netherlands and Spain). Some isolates of *Rhizoctonia* sp., the *Ceratobasidium*, was collected from the roots of *Vanda* miss Joaquim (AJ318420) and Brite Ng (AJ318429) orchids, and also from the roots of other plants such as *Rosa hybrida* cv. Linda (KC825348), strawberry *Fragaria x ananassa* (AY927319) and apples (EU002945.1). Total bases amplified from each isolate ranged from 560 to 725 bp, except from DQ097889.1 isolate with a total amplified base of 1500 bp. Maximum *BLAST* score between isolates ranged from 1005 to 1186 with homology (ident.) 99% and query cover ranged from 84 to 99%.

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Analysis on 14 other *Rhizoctonia* sp. isolates selected based on the distribution area of each different country, showed that isolates from Indonesia were highly different from those of other countries with similiarity index (IS) ranging from 45.2 to 49.9% (Figure 3). It was clear that isolates from Indonesia has been undergoing speciation and are different from isolates from other countries.

%	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	100														
2	99,4	100													
3	99,5	99,5	100												
4	99,7	99,7	99,8	100											
5	99,7	99,4	99,5	99,7	100										
6	100,0	99,5	99,7	99,8	99,8	100									
7	99,5	99,2	99,7	99,7	99,5	99,7	100								
8	96,6	99,4	99,5	99,6	96,3	99,8	96,5	100							
9	99,8	99,7	99,8	100,0	99,7	99,8	99,8	98,7	100						
10	99,7	99,7	99,8	100,0	99,7	99,8	99,8	96,6	100,0	100					
11	99,5	99,5	99,7	99,9	99,5	99,7	99,6	96,5	99,8	99,8	100				
12	99,5	99,5	99,7	99,9	99,5	99,7	99,6	96,5	99,8	99,8	99,7	100			
13	99,8	99,2	99,7	99,9	99,8	100,0	99,3	97,2	99.8	99,8	99,4	99,7	100		
14	99,8	99,7	99,8	100,0	99,7	99,8	99,8	98,7	100,0	100,0	99,8	99,8	99,8	100	
15	46,7	46,5	46,9	46,7	46,8	46,6	45,2	46,1	46,7	45,7	46,5	46,9	45,3	46,7	100

Figure 3. Matrix of similarity level percentage of the obtained *Rhizoctonia* sp. nucleotide sequences analyzed using *DNASTAR Lasergene program DM Version 3.0.25*. %: Percentage of Identity; 1: Singapore2; 2: Spain; 3: Taiwan; 4: America; 5: Argentina; 6: Netherland; 7: China; 8: Cuba; 9 : India; 10: Singapore; 11: Iran; 12: Israel; 13: Italy; 14: Japan; 15: Indonesia.

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Alignment result of nucleotide sequences of 15 *Rhizoctonia* sp. isolates (*Ceratobasidium* group) showed high occurrence of point mutations in isolates from Indonesian, which were largely insertions and substitutions. Of the 606 total bases aligned, there were 44 bases having substitution consisting of 21 transition and 23 transversion bases.

Each of these mutations had an influence on changes in amino acids formed. Of the total 194 amino acids belonging to isolates from Indonesia, some of which had very different percentage from those of other isolates. Some amino acids of isolates from Indonesia had increased their number dramatically compared to those of other isolates, i.e. Ala, Glu, Val, Gln, Lys, and Tyr by 0.7%, 1.76%, 1.8%, 2.3%, 3, 1%, and 4.73%, respectively, of total average. Several other amino acids had also decreased in number, i.e. Asp, Arg, Trp, Thr, and Leu by 0.11%, 1.53%, 2.07%, 2.25%, and 4.32%, respectively, of total average.

It appeared that the resulted amino acid changes had enormous influence in the process of adaptation to the environment in Indonesia. Results of the relationship analysis between isolates through the reconstruction of phylogenetic tree showed that isolates from Indonesia were on separated branches and far apart from 14 other isolates (Figure 4).

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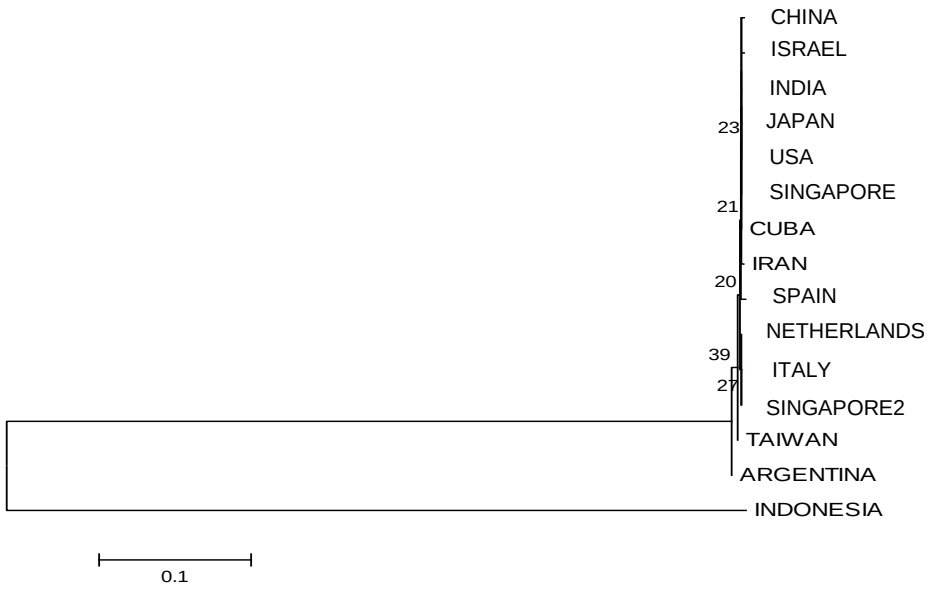

Figure 4. Reconstruction of phylogenetic trees of *Rhizoctonia* sp. isolates based on nucleotide sequences with *Maximum Parsimony* method with *Bootstrap-1000* approach

Discussions

Maximum Parsimony method with *Bootstrap-1000* approach classify the data based on specific characters (discrete character states) on which nucleotide sequences are used to obtain information about evolution through evolutionary data changes. The results of analysis were classified based on distribution on the geographical areas. A lengthy branch, which well separating between the two groups, indicated that isolates from Indonesia had considerably evolved and it led to speciation events. Such changes in sequence through mutation was supported by the ability of the mycorrhizal itself to adapt to environmental changes as well as to its host range. The adaptability of mycorrhizal is strongly influenced by the presence of amino acid changes due to mutations that alter the function of genes which they arrange.

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The reconstruction of phylogenetic tree, in addition to explaining relationship based on geographic regions, also determine the origin and history of distribution of the isolates. Trading activities of plants, fruits, and seedlings from several countries which are reported to have been infected by *Rhizoctonia* sp. is most likely the cause of its spreading. Indonesia is known as an importer country of orchid plant seedlings from Asian countries such as Thailand, Singapore, Taiwan, and China (Lakani, 2011; Somowiyarjo, 2016), and possibly have imported seedling and fruit of apple and strawberry plants from other countries such as India and Italy that had been reported infected by *Rhizoctonia* sp isolates. Proximity with Singapore in terms of geographical location is also very possible to allow distribution activities of orchids between the two countries.

This study results proved that nucleotide sequences and amino acid of *Rhizoctonia* sp. isolates from Indonesia were different with other isolates from another country (Asia, Europe, and America) due to adaptation to various environmental conditions. Furthermore, the mutations are capable of causing the *Rhizoctonia* sp. to lose its ability to infect and to associate with host plants. Another possible result of mutations is that *Rhizoctonia* sp. could have the ability to infect plants while showing pathogenic characters or otherwise become mycorrhizal endophytic.

One way to control pathogens of orchids is by using biological control, such as the mechanisms of mycorrhizal resistance induction. *Rhizoctonia solani* as OMF has a role as a biological agent in disease control (Otero *et al*., 2013) including infection of ORSV (Tong, 2014). This study results clarified the MP sequence as OMF *Rhizoctonia* sp. isolates from Indonesia based on identification of its rDNA-ITS sequences. Furthermore, this study was expected to be the basic information beneficial for the improvement of cultivation effort as well as for the development of biocontrol agents through natural orchid protection against pathogens and diseases.

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Figure 1

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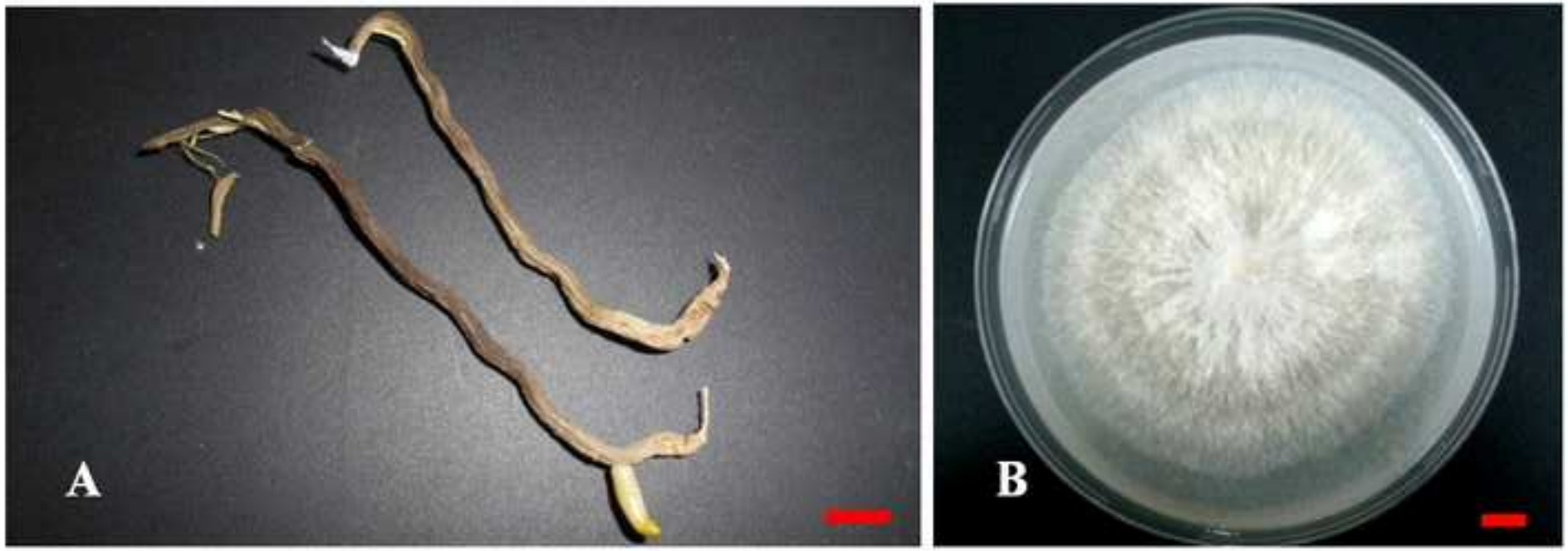

Figure 2

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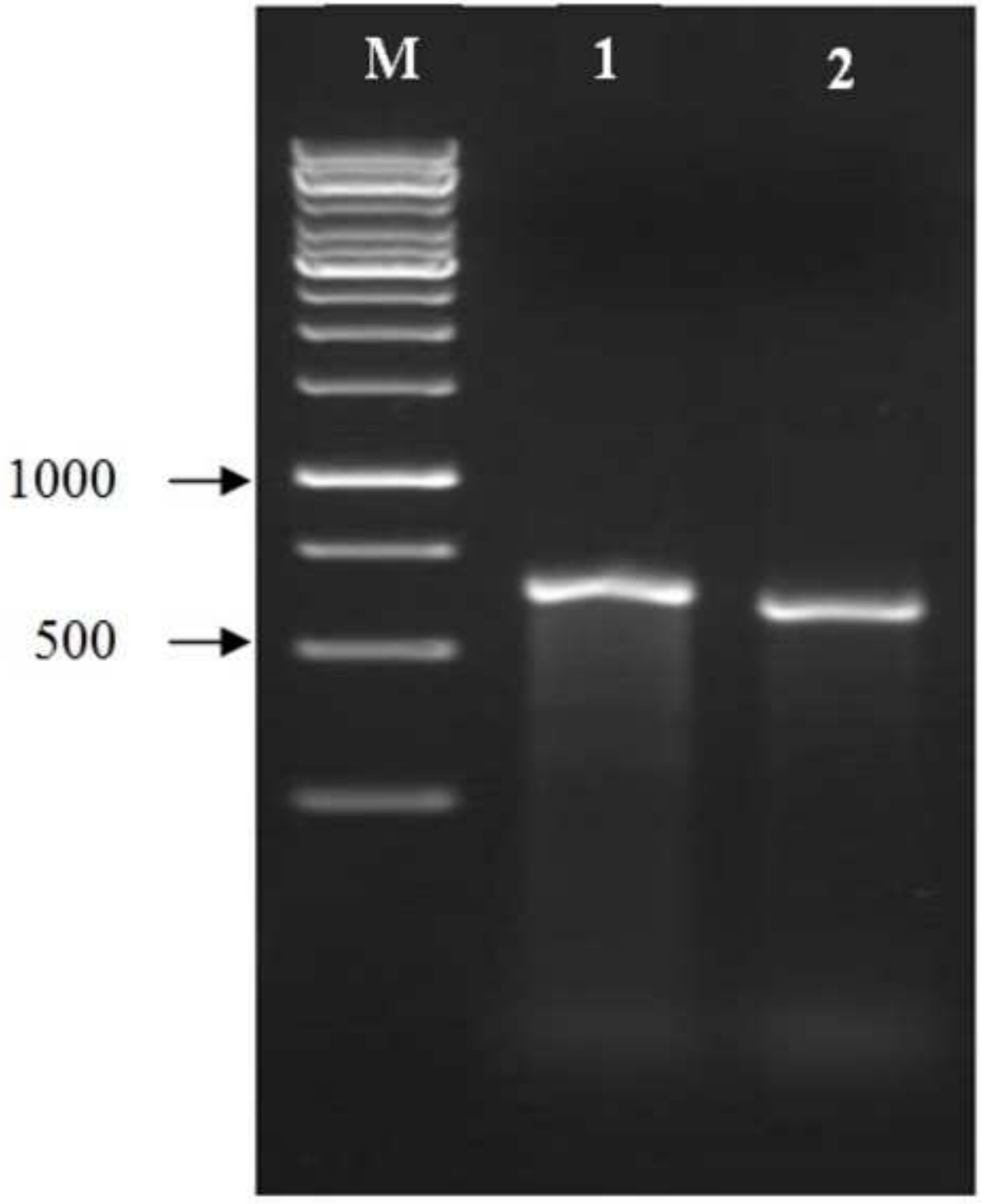

Figure 3

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%	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	100														
2	99,4	100													
3	99,5	99,5	100												
4	99,7	99,7	99,8	100											
5	99,7	99,4	99,5	99,7	100										
6	100,0	99,5	99,7	99,8	99,8	100									
7	99,5	99,2	99,7	99,7	99,5	99,7	100								
8	96,6	99,4	99,5	99,6	96,3	99,8	96,5	100							
9	99,8	99,7	99,8	100,0	99,7	99,8	99,8	98,7	100						
10	99,7	99,7	99,8	100,0	99,7	99,8	99,8	96,6	100,0	100					
11	99,5	99,5	99,7	99,9	99,5	99,7	99,6	96,5	99,8	99,8	100				
12	99,5	99,5	99,7	99,9	99,5	99,7	99,6	96,5	99,8	99,8	99,7	100			
13	99,8	99,2	99,7	99,9	99,8	100,0	99,3	97,2	99,8	99,8	99,4	99,7	100		
14	99,8	99,7	99,8	100,0	99,7	99,8	99,8	98,7	100,0	100,0	99,8	99,8	99,8	100	
15	46,7	46,5	46,9	46,7	46,8	46,6	45,2	46,1	46,7	45,7	46,5	46,9	45,3	46,7	100

Figure 4

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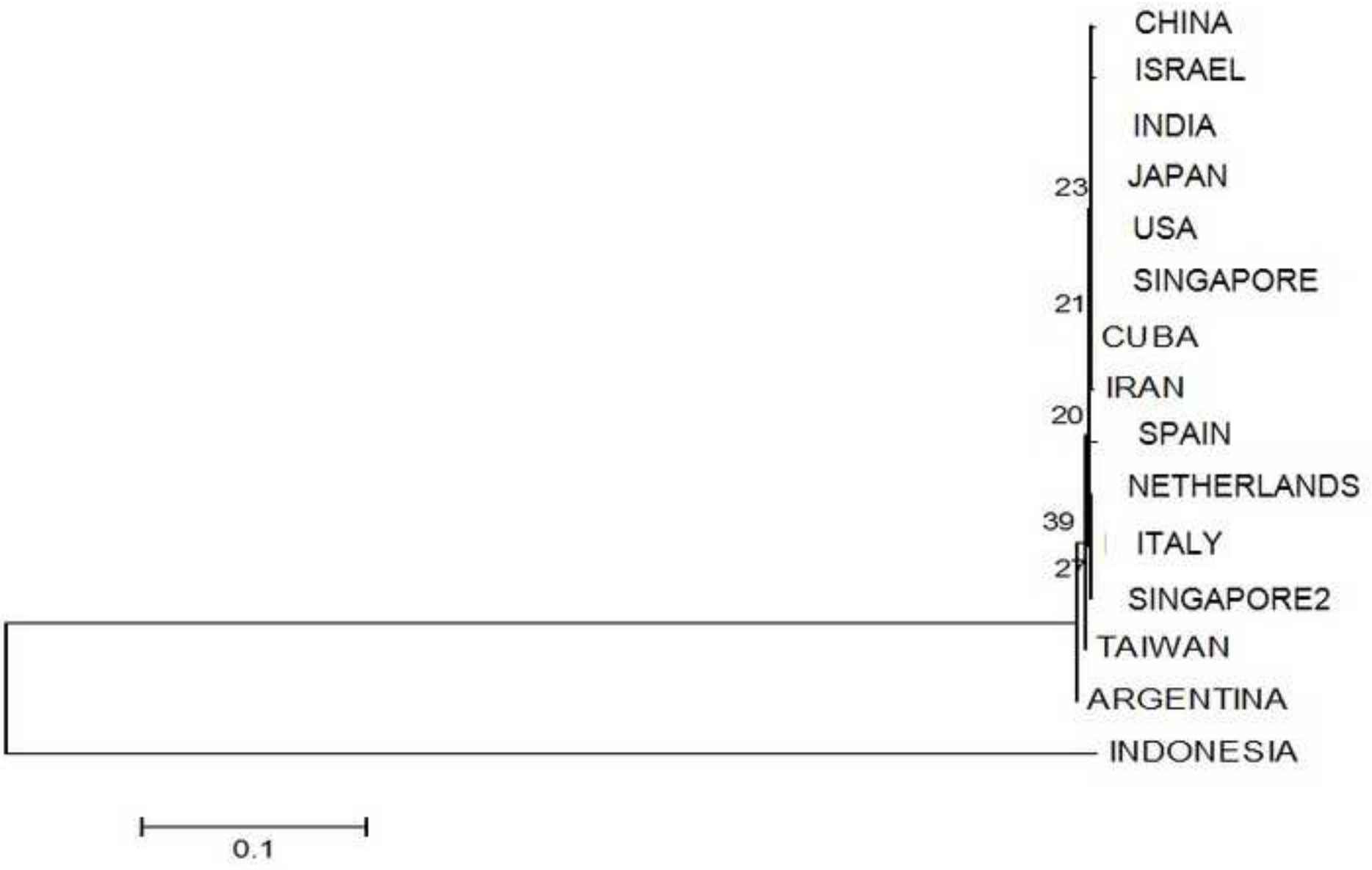

Table 1. Blast hits of *Rhizoctonia sp* (MP isolate) with *Ceratobasidium* rDNA-ITS sequence as query.

ID/accession	Organisms	Country	Totally bases	Max score
gi|70906627|gb|DQ102402.1|	Ceratobasidium sp. AG-G isolate Str14	USA	679 bp	1186
gi|70906626|gb|DQ102401.1|	Ceratobasidium sp. AG-G isolate Str16	Israel	680 bp	1186
gi|70906624|gb|DQ102399.1|	Ceratobasidium sp. AG-G isolate Str31	Israel	680 bp	1186
gi|27527695|emb|AJ318420.1|	Rhizoctonia sp. VJ15	Singapore	655 bp	1184
gi|762006709|gb|KP053814.1|	Uncultured Ceratobasidiaceae clone GX2-1	China	725 bp	1182
gi|630156424|gb|KJ495964.1|	Ceratobasidium sp. ANOF2	Taiwan	656 bp	1181
gi|528466578|gb|KC825348.1|	Rhizoctonia sp. AG-G isolate VRU-R3	Iran	694 bp	1181
gi|27527702|emb|AJ318427.1|	Rhizoctonia sp. M2ao1	Singapore	656 bp	1179
gi|440494654|gb|JX545228.1|	Uncultured Ceratobasidiaceae clone DOf-YC26	China	725 bp	1177
gi|70906625|gb|DQ102400.1|	Ceratobasidium sp. AG-G isolate Str35	Israel	681 bp	1177
gi|639127333|gb|KJ573103.1|	Ceratobasidium sp. ZeuS1-1	Taiwan	657 bp	1175
gi|70906622|gb|DQ102397.1|	Ceratobasidium sp. AG-G isolate Gm2	USA	679 bp	1175
gi|630156432|gb|KJ495972.1|	Uncultured Ceratobasidiaceae clone TANOF2	Taiwan	652 bp	1173
gi|27527708|emb|AJ318433.1|	Rhizoctonia solani	Singapore	655 bp	1171
gi|440494653|gb|JX545227.1|	Uncultured Ceratobasidiaceae clone DOf-YC11	China	725 bp	1168
gi|730911079|gb|KM488566.1|	Rhizoctonia sp. RM	Argentina	656 bp	1164
gi|27527704|emb|AJ318429.1|	Rhizoctonia sp. Abn1-2	Singapore	655 bp	1155

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Table 1. Continued

ID/accession	Organisms	Country	Totally bases	Max score
·gi|342349580|gb|JF519837.1|	Rhizoctonia sp. AG-G culture-collection CRA-COLMIA:Rh202	Italy	677 bp	1155
·gi|342349578|gb|JF519835.1|	Rhizoctonia sp. AG-G culture-collection CRA-COLMIA:Rh190	Italy	674 bp	1147
gi|154082130|gb|EU002954.1|	Uncultured Ceratobasidium clone 3b	USA	638 bp	1147
gi|154082121|gb|EU002945.1|	Uncultured Ceratobasidium clone 1g	USA	639 bp	1146
gi|312271011|gb|HM623627.1|	Ceratobasidium sp. AG-G isolate G-1B	China	632 bp	1142
gi|154082129|gb|EU002953.1|	Uncultured Ceratobasidium clone 3a	USA	637 bp	1133
·gi|71842199|gb|DQ097889.1|	Ceratobasidium sp. AG-G internal transcribed spacer 1	Japan	1500 bp	1122
gi|146186352|gb|EF536969.1|	Ceratobasidium sp. FPUB	India	617 bp	1118
gi|532733808|gb|KF171076.1|	Ceratorhiza sp. R63	Cuba	657 bp	1112
gi|532733724|gb|KF171071.1|	Ceratorhiza sp. 61	Cuba	663 bp	1112
·gi|62861810|gb|AY927319.1|	Rhizoctonia sp. AG-G isolate R11	Italy	617 bp	1112
gi|303305918|gb|HM625909.1|	Ceratobasidium sp. AG-G isolate RH169	Italy	635 bp	1107
gi|303305917|gb|HM625908.1|	Ceratobasidium sp. AG-G isolate RH168	Italy	636 bp	1105
gi|303305916|gb|HM625907.1|	Ceratobasidium sp. AG-G isolate RH167	Italy	637 bp	1105
·gi|62861820|gb|AY927329.1|	Rhizoctonia sp. AG-G isolate R25	Italy	617 bp	1105
·gi|62861814|gb|AY927323.1|	Rhizoctonia sp. AG-G isolate R16	Italy	617 bp	1105
·gi|62861818|gb|AY927327.1|	Rhizoctonia sp. AG-G isolate R22	Italy	618 bp	1103
·gi|62861811|gb|AY927320.1|	Rhizoctonia sp. AG-G isolate R13	Italy	616 bp	1103

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Table 1. Continued

ID/accession	Organisms	Country	Totally bases	Max score
gi|61612749|gb|AY738627.1|	Rhizoctonia sp. AG-G isolate R1	Italy	616 bp	1103
gi|62861839|gb|AY927348.1|	Rhizoctonia sp. AG-G isolate R56	Italy	618 bp	1101
gi|18181854|emb|AJ242897.1|	Rhizoctonia sp. C-653	Spain	659 bp	1098
gi|300303968|gb|HM597133.1|	Ceratobasidium sp. AG-G	USA	618 bp	1096
gi|224830295|gb|FJ752627.1|	Fungal sp. JIA3-1-1	China	614 bp	1085
gi|560940585|gb|KF688126.1|	Fungal sp. RTB2	India	602 bp	1083
gi|407741913|gb|JX514383.1|	Ceratobasidium sp. Ano_formo2	Taiwan	596 bp	1077
gi|83272293|gb|DQ279043.1|	Ceratobasidium sp. YA18	Netherlands	599 bp	1075
gi|62861815|gb|AY927324.1|	Rhizoctonia sp. AG-G isolate R17	Italy	592 bp	1062
gi|184186845|gb|EU605732.1|	Ceratobasidium sp. RR-2008	India	594 bp	1059
gi|67763786|dbj|AB196646.1|	Ceratobasidium sp. AG-G	Japan	560 bp	1059
gi|67763787|dbj|AB196647.1|	Ceratobasidium sp. AG-G	Japan	560 bp	1005

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