American Journal of Plant Sciences, 2016, 7, 152-158
Published Online January 2016 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2016.71016

Endophytic Fungi Associated with a Holoparasitic Plant, *Balanophora japonica* (Balanophoraceae)

Hideko Ikeda[1], Tatsuya Fukuda[2], Jun Yokoyama[3,4*]

[1]Graduate School of Science and Technology, Yamagata University, Yamagata, Japan
[2]Department of Forestry, Faculty of Agriculture, Kochi University, Nankoku, Japan
[3]Department of Biology, Faculty of Science, Yamagata University, Yamagata, Japan
[4]Institute for Regional Innovation, Yamagata University, Yamagata, Japan
Email: *jyokoyam@sci.kj.yamagata-u.ac.jp

Received 16 December 2015; accepted 25 January 2016; published 28 January 2016

Abstract

Holoparasitism is a special life cycle of flowering plants. All carbon resources are provided by photosynthetic host plants. A recent study revealed the presence of endophytic fungi in holoparasitic plants, but their ecological and evolutionary roles are still unknown. In this study, we examined endophytic fungi isolated from the holoparasitic plant *Balanophora japonica* (Balanophoraceae), collected from Kochi, Shikoku in western Japan. We isolated 23 fungal strains on inflorescences and tubers from three *B. japonica* plants at two locations and on one sample of the host plant (*Symplocos lancifolia*, Symplocaceae). Predominant isolates were *Trichoderma-Hypocrea*, *Penicillium* and *Phialemonium.* The first group was also predominant in the host plant. Fungal composition revealed in this study differed from the composition on *B. harlandii* or other root holoparasites with endophytic fungal (*Rafflesia cantleyi*) data. Those differences might be caused by various factors, including growth habits, location, phylogenetic position or host-parasite relationship.

Keywords

Balanophoraceae, *Balanophora japonica*, Endophytic Fungi, Holoparasitic Plant, *Symplocos lancifolia*, Western Japan

*Corresponding author.

How to cite this paper: Ikeda, H., Fukuda, T. and Yokoyama, J. (2016) Endophytic Fungi Associated with a Holoparasitic Plant, *Balanophora japonica* (Balanophoraceae). *American Journal of Plant Sciences*, **7**, 152-158.
http://dx.doi.org/10.4236/ajps.2016.71016

1. Introduction

Endophytic fungi are microbes that reside in plants, but without any visible symptoms on the host plant [1]-[3]. Ecological features of these fungi are still largely unknown, but some confer functions that positively affect host plants. These functions include stimulation of plant growth [4]-[6], resistance to environmental stress (e.g., drought or edaphic stresses, such as heavy metal ions; [7]-[9]) and defense mechanisms against herbivores or diseases [10] [11]. Thus, at least some of the endophytic fungi play important roles in the evolution of plants and plant-fungus interactions, and the roles could be more important than previously known. We need more insight into the interactions of endophytic fungi and their host plants to fully understand their evolutionary roles.

Holoparasitism is a special nutritional feature of flowering plants. Holoparasitic plants are echlorophyllous and all substrates (including photosynthesites as carbon sources) necessary for growth and reproduction are drawn from the host plants, with a direct plant-to-plant connection [12] [13]. As for photosynthetic flowering plants, holoparasitic plants have endophytic fungi, although reports are limited (*Rafflesia* [14]; *Cuscuta* [15]). With the addition of endophytic fungi, cost-benefit relationships in those tripartite interactions are quite complicated. If the holoparasitic plants have their own symbiotic endophytes, the hosts also provide carbon resources to the fungi and are parasitized by both plants and fungi. Symbiotic endophytes of holoparasitic plants also might support parasitization of host plants. Thus, to uncover the ecological and evolutionary roles, studies focusing on species composition of endophytic fungi in various holoparasitic plants are needed.

Balanophoraceae (Santalales) is a large family of holoparasitic plants, comprising about 17 genera and 40 species distributed mainly in tropical regions worldwide [16]. An updated description of the taxon was presented previously [17]. All members of this family fully depend on carbon resources from woody plants growing around them. Although Balanophoraceae is a major family of holoparasitic plants, there is little information about their endophytic fungi. There is only one report of *B. harlandii* Hook.f. in China [18]. The main objective of that study was to isolate from a medicinally useful plant the fungi effective against bacteria. Not all fungi isolated were examined and no comparisons were made with host plants.

In this study, we isolated and examined endophytic fungi from *Balanophora japonica* Makino (**Figure 1**). *Balanophora* is the largest genus in the Balanophoraceae family (15 spp.), distributed mainly in the Old World tropics [19]. *Balanophora japonica* is the most common of the five species of *Balanophora* in Japan. It is widely distributed in the western part of the Japanese archipelago (including the Ryukyus) and Taiwan [19] [20]. Thus, this species is appropriate for the first step in investigating the composition of endophytic fungi and their ecological and evolutionary roles in Balanophoraceae.

Figure 1. Appearance of *Balanophora japonica*. Left: A plant in Sakawa-machi. Right: A plant in Kami-shi, a host root can be seen under the tubers of the plant.

2. Materials and Methods

2.1. Plant Material

Balanophora japonica only appears above ground in late autumn, when it flowers. *Balanophora japonica* samples for subsequent fungal isolation were collected in November 2012 and 2013 from the understory of *Castanopsis* forests in Sokabegawa, Tosayamada, Kami-shi (N33˚39', E133˚39', 370 m.alt: 2012) and the Nagatani gorge, Sakawa-machi, Takaoka-gun (N33˚29', E133˚13', 370 m.alt: 2013), Kochi Prefecture, Shikoku District, in western Japan. Plants collected from the field were stored in a refrigerator until fungal isolation. For the 2012 samples, we also collected roots from a host plant and stored them in the same manner.

2.2. Fungal Isolation

After carefully removing soil clumps and plant debris by washing with tap water, the plants were sterilized with a mixed solution of sodium hypochlorite (2.5% effective chloride) and Tween X-100 (1%) for 5 min. The plants were then washed three times in sterilized water, divided into inflorescences and tubers and cut into pieces (2-3 mm). The pieces were placed on MMN-agar medium with ampicillin (100 $mg \cdot l^{-1}$) and incubated at 18˚C until fungal growth was observed. Fungal hyphae were transferred to new PDA plates and incubated at 18˚C for subsequent molecular characterization.

2.3. DNA Isolation, PCR Amplification, Sequencing and Molecular Identification

Total DNA was isolated from 200 - 300 mg of fresh hyphae collected from plates using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol. DNA was also isolated from silica-gel-dried roots of host plants. The primers ITS1 and ITS4 (**Table 1**) were used to amplify internal transcribed spacers (ITS) of nuclear rDNA regions [21]. The PCR reaction was performed using an Ex*Taq* DNA polymerase (TaKaRa) and DNA was amplified after incubation at 95˚C for 3 min, with 35 cycles of incubation at 95˚C for 0.5 min, 51˚C for 0.5 min and 72˚C for 1.5 min, with a final extension at 72˚C for 5 min. After amplification, reaction mixtures were subjected to electrophoresis in 1% agarose gels for separation of specific amplified products. Cycle sequencing reactions were performed with approximately 80 to 100 ng of purified PCR product and a BigDye Terminator Cycle Sequencing Kit v.3.1 (Applied Biosystems, USA), according to the manufacturer's instructions. Sequences were determined with an automated DNA sequencer (PRISM310; Applied Biosystems). A BLAST search [22] was conducted to identify the fungal accessions most closely related to the sequences obtained in this study. We used the DNA Data Bank of Japan (DDBJ: http://www.ddbj.nig.ac.jp) for these searches.

3. Results and Discussion

We isolated a total of 23 fungal strains from three individuals (one from Kami and two from Sakawa; **Table 2**). The most frequently isolated genus from *B. japonica* was *Trichoderma-Hypocrea* (8 of 23, 34.8%), but this genus was not isolated at Sakawa. The next frequently isolated genus was *Penicillium* (6 of 23, 26.1%), and all samples used in this study had the fungal genus as endophytes. *Phialemonium* (five strains) was isolated only from Sakawa individuals. Additional genera were *Podospora* (two strains), *Mortierella* and *Bionectia* (one strain each). A total of 13 strains were isolated from inflorescences and the largest number (5 of 6) of *Penicillium* strains and all *Phialemonium* were isolated from inflorescences. On the other hand, 10 residual strains were isolated from tubers, and six of the eight *Trichoderma-Hypocrea* strains and all *Podospora* strains were isolated from tubers.

We also isolated eight strains from a host plant of a Kami sample (identified as *Symplocos lancifolia* Siebold et Zucc.; **Table 1**). Similar to the parasite, *Trichoderma-Hypocrea* was the most common fungus isolated (3 of 8;

Table 1. List of primers used in this study.

Primer name	Sequence (5' to 3')	Reference
ITS1	TCCGTAGGTGAACCTGCGG	[21]
ITS4	TCCTCCGCTTATTGATATGC	[21]

Table 2. List of endophytic fungi isolated from *B. japonica* and its host plant.

Strain ID	Accession of the highest score	Accession No.	Identity	Identification at generic level
T001-F1-1	*Penicillium thomii* FRR 2077	NR_077159	99%	*Penicillium*
T001-F1-2A	*Penicillium thomii* FRR 2077	NR_077159	99%	*Penicillium*
T001-F1-3	*Penicillium* sp. 3 JJK-2011	HM469421	99%	*Penicillium*
T001-F2-1	*Penicillium* sp. 3 JJK-2011	HM469421	99%	*Penicillium*
T001-F2-3	*Penicillium* sp. 11MA10	JX270445	99%	*Penicillium*
T001-R1-1A	*Podospora* sp. XSD-39	EU273519	99%	*Podospora*
T001-R1-1B	*Podospora* sp. XSD-39	EU273519	99%	*Podospora*
T001-R2-1	*Trichoderma spirale* DAOM 183974	NR_077177	99%	*Trichoderma-Hypocrea*
T001-R2-2	*Trichoderma spirale* isolate F28	JF439515	100%	*Trichoderma-Hypocrea*
T001-F2-4	*Hypocrea lixii* isolate FZ1302	HQ259308	100%	*Trichoderma-Hypocrea*
T003-F1-1	Ascomycota sp. UNEX FECRGA 2012E270	KP899441	99%	*Phialemonium*
T003-F2-1	*Bionectria ochroleuca* isolate XSD-89	EU326186	100%	*Bionectria*
T003-F2-2	Ascomycota sp. UNEX FECRGA 2012E270	KP899441	99%	*Phialemonium*
T003-R2-2	*Penicillium* sp. F03	JF439497	100%	*Penicillium*
T004-F1-1	Ascomycota sp. UNEX FECRGA 2012E270	KP899441	99%	*Phialemonium*
T004-F1-2	*Hypocrea lixii* isolate FZ1302	HQ259308	99%	*Trichoderma-Hypocrea*
T004-F2-1	Ascomycota sp. UNEX FECRGA 2012E270	KP899441	99%	*Phialemonium*
T004-F2-2	Ascomycota sp. UNEX FECRGA 2012E270	KP899441	99%	*Phialemonium*
T004-R2-1	Uncultured fungus clone HI38	JX457015	100%	*Trichoderma-Hypocrea*
T004-R2-2	Uncultured fungus clone HI38	JX457015	99%	*Trichoderma-Hypocrea*
T004-R1-1	*Trichoderma tawa* strain IPBCC07_545	KC847191	100%	*Trichoderma-Hypocrea*
T004-R1-2	*Trichoderma* sp. TR094	HQ608118	99%	*Trichoderma-Hypocrea*
T003-R2-1	*Mortierella* sp. L-4	KJ735027	99%	*Mortierella*
HT002-R1-1A	*Penicillium* sp. CL	KM520352	99%	*Penicillium*
HT002-R1-1B	*Umbelopsis ramanniana* isolate TR145	HQ608138	99%	*Umbelopsis*
HT002-R1-2	*Penicillium simplicissimum* voucher CC 19-02	KF359583	99%	*Penicillium*
HT002-R1-3	*Podospora* sp. XSD-39	EU273519	99%	*Podospora*
HT002-R2-1	*Trichoderma spirale* isolate F28	JF439515	100%	*Trichoderma-Hypocrea*
HT002-R2-2	*Hypocrea koningii* isolate F50	JF439478	99%	*Trichoderma-Hypocrea*
HT002-R3-1	*Podospora* sp. XSD-39	EU273519	100%	*Podospora*
HT002-R3-2	*Hypocrea koningii* isolate F50	JF439478	99%	*Trichoderma-Hypocrea*

Note: T001, T003, and T004 are individual IDs of B. japonica. T001 was originated from Kami and the others from Sakawa. HT002 is an ID of a host plant of *B. japonica* (*Symplocos lancifolia* from Kami). The subsequent letters "F" and "R" indicate the parts of plants used for isolation ("F" refers to inflorescences and "R" to tubers for *B. japonica* and roots for a host plant).

37.5%), and *Penicillium* and *Podospora* were the least common (2 of 8; 25%). *Umbelopsis* (1 strain) was the only fungus that was isolated from a host plant, but not from *B. japonica*. All results for molecular identifications will appear in DDBJ/EMBL/GenBank International DNA Data Bank under the accession nos. LC109274-LC109304.

All isolated fungal genera are known as common soil fungi and frequently found in various woody plant species as endophytes [23]-[25]. The similarity in fungal composition on *B. japonica* and its host suggested either a similar process of acquiring endophytic fungi from soil, horizontal transmission from hosts to parasites or both. In the case of *Penicillium*, however, most isolates were obtained from inflorescences. Detailed identification indicated that the species from host roots differed from those isolated from parasites. These facts suggest that most endophytic *Penicillium* strains were acquired after the aboveground parts of *B. japonica*.

This interpretation did not coincide with the *B. harlandii* results from China, as Tu *et al*. [18] did not isolate fungi from flowers or leaves. We could not identify any reason for these differences, but factors, such as age and longevity of aboveground parts or differences among species, might be related. Further examinations, particularly time-series analyses of fungal infections, should be conducted.

Although the major infected parts differed from each other, *Penicillium* was the only endophytic fungus in common with results from the previous study of *B. harlandii* [18]. However, the dominance status differed between the two species. *Penicillium* was one of the major fungi isolated, while only 3 of the 28 identified strains were *Penicillium* in *B. harlandii*. The major fungus found in China was the basidiomycotan anamorphic fungus *Rhizoctonia* [18]. This difference might be caused by differences in how the hosts are used. The host of *B. harlandii* is *Ficus* spp. (Moraceae), while *B. japonica* mainly uses *Symplocos* spp. as the host [20]. The endophytic assemblage of *Ficus* spp. [26] [27] indicated the presence of some fractions of “sterile forms” that might correspond to *Rhizoctonia*. Tu *et al*. [18] characterized the anamorph genus as different from conidiospores. Further studies, especially exhaustive molecular identification of fungi, are needed to elucidate the relationships between fungi and hosts in different parasitic plants.

The only example of a root holoparasite, other than Balanophoraceae, was *Rafflesia cantleyi* [14]. However, the results of *Rafflesia* were completely different from those of Balanophoraceae. Major isolates were *Colletotrichum* spp. that were never isolated from *Balanophora* (Tu *et al*. [18] and this study). *Colletotricum* is also a major endophytic fungus isolated from various plants [28] [29]. This fact might be caused by differences in host taxa (*Tetrastigma* [Vitaceae] is a host of *Rufflesia* spp.), distribution range (tropical vs. temperate evergreen forests) and/or phylogenetic position (Malpighiales vs. Santalales). Additional studies are required to identify the factors that contribute to these differences through an investigation of fungal composition of other holoparasitic species.

In this study, we could not identify roles for endophytic fungi of *B. japonica* in relation to its parasitic lifestyle. High similarity at the generic level indicated that *B. japonica* might obtain beneficial effects similar to those of host plants. In particular, the predominance of *Trichoderma-Hypocrea* might affect root growth stimulation [5] [6] and, thus, is advantageous to both hosts and parasites. *Trichoderma-Hypocrea* was not isolated in this study. This variation among *B. japonica* individuals, in terms of fungal composition, might provide a good opportunity to test the effects of endophytes. We only found a hint of tripartite relationships among host plants, parasitic plants and endophytic fungi. Further study is required to elucidate these relationships and the roles of each organism.

Acknowledgements

We thank Y. Kumekawa, K. Matsuyama, Y. Muramatsu, M. Muroi, K. Ohga, and N. Yokoyama for their help in the course of this study. This study was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, JAPAN (KAKENHI: No. 26291076 for J.Y. and T.F.)

The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: http://www.textcheck.com/certificate/A8VLeD.

References

[1] Carroll, G. (1988) Fungal Endophytes in Stems and Leaves: From Latent Pathogen to Mutualistic Symbiont. *Ecology*, **69**, 2-9. http://dx.doi.org/10.2307/1943154

[2] Schardl, C.L. and Phillips, T.D. (1997) Protective Grass Endophytes. Where Are They from and Where Are They Going? *Plant Disease*, **81**, 430-438. http://dx.doi.org/10.1094/PDIS.1997.81.5.430

[3] Stone, J.K., Bacon, C.W. and White, J.F. (2000) An Overview of Endophytic Microbes: Endophytism Defined. In: Bacon, C.W. and White, J.F., Eds., *Microbial Endophytes*, Marcel Dekker, New York, 3-29.

[4] Verma, A., Verma, S., Sudha, Sahay, N., Bütehorn, B. and Franken, P. (1999) *Piriformospora indica*, a Cultivable Plant-Growth-Promoting Root Endophyte. *Applied and Environmental Microbiology*, **65**, 2741-2744.

[5] Bae, H., Sicher, R.C., Kim, M.S., Kim, S.-H., Strem, M.D., Melnick, R.L. and Bailey, B.A. (2009) The Beneficial Endophyte *Trichoderma hamatum* Isolate DIS 219b Promotes Growth and Delays the Onset of the Drought Response in *Theobroma cacao*. *Journal of Experimental Botany*, **60**, 3279-3295. http://dx.doi.org/10.1093/jxb/erp165

[6] Hermosa, R., Viterbo, A., Chet, I. and Monte, E. (2012) Plant-Beneficial Effects of *Trichoderma* and of Its Genes. *Microbiology*, **158**, 17-25. http://dx.doi.org/10.1099/mic.0.052274-0

[7] Rodriguez, R.J., Redman, R.S. and Henson, J.M. (2004) The Role of Fungal Symbioses in the Adaptation of Plants to High Stress Environments. *Mitigation and Adaptation Strategies for Global Change*, **9**, 261-272. http://dx.doi.org/10.1023/B:MITI.0000029922.31110.97

[8] Rodriguez, R.J. and Redman, R.S. (2008) More than 400 Million Years of Evolution and Some Plants Still Can't Make It on Their Own: Plant Stress Tolerance via Fungal Symbiosis. *Journal of Experimental Botany*, **59**, 1109-1114. http://dx.doi.org/10.1093/jxb/erm342

[9] Worchel, E.R., Giauque, H.E. and Kivlin, S.N. (2013) Fungal Symbionts Alter Plant Drought Response. *Miclobial Ecology*, **65**, 671-678. http://dx.doi.org/10.1007/s00248-012-0151-6

[10] Arnold, A.E., Mejia, L.C., Kyllo, D., Rojas, E.I., Maynard, Z., Robbins, N. and Herre, E.A. (2003) Fungal Endophytes Limit Pathogen Damage in a Tropical Tree. *Proceeding of the National Academy of Sciences of the United States of America*, **100**, 15649-15654. http://dx.doi.org/10.1073/pnas.2533483100

[11] Czarnoleski, M., Pawlik, K., Olejniczak, P., Kozłowski, J. and Lembicz, M. (2012) An Endophytic Fungus Reduces Herbivory in Its Recently Colonised Grass Host: A Food-Choice Experiment on Common Voles, Weeping Alkaligrass and *Epichloë typhina*. *Plant Ecology*, **213**, 1049-1053. http://dx.doi.org/10.1007/s11258-012-0064-y

[12] Press, M.C. and Graves, J.D. (Eds.) (1995) Parasitic Plants. Chapman & Hall, London, 292.

[13] Nickrent, D.L. and Musselman, L.J. (2004) Introduction to Parasitic Flowering Plants. *The Plant Health Instructor*. http://www.apsnet.org/edcenter/intropp/PathogenGroups/Pages/ParasiticPlants.aspx http://dx.doi.org/10.1094/PHI-I-2004-0330-01

[14] Refaei, J., Jones, E.B.G., Sakayaroj, J. and Santhanam, J. (2011) Endophytic Fungi from *Rafflesia cantleyi*: Species Diversity and Antimicrobial Activity. *Mycosphere*, **2**, 429-447.

[15] Suryanarayanan, T.S., Senthilarasu, G. and Muruganandam, V. (2000) Endophytic Fungi form *Cuscuta reflexa* and Its Host Plants. *Fungal Diversity*, **4**, 117-123.

[16] Stevens, P.F. (2001) Angiosperm Phylogeny Website. Version 12, July 2012 [and More or Less Continuously Updated Since]. http://www.mobot.org/MOBOT/research/APweb/

[17] Su, H.J., Hu, J.M., Anderson, F.E., Der, J.P. and Nickrent, D.L. (2015) Phylogenetic Relationships of Santalales with Insights into the Origins of Holoparasitic Balanophoraceae. *Taxon*, **64**, 491-506. http://dx.doi.org/10.12705/643.2

[18] Tu, X., Zhang, Y., Han, Q., Yu, C. and An, R. (2011) Isolation and Screening of Endophytic Antibacteria from *Balanophora harlandii* Hook.f. *Chinese Agricultural Science Bulletin*, **27**, 309-312.

[19] Hansen, B. (1972) The Genus *Balanophora*, a Taxonomic Monograph. *Dansk Botanisk Arkiv*, **28**, 1-188.

[20] Su, H.J., Murata, J. and Hu, J.M. (2012) Morphology and Phylogeny of Two Holoparasitic Plants, *Balanophora japonica* and *Balanophora yakushimensis* (Balanophoraceae), and Their Hosts in Taiwan and Japan. *Journal of Plant Research*, **125**, 317-326.

[21] White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990) Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: Innis, M., Gelfand, D., Sninsky, J. and White, T.J., Eds., *PCR Protocols*: *A Guide to Methods and Application*, Academic Press, San Diego, 315-322. http://dx.doi.org/10.1016/b978-0-12-372180-8.50042-1

[22] Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs. *Nucleic Acids Research*, **25**, 3389-3402. http://dx.doi.org/10.1093/nar/25.17.3389

[23] Hoff, J.A., Klopfenstein, N.B., McDonald, G.I., Tonn, J.R., Kim, M.-S., Zambino, P.J., Hessburg, P.F., Rogers, J.D., Peever, T.L. and Carris, L.M. (2004) Fungal Endophytes in Woody Roots of Douglas-fir (*Pseudotsuga menziesii*) and Ponderosa Pine (*Pinus ponderosa*). *Forest Pathology*, **34**, 255-271. http://dx.doi.org/10.1111/j.1439-0329.2004.00367.x

[24] Summerbell, R.C. (2005) Root Endophyte and Mycorrhizosphere Fungi of Black Spruce, *Picea mariana*, in a Boreal Forest Habitat: Influence of Site Factors on Fungal Distributions. *Studies in Mycology*, **53**, 121-145. http://dx.doi.org/10.3114/sim.53.1.121

[25] Gazis, R. and Chaverri, P. (2010) Diversity of Fungal Endophytes in Leaves and Stems of Wild Rubber Trees (*Hevea brasiliensis*) in Peru. *Fungal Ecology*, **3**, 240-254. http://dx.doi.org/10.1016/j.funeco.2009.12.001

[26] Suryanarayanan, T.S. and Vijaykrishna, D. (2001) Fungal Endophytes of Aerial Roots of *Ficus benghalensis*. *Fungal Diversity*, **8**, 155-161.

[27] Maheswari, S. and Rajagopal, K. (2011) Biodiveristy of Endophytic Fungi Associated with *Ficus religiosa* and *F. benghalensis*. *Mycologia Balcanica*, **8**, 169-172.

[28] Photita, W., Taylor, P.W.J., Ford, R., Hyde, K.D. and Lumyong, S. (2005) Morphological and Molecular Characterization of *Colletotrichum* Species from Herbaceous Plants in Thailand. *Fungal Diversity*, **18**, 117-133.

[29] Gonzaga, L.L., Costa, L.E.O., Santos, T.T., Araújo, E.F. and Queiroz, M.V. (2014) Endophyic Fungi from the Genus *Colletotrichum* Are Abundant in the *Phaseolus vulgaris* and Have High Genetic Diversity. *Journal of Applied Microbiology*, **118**, 485-496. http://dx.doi.org/10.1111/jam.12696