International Journal of Agricultural Technology 2014 Vol. 10(3): 607-616
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Genetic Diversity of *Beauveria* sp. Isolated in Thailand

Poeaim, S.[1*], Hangtrakul, S.[1] and Soytong, K.[2]

[1]Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok 10520, Thailand, [2]Department of Pest Management Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok 10520, Thailand.

Poeaim, S**.**, Hangtrakul, S**.** and Soytong, K**. (**2014**).** Genetic diversity of *beauveria* sp. isolated in Thailand. International Journal of Agricultural Technology 10(3):607-616.

Abstract The entomopathogenic fungus, *Beauveria* sp. was proposed as a biological control agent. In this study, 29 isolates of *Beauveria* sp. were collected from insects and soil in Thailand. Diversity has been investigated by using morphological and molecular techniques. Identification based on morphological characteristics could not identify correctly down to species level. Thus, genetic diversity of *Beauveria* sp. were detected using sequencing, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of internal transcribed spacer region (ITS1-5.8S-ITS2) and small portion of 18S and 28S rDNA (ribosomal DNA) and random amplified polymorphic DNA (RAPD) techniques. The ITS1/ITS4 and PN3/PN16 primers detected a unique fragment of approximately 600 and 900 base pairs, respectively. Sequencing data conformed that all isolates are *Beauveria* and were clustered into two main groups; *Beauveria bassiana* and *Beauveria brongniartii*. Based on the RFLP patterns, the isolates were divided into two distinct groups as observed using *Alu*I and *Hha*I (*Asp*LEI). PCR-RFLP technique successfully distinguished 25 *B. bassiana* isolates from 4 *B. brongniartii* isolates. Random amplified polymorphic DNA (RAPD) technique was used to study genetic relationship among the 14 isolates. Fifty decamer primers were screened and eleven primers were able to amplify reproducible fragments with easily recordable bands. The simple matching coefficient was used to construct a UPGMA dendrogram. Pairwise estimates of genetic similarities ranged from 0.568 to 0.935. However, RAPD technique was not able to distinguish some isolates of *B. bassiana*. For the molecular markers, this diversity was not associated with its efficiency for *Plutella xylostella* control.

Keywords: Genetic diversity, RAPD, RFLP, *Beauveria* sp., Entomopathogenic fungi

Introduction

The entomopathogenic fungus *Beauveria* sp. (Ascomycota: Hypocreales) causes the disease known as the white muscardine disease in insects has been adopted successfully to control a number of important pests. Growth of the fungus inside the insect causes death by attrition and by disruption of physiological processes of the insect. *Beauveria* sp. is classiflied based on the

[*] **Corresponding author:** Poeaim, S.; **E-mail:** poeaim@hotmail.com

shape of their conidia and the placement of conidia on the conidiogenous apparatus (St. Leger *et al*., 1992; Glare and Inwood, 1998). Recently, genetic variability within *Beauveria* spp. has been investigated by several molecular genetic techniques that included restriction fragment length polymorphism; RFLP (Neuveglise *et al*., 1994; 1997; Glare and Inwood, 1998; Coates *et al*., 2002) random amplified polymorphism DNA; RAPD (Bidochka *et al*., 1994; Piatti *et al*., 1998) strain specific sequencing characteristized amplified region; SCAR (Castrillo *et al*., 2003) and amplified fragment length polymorphism; AFLP (de Muro *et al*., 2003).

Molecular markers have been utilized to assess genetic diversity among isolates of *Beauveria* spp. and other entomopathogenic fungi, thereby providing means to identify strains of interest, study on population structure and the potential of these microorganisms for biological control. For selecting efficacious genotypes for biological control programs, it is important to have an understanding of the population genetics represented. To date, there have been few studies about the genetic diversity of *Beauveria* sp. isolated from Thailand. The purpose of this study was to investigate the genetic variability various *Beauveria* sp. that were isolated from insects and soil in Thailand using traditional (morphological and cultural features) and molecular techniques (DNA sequencing, PCR-RFLP and RAPD).

Materials and methods

Source of fungal isolates

There were 29 isolates of *Beauveria* sp. studied, 16 were isolated from insects and 6 were isolated from soil samples from different regions in Thailand, 1 commercial isolate and other isolates but data on origin are not available. All fungal isolates studied are listed in table 1. The fungi from insects were isolated by incubating or streaking sample on the surface of DG18 medium. Soil samples were processed by diluting 10 g in 100 ml of sterile water added with 0.1% Tween-80. Then 100 µl of the soil dilution (10^{-1}, 10^{-2} and 10^{-3}) was spread on the surface of DG18 media. The plates were incubated and when single colony of *Beauveria* sp. developed, it was transferred to potato dextrose agar (PDA) or Sabouraud dextrose agar plus 1% yeast extract (SDAY) for purification.

Morphological characteristics

The identification was done by observation of conidia, conidiophores, colony and mycelia morphology. The morphology of conidiophores and conidia was checked to confirm species, using slide culture technique. Agar blocks (1×1 cm) of media were cut out and placed on glass slides which were laid on the bottom of a sterile Petri dish or other moist chamber, and then the agar block was inoculated with the isolates of *Beauveria* sp. They were grown at 25°c for 5-7 days. These agar blocks provide good surface growth on the coverslip for microscopic observation of sporulation. For colony characteristics, all were propagated and maintained with a 5 mm diameter mycelia plug taken from the growing edge of a 3-5 days old culture grown on PDA plates for 28 days at room temperature.

DNA extraction and amplifications

Mycelia and conidia from each isolate were plated on PDA and single spore colony was grown on potato dextrose broth (PDB), incubated on shaker (150 rpm) at room temperature for 5-7 days. Mycelium was recovered by centrifugation and filtration through Whatman No. 1 filter paper, washed twice with sterilized water, adding liquid nitrogen and ground until a powder mycelium was obtained. Genomic DNA from each isolated were extracted with Dneasy Plant Mini Kit® (QIAGEN) following the manufacture's instructions.

DNA sequencing and RFLP marker

The ITS1-5.8S-ITS2 regions from 29 isolates of rDNA were amplified using ITS1)5'TCGGTAGGTGAACCTGCGG3'(/ ITS4)5'TCCTCCGCTTATTGATATGC3' ((White *et al*., 1990) and PN3 (5'CCGTTGGTGAACCAGCGGAGGGATC3')/ PN16 (5'TCCCTTTCAACAATTTCACG3') (Wada *et al*., 2003). PCR amplification was performed in a total volume of 25 µl containing 200 ng of genomic DNA, 200 µM dNTPs, 0.8 *p*M each primer, 1X PCR buffer, 2.5 mM $MgCl_2$, and 1U *Taq* DNA polymerase (Biolabs, England). The thermocycler program included an initial denaturation at 95°c for 5 mins, followed by 35 cycles of denaturation at 94°c for 1.30 mins, annealing at 55°c for 2 mins, and extension at 72°c for 3 mins, and a final extension at 72°c for 5 mins. PCR products were separated by electrophoresis along with 100 bp of DNA marker. The PCR-amplified ITS fragments were purified and then used directly for sequencing.

Twelve restriction endonucleases (*Acc*II (*Fbl*I), *Alu*I, *Bam*HI, *Bsn*I (*Hae*III), *Hha*I (*Asp*LEI), *Mbo*I (*Bss*MI), *Msp*I (*Hpa*II), *Eco*RI, *Sal*I, *Sfr*274I

(*Xho*I), *Sma*I and *Bgl*I) were screened to detect polymorphic restriction sites of the PCR product amplified from 29 different isolates. Digestion of the PCR products was carried out and incubated according to the manufacturer's instructions. After digestion, the restriction fragments produced were separated by electrophoresis in 1 % agarose gels in 1X TBE buffer along with 100 bp of DNA marker.

RAPD marker

The 14 isolates of *Beauveria* sp. were amplified using RAPD primers (Decamer) as described by William *et al.* (1990). The twenty microliter of mixture was consisted of 200 ng of genomic DNA, 200 µM dNTPs, 1 *p*M each primer, 1X PCR buffer, 2.5 mM $MgCl_2$, and 1U *Taq* DNA polymerase (Biolabs, England). The thermocycler program included an initial denaturation at 94°c for 5 mins, followed by 45 cycles of denaturation at 94°c for 1 mins, annealing at 36°c for 1 mins, and extension at 72°c for 1.30 mins, and a final extension at 72°c for 10 mins. PCR products were seperated by electrophoresis on 2% agarose gel in 1X TBE buffer along with 100 bp of DNA marker. The gel were stained with 10 µg/ml ethidium bromide solution about 10 min, then rinsed the gel with sterile water about 10 min. The gel was examined under UV light transilluminator and photographed using gel documentation system.

Data scoring and analysis of RAPD: Clear and well resolved bands of the samples were compared with each other and DNA fragments were scored as present (1) or absent (0) from each primer. The data were used to estimate genetic similarity among the genotypes based on similarity index. This proximity matrix was cluster analysis derived from the dendrogram using unweighted pair group method with arithmetic mean (UPGMA), and all these computations were carried out using NTSys Version 2.0e.

Fungal virulence, the mature conidia from the culture of each isolate was separately suspended to obtain a density of 1 X 10^6 conidia/mL. Ten microliters of fungal suspension were used by dripping topically on the larval of *Plutella xylostella*. The numbers of dead larval were recorded and calculate the rate of mortality.

Results and discussion

The colony characteristics observed were white to pale-yellow mycelium bearing masses of powdery spores. Morphological features of colonies were studied and could be separated into five groups on the basis of colony color and surface (data not shown). For PDA media, the hyphae and conidia in *Beauveria*

are delicate and hyaline (none pigmented). The conidia morphological under bright field light microscope showed that all of conidia are globose to ellipsoidal in shape and conidiophores were flask-shaped. Conidia sizes varied in width 1.91±0.42 µm (Bb012) to 3.34±0.22 µm (Bb009) and length 3.11±0.39 µm (Bb015) to 4.50±0.37 µm (Bb028) Length/width ratio of the conidia was calculated and generated into two groups: isolates with length/width ratio < 2 is low ratio group and isolates with length/width ratio > 2 is high ratio group (Bb012, Bb013, Bb027 and Bb028) (Table 1). All the fungal isolates in this work were identified as *Beauveria* using morphological characters of the conidiophores.

However, identification based on morphological characteristics could not identify correctly down to species level, thus, genetic diversity of *Beauveria* were detected using internal transcribed spacer region (ITS1-5.8S-ITS2) and small portion of 18S and 28S rDNA (ribosomal DNA) sequencing. ITS1/ITS4 and PN3/PN16 primers were detected a unique fragment of approximately 600 and 900 bp, respectively. The PCR products were sequenced and compared to others in GenBank using BlastN. Sequencing data conformed that all sample isolated are *Beauveria* and were clustered into two main groups; *B. bassiana* and *B. brongniartii*. Moreover, *B. bassiana* were subdivided into 2 groups: Bb011, Bb015, Bb016 and Bb025, whose sequences were different from the other *B. bassiana* isolates (data not shown). In additions, isolates with low and high length/width ratio were placed within a cluster of isolates *B. bassiana* and *B. brongniartii*, respectively. Some photo of colony, conidiophores and conidia of low length/width ratio (Bb001) and high length/width ratio (Bb012) which was cultured on PDA are shown in figures 1A-B and 1C-D, respectively.

PCR products were digested with 12 restriction endonucleases: *Acc*II (*Fbl*I), *Alu*I, *Bam*HI, *Bsn*I (*Hae*III), *Hha*I (*Asp*LEI), *Mbo*I (*Bss*MI), *Msp*I (*Hpa*II), *Eco*RI, *Sal*I, *Sfr*274I (*Xho*I), *Sma*I and *Bgl*I. All of them could be digested with *Alu*I, *Bsn*I (*Hae*III), *Hha*I (*Asp*LEI) and *Msp*I. *Alu*I (Figure 2A) and *Hha*I (*Asp*LEI) (Figure 2B) showed different patterns of *B. bassiana* and *B. brongniartii* (Bb012 and Bb013), but had monomorphic band pattern using *Bsn*I (*Hae*III) and *Msp*I. Based on the RFLP patterns, the isolates were divided into two distinct groups and successfully distinguished 25 *B. bassiana* isolates from 4 *B. brongniartii* isolates. However, the diversity by ITS-RFLP within *Beauveria* is not related to host or geographic location.

Initially, 50 decamer primers were screened, out of which, only 11 primers showed reproducible fragments with easily recordable bands and gave polymorphisms between the different (14) isolates of *Beauveria* sp. (Table 2). These distinguishable banding patterns can be successfully identified, especially in primer OPA16 which could amplify polymorphism bands. The

number of RAPD bands per primer ranged from 4 (OPA08) to 12 (OPA16 and OPA17) with an average of 7.45, size of the amplification products ranged from 200 (OPA18) to 1400 bp (OPA17). Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level.

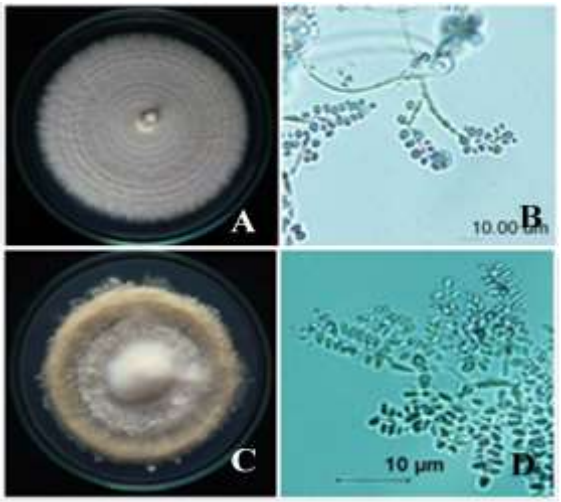

Fig. 1. Some photo of colony, conidiophores and conidia of *Beauveria bassiana* (A-B) and *Beauveria brongniartii* (C-D) were cultured on potato dextrose agar (PDA)

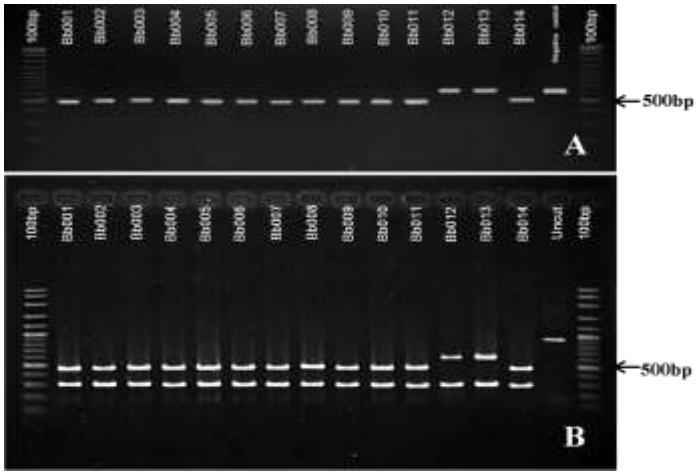

Fig. 2. PCR-RFLP patterns in ITS1-5.8S-ITS2 with ITS1/ITS4 primers using *Alu*I (A) and in ITS1-5.8S-ITS2 and small portion of 18S and 28S rDNA with PN3/PN16 primers using *Hha*I (*Asp*LEI)

The simple matching coefficient among 14 isolates based on the RAPD fragments was used to construct a dendrogram (Figure 3) by UPGMA analysis. The isolates were discriminated into three groups. The first major group consisted of 11 isolates of *B. bassiana* and the second group has two isolates (Bb011 and Bb013). Noteworthy, Bb012 is different as compared to the other strains. Pairwise estimates of genetic similarity among the 14 isolates ranged from 0.568 to 0.935. The highest genetic similarity of 0.935 was seen in Bb004 and Bb006 that are more closely related. The lowest genetic similarity of 0.568 was seen in Bb002 and Bb012 (Table 3). The current study and other studies strongly suggest that *B. bassiana* is an aggregate of species, rather than a single species (St. Leger *et al*., 1992; Rehner and Buckley, 2005).

Table 1. Morphological description of the 29 isolates of *Beauveria* sp. used in this study

Isolates	Host	Geographic Origin	Conidia size (μm)		Length/Width Ratio	Conidia Ratio
			Length	Width		
			Mean	Mean		
Bb001	NA	NA	3.57±0.30	3.08±0.25	1.15±0.06	< 2
Bb002	NA	NA	3.67±0.51	2.91±0.36	1.27±0.18	< 2
Bb003	NA	NA	3.25±0.39	2.64±0.42	1.25±0.15	< 2
Bb004	NA	NA	3.84±0.33	2.99±0.29	1.29±0.14	< 2
Bb005	NA	NA	3.68±0.40	3.08±0.32	1.20±0.17	< 2
Bb006	NA	NA	3.41±0.26	2.66±0.30	1.28±0.11	< 2
Bb007	Coleoptera	Chiangrai	3.43±0.35	2.78±0.34	1.23±0.11	< 2
Bb008	Coleoptera	Chiangrai	3.84±0.35	3.08±0.38	1.35±0.15	< 2
Bb009	Isoptera	Chiangrai	3.93±0.06	3.34±0.22	1.25±0.08	< 2
Bb010	Coleoptera	Chiangrai	3.31±0.32	2.65±0.30	1.25±0.09	< 2
Bb011	Coleoptera	Chiangmai	3.25±0.25	2.81±0.23	1.15±0.06	< 2
Bb012	Coleoptera	Chiangmai	4.21±0.45	1.91±0.42	2.27±0.36	> 2
Bb013	Unidentified	Chiangmai	4.16±0.47	1.94±0.33	2.20±0.48	> 2
Bb014	Coleoptera	Chiangmai	3.70±0.44	2.78±0.32	1.34±0.16	< 2
Bb015	Hymenoptera	Chiangmai	3.11±0.39	2.47±0.29	1.26±0.11	< 2
Bb016	Lepidoptera	Chiangmai	3.21±0.35	2.66±0.29	1.20±0.10	< 2
Bb017	soil	Kanchanaburi	3.82±0.75	3.02±0.39	1.25±0.13	< 2
Bb018	soil	Ratchaburi	3.81±0.38	3.23±0.34	1.18±0.08	< 2
Bb019	soil	Samutsongkhram	3.29±0.30	2.89±0.32	1.14±0.06	< 2
Bb020	soil	Saraburi	3.43±0.20	2.99±0.26	1.15±0.06	< 2
Bb021	soil	Jantaburi	3.41±0.25	2.73±0.26	1.25±0.08	< 2
Bb022	soil	Sakaew	3.48±0.23	2.91±0.25	1.20±0.06	< 2
Bb023	Coleoptera	Nakhonratchasima	3.86±0.66	3.04±0.42	1.25±0.13	< 2
Bb024	Araneae	Jantaburi	3.68±0.56	2.97±0.47	1.24±0.12	< 2
Bb025	Coleoptera	Chiangmai	3.52±0.18	2.84±0.22	1.24±0.10	< 2
Bb026	Commercial	Nakhonsawan	3.42±0.28	2.71±0.23	1.26±0.11	< 2
Bb027	Coleoptera	Chiangmai	4.41±0.33	2.14±0.19	2.06±0.19	> 2
Bb028	Coleoptera	Chiangmai	4.50±0.37	2.22±0.22	2.03±0.17	> 2
Bb029	Coleoptera	Chiangmai	3.39±0.31	2.82±0.31	1.20±0.05	< 2

The virulence of both *B. bassiana* and *B. brongniartii* against the *Plutella xylostella* was also determined. Bioassay results showed considerable variability among some *Beauveria* isolates' aggressiveness to the *P. xylostella* larvae, in which the insect mortality ranged from 0 to 50 %. The non-virulent and low-virulent isolates spanned in three groups (data not shown). Based on the RAPD patterns, the diversity within *Beauveria* is not related to host, geographic location or virulence. No correlation between pathogenicity and grouping according to RAPD pattern was found and relatedness of the original insect host according to Piatti *et al*. (1998). In some cases, genetic diversity has been linked with pathogenicity (Neuvéglise *et al*., 1997). Indeed, to validate the association of these markers, a higher number of *Beauveria* isolates should be screened.

Table 2. Primer codes and sequences of the RAPD primers used and fragment sizes of the generated RAPD markers

Primer	Sequence(5'-3')	Fragment size (bp)
OPA08	GTGACGTAGG	300, 800, 1000, 1200
OPA16	AGCCAGCGAA	250, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300
OPA17	GACCGCTTGT	400, 500, 700, 800, 1000, 1100, 1150, 1200, 1250, 1300, 1350, 1400
OPA18	AGGTGACCGT	200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200
OPA19	CAAACGTCGG	300, 400, 500, 600, 700, 800, 1000
OPA20	GTTGCGATCC	400, 500, 600, 1100, 1200
OPB01	GTTTCGCTCC	400, 500, 600, 700, 900, 1000, 1300
OPB07	GGTGACGCAG	400, 600, 700, 800, 1000, 1100, 1200
OPB08	GTCCACACGG	500, 600, 800, 900, 1100, 1200
OPB10	CTGCTGGGAC	500, 800, 1000, 1050, 1150
OPB17	AGGGAACGAG	400, 700, 800, 1200, 1250, 1300

Conclusion

Twenty nine of *Beauveria* sp. isolated from different hosts, soil samples and geographic origins were observed for their morphology and genetic diversity. Based on molecular technique, sequencing analysis of ITS regions of rDNA, ITS-RFLP and RAPD, it was find out that they can be clustered into two main groups (*Beauveria bassiana* and *Beauveria brongniartii*). In relation to differences in molecular markers, this diversity was not associated with its efficiency on *Plutella xylostella* control. Future studies will be directed on characterizing many species in *Beauveria* spp. isolated from widely scattered locations and from the same or very similar arthropod-host species. The

information on genetic diversity of *Beauveria* sp. will be important in understanding the population structure of *Beauveria* spp. in Thailand.

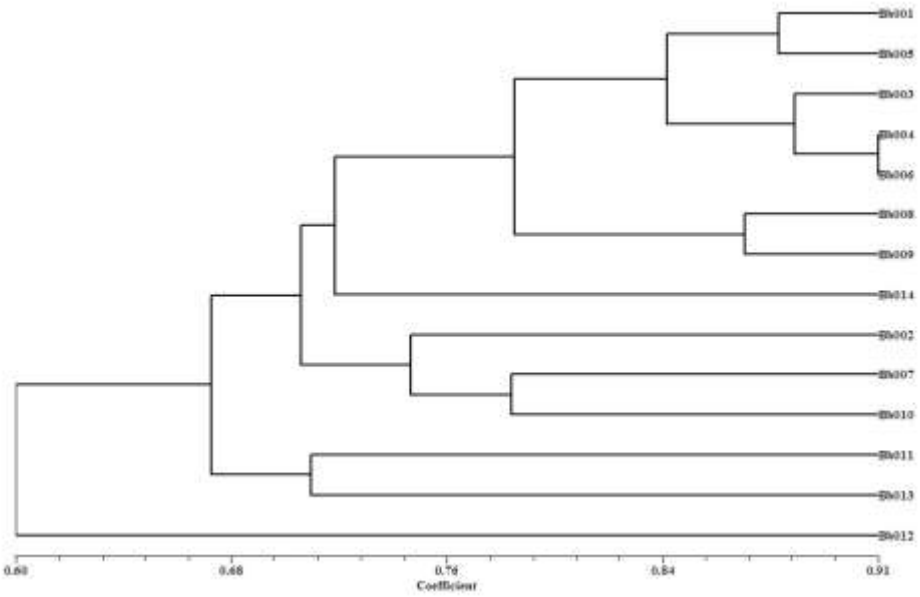

Fig. 3. Dendrogram of 12 isolates of *Beauveria bassiana* (Bb001-011 and Bb014) and 2 isolate of *Beauveria brongniartii* (Bb012 and 013) constructed from RAPD data using UPGMA method based on the simple matching similarity coefficient

Table 3. Matrix of similarity among 14 isolates of *Beauveria* sp. based on RAPD marker

Isolates	Bb001	Bb002	Bb003	Bb004	Bb005	Bb006	Bb007	Bb008	Bb009	Bb010	Bb011	Bb012	Bb013	Bb014
Bb001	1.000													
Bb002	0.791	1.000												
Bb003	0.904	0.791	1.000											
Bb004	0.917	0.747	0.917	1.000										
Bb005	0.898	0.800	0.816	0.854	1.000									
Bb006	0.882	0.762	0.902	0.935	0.833	1.000								
Bb007	0.759	0.757	0.785	0.722	0.721	0.659	1.000							
Bb008	0.829	0.667	0.829	0.897	0.800	0.844	0.727	1.000						
Bb009	0.827	0.721	0.808	0.826	0.878	0.784	0.783	0.901	1.000					
Bb010	0.729	0.692	0.760	0.782	0.659	0.766	0.786	0.787	0.771	1.000				
Bb011	0.745	0.658	0.681	0.687	0.750	0.674	0.659	0.673	0.745	0.744	1.000			
Bb012	0.679	0.568	0.698	0.703	0.640	0.750	0.596	0.726	0.698	0.653	0.688	1.000		
Bb013	0.720	0.585	0.680	0.724	0.745	0.714	0.659	0.785	0.820	0.717	0.733	0.667	1.000	
Bb014	0.762	0.644	0.781	0.782	0.747	0.777	0.667	0.804	0.800	0.701	0.716	0.729	0.752	1.000

Acknowledgements

This work was supported by a grant from King Mongkut's Institute of Technology Ladkrabang for fiscal year 2008-2009. We are also grateful to Mrs. Pimpun Sommartya from National Biological Control Research Center, Biological Control Section (Entomology and Zoology Research Group) of the Department of Agriculture, Ministry of Agriculture and Cooperatives and Dr. Ohnmar Myo Aung for providing the partial isolates of *Beauveria* sp. We thank anonymous reviewers for their helpful comments on the manuscript.

References

Bidochka, M. J., McDonald, M. A., St Leger, R. J. and Roberts, D. W. (1994). Differentiation of species and strains of entomopathogenic fungi by random amplification of polymorphism dna (rapd). Current Genetics 25:107-113.

Castrillo, L. A., Vandenberg, J. D. and Wraight, S. P. (2003). Strain-specific detection of introduced *beauveria bassiana* in agricultural fields by use of sequence-characterized amplified region markers. Journal of Invertebrate Pathology 82:75-83.

Coates, B. S., Hellmich, R. L. and Lewis, L. C. (2002). *Beauveria bassiana* haplotype determination based on nuclear rdna internal transcribed spacer PCR-RFLP. Mycological Research 106:40-50.

de Muro, M. A., Mehta, S. and Moore, D. (2003). The use of ampliled fragment length polymorphism for molecular analysis of *beauveria bassiana* isolates from Kenya and other countries, and their correlation with host and geographical origin. FEMS Microbiology Letters. 229:249-257.

Glare, T. R. and Inwood, A. J. (1998). Morphological and genetic characterisation of *beauveria* spp. from New Zealand. Mycological Research 102:250-256.

Mitina, G. V., Tokareva, Y. S., Movilab, A. A. and Yli-Mattila, T. (2011). Polymorphism of *beauveria bassiana* (deuteromycota: hyphomycetes) strains isolated from *ixodes ricinus* (acari: ixodidae) in Moldova. Ticks and Tick-borne Diseases 2:50-54.

Neuvéglise, C. Y., Brygoo, Y. and Riba, G. (1994). Comparative analysis of molecular and biological characteristics of strains of *beauveria brongniartii* isolated from insects. Mycological Research 98:322-328.

Neuvéglise, C., Brygoo, Y. and Riba, G. (1997). 28s rdna group-i introns: a powerful tool for identifying strains of *beauveria brongniartii*. Molecular Ecology 6:373-381.

Piatti, P., Cravanzola, F., Bridge, P. and Ozino, O. (1998). Molecular characterization of *beauveria brongniartii* isolates obtained from melolontha in valle d'aosta (italy) by rapd-pcr. Letters in Applied Microbiology 26:317-324.

Rehner, S. A. and Buckley, E. P. (2005). A *beauveria* phylogeny inferred from nuclear its and ef1-α sequences: evidence for cryptic diversification and links to *cordyceps* teleomorphs. Mycologia 97:84-98.

St Leger, R. J., Allee, L. L., May, B., Staples, R. C. and Roberts, D. W. (1992). World-wide distribution of genetic variation among isolates of *beauveria* spp. Mycological Research 96:1007-1015.

Wada, S. and Horita, M. 2003. Discrimination of Japanese isolates of *beauveria brongniartii* (deuteromycotina: hyphomycetes) by rflp of the rdna-its regions. Applied Entomology and Zoology 38:551-557.

White, T. J., Bruns, T., Lee, S. and Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. In Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (Eds), PCR protocols: a guide to methods and applications. New York: Academic Press, Inc. pp. 315-322.

William, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. U. (1990). DNA polymorphism amplified by arbitrary primer are useful as genetic markers. Nucleic Acid Research 8:6531-6535.

(Received 4 April 2014; accepted 30 April 2014)