“Shall I refuse my dinner because I do not fully understand the process of digestion?”

— Oliver Heaviside

Comparing the effectiveness of protein coding genes to the ITS gene as DNA barcode markers for species-level identification of *Penicillium* and *Aspergillus* fungal isolates

by

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ABSTRACT

Penicillium and *Aspergillus* are important genera of fungi in the order *Eurotiales* that are species-rich and are found living ubiquitously. The ability to identify these fungal species allows researchers to collate and access valuable information associated with these fungi. In recent years, a molecular technique known as DNA barcoding has become a common method used for fungal identification. The internal transcribed spacer (ITS) gene was described in 2012 as the universal DNA barcode marker for fungal identifications but has limited sequence variability to identify species of *Penicillium* and *Aspergillus*. Protein coding genes; benA and calmodulin may be more effective barcode markers for identifying *Penicillium* and *Aspergillus* species, respectively, due to higher interspecific variations in the gene sequences. In the present study, we compare whether it is more effective to identify *Penicillium* and *Aspergillus* fungal isolates to the species level using protein coding genes or the ITS gene as barcode markers.

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I would like to express my sincere gratitude to both my supervisors, Christopher Gray and John Johnson, for being extremely understanding and accommodating from the beginning to the end of this project. I thank all the members of the NPRG for being so supportive, kind and helpful to me at all times. Last but certainly not least, to my friends and Canadian families, thank you for consistently being my pillars of strength and helping me manoeuvre around the obstacles thrown my way.

STATEMENT OF RESEARCH CONTRIBUTIONS

The fungal isolates used in this study were previously collected, isolated and cryopreserved by members of the Natural Products Research Group (NPRG) at University of New Brunswick (UNB). DNA from these fungi were also previously extracted, amplified using the ITS gene and sent to Genome Québec for sequencing by members of the NPRG. I subcultured the fungal isolates in this study, extracted and amplified DNA from the hyphae of these fungal isolates, sent the amplicons to Genome Québec for sequencing and obtained the gene sequences upon completion of sequencing. I then uploaded the genes sequences into the Basic Local Alignment Search Tool (BLAST) in GenBank, obtained and analysed the search outputs from BLAST and determined the identity of the fungal isolates.

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List of Nomenclature, Symbols or Abbreviations

BLAST: Basic local alignment search tool

bp: Base pairs

DNA: Deoxyribonucleic acid

ITS: Internal transcribed spacer

MEA: Malt extract agar

NPRG: Natural Products Research Group

PCR: Polymerase chain reaction

PDA: Potato dextrose agar

RNA: Ribonucleic acid

UNB: University of New Brunswick

Introduction

Penicillium and *Aspergillus* are important fungal genera in the order *Eurotiales* that are species-rich[1–5] and are found living ubiquitously.[1,3–18] Taxonomic identification of *Penicillium and Aspergillus* is important as it allows researchers to discover and collate existing and important information that are associated with these fungi.[2,19]

For decades, taxonomic identifications of fungi were performed based on the morphological characteristics of the fungal species but in recent years, molecular techniques based on polymerase chain reaction (PCR) amplification and sequencing of genomic deoxyribonucleic acid (DNA) of fungi, are commonly used.[2,20–22] DNA barcoding is a sequence-based identification that targets a specific region of the ribosomal ribonucleic acid (RNA) that is used to identify fungi to the species level.[2,19,22,23] This method uses short DNA gene sequences (typically between 400 to 800 bp)[20,23,24] that vary between species[23,24] and compares them to existing gene sequences for species identification. In 2012, the Internal Transcribed Spacer (ITS) gene (Figure 1) of the ribosomal RNA was declared the universal DNA barcode marker for identifying fungi.[20] The ITS gene was chosen as the universal barcode marker due to its ease of amplification with PCR,[6,20,25] high interspecific variations in gene sequences,[2,20,25] and having the highest sequencing success rate (73%) across a wide range of fungi.[20] As a result, the fungal ITS gene is frequently used as the barcode marker to identify fungal species. To identify fungal species, the ITS gene is amplified, sequenced and compared against existing sequences in sequence databases such as the widely used public repository, GenBank.[24,25] GenBank employs a Basic Local Alignment Search Tool

(BLAST) which compares the unknown sequence to the sequences in the database and the fungal species is determined by the researcher's interpretation of the BLAST search results. This interpretation is based on the level of similarity between the unidentified sequence and the ITS gene sequences that exist in the database at the time of search using a threshold similarity match value.[2,20,24,24,25]

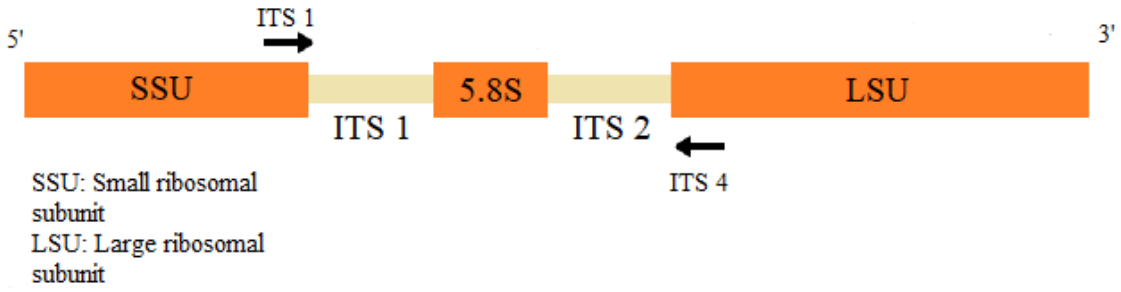

Figure 1. The internal transcribed spacer (ITS) gene structure of a fungi with the forward primer ITS1 and reverse primer ITS4 adapted from Sibero et al..[26]

Even though the ITS gene is widely used as the barcode marker for the identification of fungi, only a small percentage of the possible 1.5 to 5.1 million species of fungi have been sequenced using this gene.[25,27–30] Over 170,000[2,20] ITS gene sequences have been deposited into GenBank but many of these sequences have insufficient or incorrectly annotated taxonomic identifications which may lead to incorrect identifications of fungal species.[2,4,25] As there is not a standardised threshold similarity value for interpreting BLAST search results, researchers today are still using arbitrary threshold values to interpret BLAST search results in order to identify fungal species.[2,20,31–34] In most fungal studies, the default threshold value is at 97% but the choice of threshold remains subjective to the researcher's discretion.[2,30,35–38] Moreover, the ITS gene has limitations[3–5,39] when it comes to identifying species-rich genera like

those of *Penicillium* and *Aspergillus*.[2–5] It has been documented that different species within these genera of fungi share ITS sequences and therefore lack variations in their gene sequences.[3,5,20] Because DNA barcoding relies on the interspecific variation in gene sequences in order to discriminate between species, it is challenging to identify *Penicillium* and *Aspergillus* using the ITS gene if there is little to no interspecific variation in their gene sequences.[2,6,8,20,40,41] Consequently, other barcode markers are required to supplement the identification of these highly speciose genera of fungi.[2,20,23]

Protein coding genes (Figures 2 and 3) may be more effective DNA barcode markers than the ITS gene at determining taxonomic relationships in the phylum *Ascomycota* which includes the genera *Penicillium* and *Aspergillus*.[20,23,42,43] This is possibly because protein coding genes occasionally mutate quicker than the ITS gene subsequently developing higher interspecific variation in the gene sequences between fungal species.[2,44] BenA and calmodulin are protein coding genes that have been found to be effective at species-level identification of *Penicillium* and *Aspergillus*, respectively.[2,3,5,40,45,46] Of the two protein coding genes, benA is more useful as a DNA barcode marker for fungal identification of *Penicillium* species whilst calmodulin works well for identifying species of *Aspergillus*.[2,3,5,47,48]

Figure 2. The benA gene structure of a fungi showing forward primer bt2a and reverse primer bt2b as adapted from Glass and Donaldson.[49]

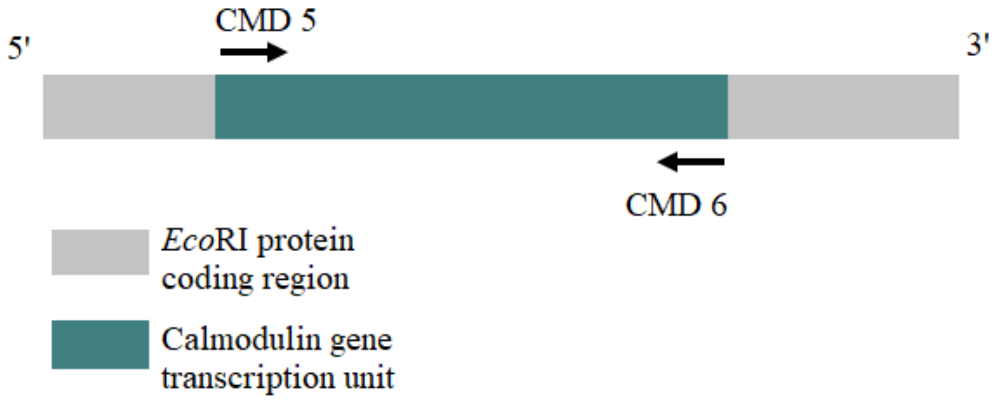

Figure 3. The calmodulin gene structure of a fungi showing forward primer CMD5 and reverse primer CMD 6 as adapted from Rasmussen et al..[50]

The objective of this research was to compare the effectiveness of protein coding genes to the ITS gene as DNA barcode markers for species-level identification of *Penicillium* and *Aspergillus* fungal isolates.

Materials and Methods

Extraction and amplification of DNA from *Penicillium* and *Aspergillus* fungal isolates

Penicillium (n=24) and *Aspergillus* (n=6) fungal isolates that were previously isolated by members of the Natural Products Research Group (NPRG) that were identified only to the genus level or were not successfully sequenced (no sequences obtained) were selected to be used for this study. Hyphae from a few of the selected fungal isolates were subcultured onto two types of agar; fresh malt extract agar (MEA) and fresh potato dextrose agar (PDA) to observe which type of agar promoted more flocculent hyphae growth for collection. PDA was determined to be the more suitable agar and hyphae from each of the 30 fungal isolates were subcultured onto PDA. The endophytes were kept in ambient light and room temperature (20–22°C) and were allowed to grow until there were sufficient growth of hyphal material from each isolate to be collected for DNA extraction.

A small amount of fungal hyphal material from each isolate was placed into individual 1.5 mL Eppendorf tubes. DNA extraction was carried out using a QIAGEN® DNEasy Plant Mini Kit following the manufacturer's protocol. A maximum of three attempts of DNA extractions were carried out for each fungal isolate. Upon completion of DNA extraction, the benA gene of each *Penicillium* fungal isolate was amplified (PCR) with primer pairs bt2a and bt2b whereas the calmodulin gene of each *Aspergillus* fungal isolate was amplified (PCR) with primer pairs CMD5 and CMD6 (Table 1).

Table 1. Protein coding gene primers and primer sequences used for PCR amplification and sequencing of *Penicillium* and *Aspergillus* fungal isolates.

Genus	Gene	Primer	Primer Sequence (5'-3')	Direction
Penicillium	benA	bt2a	GGT AAC CAA ATC GGT GCT GCT TTC	Forward
		bt2b	ACC CTC AGT GTA GTG ACC CTT GGC	Reverse
Aspergillus	calmodulin	CMD5	CCG AGT ACA AGG AGG CCT TC	Forward
		CMD6	CCG ATA GAG GTC ATA ACG TGG	Reverse

The thermal cycling parameters (35 repeated cycles of PCR) used to amplify the benA gene were the following: initial activation at 95°C for five minutes (only the first cycle), DNA denaturation at 95°C for one minute, annealing of primers at 55°C for 30 seconds and extension of new DNA strands at 72°C for one minute and a final extension of DNA strands at 72°C for 10 minutes (after the final cycle).[3,41] The thermal cycling parameters (42 repeated cycles of PCR) used to amplify the calmodulin gene were the following: initial activation at 94°C for one minute (only for the first cycle), DNA denaturation at 94°C for one minute, annealing of primers at 55°C for 30 seconds, extension of new DNA strands at 72°C for 90 seconds and a final extension of DNA strands at 72°C for 10 minutes (after the final cycle).[3,41]

Gel electrophoresis and DNA sequencing of PCR amplicons

Gel electrophoresis was performed on the PCR products (amplicons) to determine whether PCR amplification was successful. The gel used for gel electrophoresis in this study was an agarose gel. The first well of each lane was loaded with 5 µL of 1000 bp Invitrogen DNA ladder and 15 µL of each PCR product was loaded into subsequent wells. The gel underwent electrophoresis at 120V for 25 minutes. After electrophoresis, the agarose gel was then imaged using the Gel Doc XR+ system and Image Lab software by BIO-RAD. The image of the agarose gel was analysed using Image Lab to verify

successful PCR amplifications by the distinct presence of bands on the agarose gel image. Amplicons that produced a distinct band on the agarose gel were prepared for sequencing according to the protocol set by Genome Québec and were sent to Genome Québec for sequencing.

Species identification of *Penicillium* and *Aspergillus* fungal isolates

FASTA formatted sequences were downloaded from Genome Québec after sequencing was completed. The obtained sequences were collated and uploaded into the Basic Local Alignment Search Tool (BLAST) in GenBank, a public sequence database on the National Centre for Biotechnology Information (NCBI) website which compares the uploaded sequences to the existing sequences in GenBank at the time of search. Additionally, previously obtained ITS gene sequences of *Penicillium* and *Aspergillus* fungal isolates that were sequenced by members of the NPRG and identified only to the genus level, were uploaded into BLAST again to compare these sequences to the current sequences that exist in GenBank.

After obtaining the search outputs from BLAST, the identity of the fungal isolates in this study were determined based on the percentage similarity match value between the uploaded sequence and the existing sequences in GenBank. As there is no standard threshold similarity match value, a commonly used threshold value of ≥97% was used to interpret BLAST search results.[2,30,35–38] The fungal isolates were identified to the species or genus level based on the percentage similarity match value using the threshold value and the consistency of sequence matches with the same fungal species or genus.

Results

DNA amplification was successful if there was a presence of a distinct white band on the agarose gel (Figure 4). Empty lanes with no distinct bands indicate that no PCR amplicons were present and that PCR amplification was not successful.

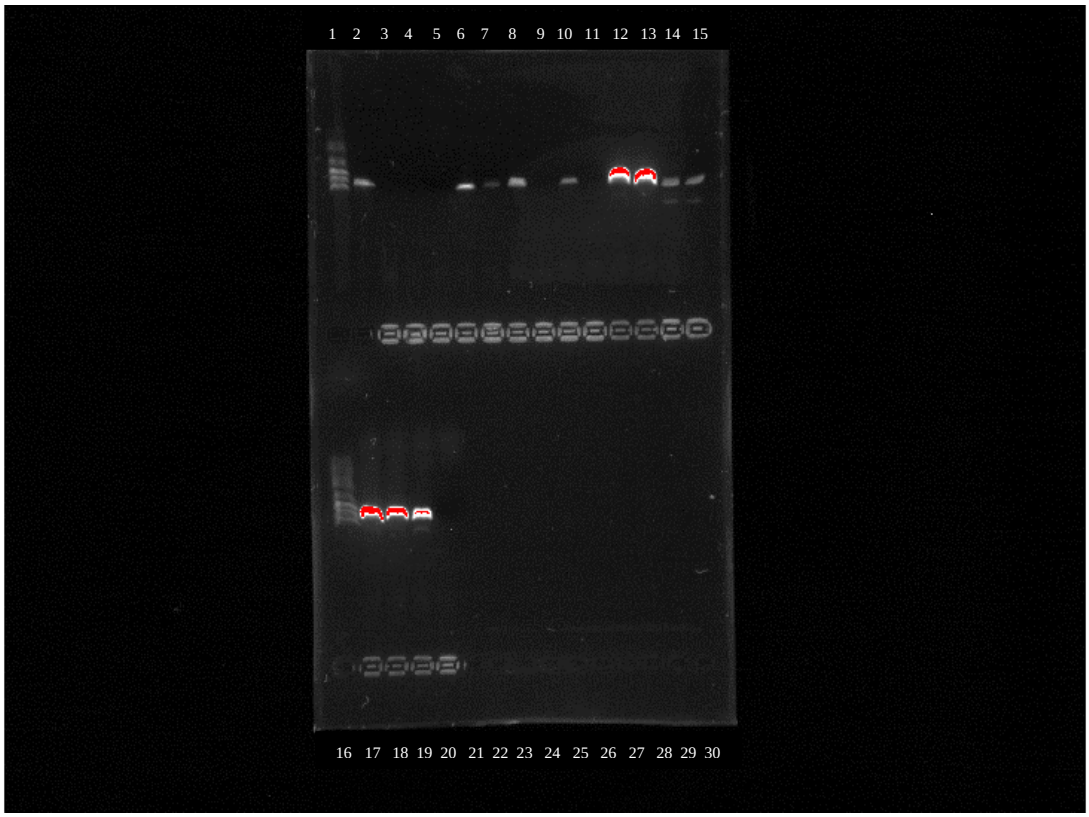

Figure 4. An example of an agarose gel after completion of gel electrophoresis showing distinct white or red (automatic software detection of abundant amplicons) bands indicating the presence of *Penicillium* (lanes 2, 6 to 8, 10 and 12 to 15) and *Aspergillus* (lanes 17 to 19) PCR amplicons. Empty lanes (3, 5, 9, 11, 13, 14, 16, 18 and 20) indicate that no PCR amplicons were present and no amplicons were loaded into wells 21 to 30. The 1000 bp DNA ladder in lanes 1 and 16 indicate the base pair length of the PCR amplicons with the expected length between between 400 to 600 bp.

Successfully amplified DNA were sequenced and the sequences were retrieved from Genome Québec in a FASTA format. These sequences were uploaded into BLAST that compares the uploaded sequence to the existing sequences in GenBank and the search outputs were analysed and interpreted. The identity of the fungal species was determined based on the percentage similarity match value using a threshold value of ≥97%. The results from the analyses of BLAST search outputs comparing the species identification results from DNA barcoding using the benA gene to the ITS gene for *Penicillium* fungal isolates and using the calmodulin gene to the ITS gene for *Aspergillus* fungal isolates are presented in Tables 2 and 3. As there may be multiple existing sequences that match the unknown sequence uploaded into BLAST with the same taxonomic identity, only the maximum sequence similarity percentage is represented in Tables 2 and 3.

Of the 24 *Penicillium* fungal isolates that were sequenced with the ITS gene, five isolates were identified to the species-level, 14 isolates were identified to the genus-level and five fungal isolates were not successfully sequenced with the ITS gene (Table 2). The five fungal isolates that were identified to the species level were *P. thornii* (two isolates), *P. expansum* (two isolates), *P. minioluteum* (one isolate) and *A. fumigatus* (one isolate). The 14 fungal isolates that were identified to the genus level belonged to the genus *Penicillium.* On the other hand, six out of six fungal isolates belonging to the genus *Aspergillus* were identified to the species level as *A. fumigatus* when sequenced with the ITS gene (Table 3).

Of the 24 *Penicillium* fungal isolates that were sequenced with the benA gene, 12 isolates were identified to the species-level, eight isolates were identified to the genus-level and four fungal isolates were not successfully sequenced with the benA gene (Table 2). The 12 fungal isolates that were identified to the species level were *P. bissettii* (six isolates),

Table 2. Comparison of the identification of *Penicillium* fungal isolates using the universal fungi barcode marker, ITS and protein coding gene, benA.

		ITS[B]		BenA[C]	
Isolate Code	Morphology[A]	Fungal Taxa	Maximum Sequence Similarity (%)	Fungal Taxa	Maximum Sequence Similarity (%)
TC2-020	*Penicillium* sp.	*P. thornii*	100.00	n/d[D]	n/d[D]
KP2-033H	*Penicillium* sp.	*Penicillium* spp.	100.00	*P. bissettii*	100.00
KP2-033B	*Penicillium* sp.	*P. expansum*	100.00	*P. expansum*	100.00
KP2-033A	*Penicillium* sp.	n/d[D]	n/d[D]	*P. minioluteum*[E]	100.00
KP2-025C	*Penicillium* sp.	n/d[D]	n/d[D]	*Penicillium* spp.	98.60
KP2-025B	*Penicillium* sp.	n/d[D]	n/d[D]	*Penicillium* spp.	94.12
KP2-001F	*Penicillium* sp.	*Penicillium* spp.	100.00	*P. minioluteum*[E]	100.00
KP1-175M	*Penicillium* sp.	*Penicillium* spp.	100.00	*Penicillium* spp.	98.38
KP1-175L	*Penicillium* sp.	*Penicillium* spp.	100.00	n/d[D]	n/d[D]
KP1-175G	*Penicillium* sp.	*P. minioluteum*[E]	100.00	*P. melinii*	100.00
KP1-135F	*Penicillium* sp.	*Penicillium* spp.	100.00	n/d[D]	n/d[D]
KP1-131Y	*Penicillium* sp.	*A. fumigatus*	100.00	*Penicillium* spp.	98.57
KP1-131L	*Penicillium* sp.	*P. thornii*	99.80	*Penicillium* spp.	96.16
KP1-131CC	*Penicillium* sp.	n/d[D]	n/d[D]	*Penicillium* spp.	94.19
KP1-123B	*Penicillium* sp.	*Penicillium* spp.	100.00	*P. bissettii*	100.00
KP1-123A	*Penicillium* sp.	*Penicillium* spp.	100.00	*P. bissettii*	100.00
KP1-091A	*Penicillium* sp.	*Penicillium* spp.	100.00	*P. bissettii*	100.00
KP1-075B	*Penicillium* sp.	*Penicillium* spp.	100.00	*P. bissettii*	100.00
KP1-063J	*Penicillium* sp.	*Penicillium* spp.	100.00	n/d[D]	n/d[D]
KP1-045A	*Penicillium* sp.	*Penicillium* spp.	100.00	*Penicillium* spp.	98.61
KP1-025B	*Penicillium* sp.	n/d[D]	n/d[D]	*Penicillium* spp.	98.61
KP1-017E	*Penicillium* sp.	*Penicillium* spp.	100.00	*P. bissettii*	100.00
KP1-013B	*Penicillium* sp.	*Penicillium* spp.	100.00	*P. melinii*	100.00
CT1-006B	*Penicillium* sp.	*Penicillium* spp.	99.20	*P. spinulosum*[F]	100.00

[A]Morphology indicates species identification based on the physical structures of fungi. [B]ITS and [C]BenA indicates species identification using DNA barcoding. [D]No sequences were obtained. [E]Synonymous to *Talaromyces minioluteus.* [F]Synonymous to *P. trzebinskii* and *P. roseomaculatum*.

Table 3. Comparison of the identification of *Aspergillus* fungal isolates using the universal fungi barcode marker, ITS and protein coding gene, calmodulin.

		ITS[B]		Calmodulin[C]	
Isolate Code	Morphology[A]	Fungal Taxa	Maximum Sequence Similarity (%)	Fungal Taxa	Maximum Sequence Similarity (%)
KP2-025D	*Aspergillus* sp.	*A. fumigatus*	100.00	*T. amastolkiae*	98.51
KP2-001C	*Aspergillus* sp.	*A. fumigatus*	100.00	*A. fumigatus*	100.00
KP1-131T	*Aspergillus* sp.	*A. fumigatus*	100.00	*A. fumigatus*	100.00
KP1-131Q	*Aspergillus* sp.	*A. fumigatus*	100.00	*A. fumigatus*	100.00
KP1-131AA	*Aspergillus* sp.	*A. fumigatus*	100.00	*A. fumigatus*	100.00
KP1-063N	*Aspergillus* sp.	*A. fumigatus*	100.00	*A. fumigatus*	100.00

[A]Morphology indicates species identification based on the physical structures of fungi. [B]ITS and [C]Calmodulin indicates species identification using DNA barcoding.

P. expansum (one isolate), *P. minioluteum* (two isolates), *P. melinii* (two isolates) and *P. spinulosum* (one isolate). The eight fungal isolates that were identified to the genus level belonged to the genus *Penicillium*.

Sequencing of the calmodulin gene of six *Aspergillus* fungal isolates led to the species identification of all six of these fungal isolates (Table 3). The six fungal isolates that were identified to the species level were *A. fumigatus* (five isolates) and for the case of one isolate, it was identified as *Talaromyces amastolkiae* (one isolate) but was morphologically identified as belonging to the genus *Aspergillus*.

Discussion

Penicillium and *Aspergillus* are amongst the most species-rich and abundant genera of fungi distributed worldwide with over 400[5,47] and 800[51,52] species described, respectively.[1,3–18,53] Identifying *Penicillium* and *Aspergillus* to the species level is important for researches to access valuable information[52,54–56] associated with these genera of fungi as *Penicillium* and *Aspergillus* contribute to economical, biotechnological and medical aspects of human life.[2,4,11,12,57–66]

Species identification of fungi was originally based on morphological characteristics[2,5,39,46,47,63,67] but this method of identification is difficult even for expert taxonomists[47,68] when it comes to species of *Penicillium* and *Aspergillus*.[68–70] As shown in this study, morphological examinations of *Penicillium* and *Aspergillus* fungal isolates only identified them to the genus level. Due to the limited ability to identify species using morphology, DNA barcoding is increasingly used for the identification of these fungal endophytes to the species level.[68,69,71–73] DNA barcoding distinguishes species based on the diversity in the short DNA sequences (typically 400-800 bp)[20,23,24,74] of target DNA genes (i.e. ITS, benA or calmodulin) in fungi species.[68]

Previously, members of the Natural Products Research Group (NPRG) worked to identify fungal endophytes using both morphological examinations and DNA barcoding of the ITS gene but in the case of the 30 fungal isolates studied in this research, they could not identify these endophytes beyond the genus level. However, when the ITS gene sequences obtained from this previous analysis were uploaded into BLAST and searched against the current existing sequences in GenBank in order to have the most recent

analyses and species identification, 11 out of 30 of the fungal isolates that were previously identified to the genus level, were successfully identified to the species level. One reason that could explain this difference is perhaps during the previous analysis of the BLAST search outputs for species identification, the researchers were reserved in interpreting the search outputs and identifying the species of the fungal isolates. Another possible reason to explain this difference could be due a more current GenBank database at the time these sequences were searched using BLAST in this study. New sequences are often uploaded into the GenBank database[31] thus when the previously obtained ITS sequences were searched in this study, the ITS sequences of 11 fungal isolates had matching sequences above the threshold value (97%) in GenBank and were identified to the species level. The ability to identify the species of fungal isolates that were previously unidentified using the same gene sequences indicates that species identification based on the analysis of BLAST results depends on the existing sequences in the GenBank database at the time of search thus the species identity of fungal isolates identified using DNA barcoding may need to be updated over time.

Despite the universal effectiveness of the ITS gene in discriminating species of fungi, this barcode marker may be less effective than protein coding genes when it comes to discriminating genera of fungi that are species-rich as these fungi have been documented to share ITS gene sequences.[2,6,8,20,40,41] Protein coding genes (benA and calmodulin) were used in this study as they can be better DNA barcode markers than the ITS gene at identifying *Penicillium* and *Aspergillus* species.[20,23,42] Previously, 24 *Penicillium* fungal isolates were only identified to the genus level with the ITS gene but after the ITS gene sequences of these fungal isolates were searched again using BLAST

in this study, five *Penicillium* fungal isolates were able to be identified to the species level. When the same 24 *Penicillium* fungal isolates were sequenced with the benA gene, 12 *Penicillium* fungal isolates were identified to the species level. Although gene sequences are often added into the GenBank database, these sequences were compared to similar existing sequences in the GenBank database during the time of search and from the analyses of the results, showed that using benA as a barcode marker allowed the identification of more *Penicillium* fungal isolates in this study than the ITS gene. However, some of the species of fungal isolates that could be identified using the ITS gene could not be identified to the species level or did not obtain sequences when using the benA gene and vice versa.

Of the 12 *Penicillium* fungal isolates that were identified to the species-level using benA as a barcode marker, half of these 12 fungal isolates were identified as *P. bissettii*. In comparison, when sequenced using the ITS gene, the fungal isolates that were identified as *P. bissettii* when sequenced with the benA gene, were only identified to the genus level with the ITS gene.

An anomaly that occurred in this research, was the different identifications of a *Penicillium* fungal isolate, KP1-175G when sequenced with the ITS and benA genes (Table 2). When sequenced with the ITS gene, this fungal isolate was identified as *P. minioluteum* but when sequenced with the benA gene, the isolate was identified as *P. melinii* (Table 2). These two species are distinct species of fungi and the names *P. minioluteum* and *P. melinii* are not synonymous. The different identifications for this fungal isolate could be due to incorrect annotations or identifications of existing GenBank sequences as the sequences uploaded onto GenBank are often only annotated

based on computational results (i.e. BLAST) and are not verified or characterised.[31] Other alternate databases such as UNITE[74] have been curated to address the issue of incorrect annotations of sequences in GenBank but these databases usually target the gene sequences of the universal barcode marker, ITS and not for protein coding genes thus in this study, GenBank was used as it contains both ITS and protein coding gene sequences. The issue of incorrect annotations of sequences is exacerbated with the frequent addition of new sequences to public genetic databases such as GenBank.[31] Inaccurate taxonomic identifications of DNA sequences can happen for many reasons including accidental amplification of contaminant DNA,[75] incorrect identification of organisms based on computational results (BLAST)[31,75] and the use of arbitrary threshold similarity match values[31] when analysing BLAST search results for species annotations.

In the case of the six *Aspergillus* fungal isolates that were identified to the species-level in this research, the ITS gene and calmodulin gene have similar effectiveness as barcode markers in identifying these fungal isolates. Both these barcode markers allowed the identification of all six *Aspergillus* fungal isolates. When sequenced using the ITS gene, all six of the fungal isolates were identified as *A. fumigatus* but when sequenced with the calmodulin gene, five of the fungal isolates were identified as *A. fumigatus* but one of the fungal isolate, KP2-025D was identified as *Talaromyces amastolkiae*.

Unlike the other *Aspergillus* fungal isolates in this research, the fungal isolate KP2-025D, was identified to the genus level as *Aspergillus* using morphological examinations and further specified using DNA barcoding of the ITS gene as *A. fumigatus* (Table 3). When sequenced using the protein coding gene, calmodulin, the same fungal

isolate was identified as *Talaromyces amastolkiae* (Table 3). Both of these fungi species, *T. amastolkiae* and *A. fumigatus* belong to the same order of fungi, *Eurotiales* but have different morphological, physiological and phylogenetic characteristics.[2–5,8,46,76] *Talaromyces amastolkiae* was described in 2012[77] and is a sexual morph of *Penicillium* spp.[78,79] but have not been described as an intimate relative of *A. fumigatus*. This indicates that there may be limitations to using protein coding genes as a barcode marker than the ITS gene as a barcode marker. There are more existing ITS gene sequences than protein coding genes sequences in the GenBank database which is the database used in this research thus limits the number of comparisons that can be made between the unknown sequence and the existing sequences for the identification of fungal species.[20,31] Furthermore, there is no curated database for protein coding gene sequences to address the issue of incorrectly annotated or identified sequences and the identification of species using DNA barcoding is dependent on the existing sequences in GenBank.[80]

Conclusion

This research shows that the benA gene is a more effective DNA barcode marker than the ITS gene when it comes to species-level identification of *Penicillium*.[81,82] As can be seen in Table 2, only five of 24 *Penicillium* fungal isolates could be identified to the species level when the ITS gene of each fungal isolate was sequenced whereas when the benA gene was sequenced, 12 of the same 24 *Penicillium* fungal isolates were identified to the species-level. In contrast, when the ITS gene and protein coding gene, calmodulin of *Aspergillus* fungal isolates in this research were sequenced, sequencing of both genes allowed the identification of all six *Aspergillus* fungal isolates to the species level but one of the fungal isolates identified using the calmodulin gene was a species from a different genus, *Talaromyces*.

Even though the ITS gene was declared the universal DNA barcode marker for fungal identification,[20] there are limitations to the ability of this barcode marker to identify fungal species.[6] Sequencing of the ITS led to the identification of less *Penicillium* fungal isolates than the protein coding gene, benA, as this research has shown. Although protein coding genes may be more effective, there are curated databases for the ITS gene that addresses incorrectly annotated or identified sequences as well as more ITS gene sequences in GenBank than protein coding gene sequences thus species identifications may be limited when using protein coding genes as barcode markers.

As effective as DNA barcode markers are when it comes to the identification of fungal species, there are still major problems with this molecular technique with the biggest being incorrect identifications of sequences in genetic databases.[31,80,83] Accurate identification of *Penicillium* and *Aspergillus* species is important as it allows researchers

to access valuable information about these fungi thus caution should be taken when attempting to identify species within these genera to avoid false identifications.[84]

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Appendix

Appendix 1. Gene sequences obtained from sequencing of the benA gene with primer pairs bt2a and bt2b of *Penicillium* fungal isolates.

KP2-033H

521bp

TAACCAAATCGGTGCTGCTTTCTGGTACGTGCCACGCCCACCAAAAAACTGT

CCAACACAACAAAGCAGCCCCTCAATTGCGAGTCTGAATGGACAAGATGTAC

TAACTCGAACTGCAGGCAGACCATTGCTGGTGAGCACGGCCTTGACGGCGAT

GGCCAGTAAGTTTCTTCGATACCCTTCGACAAAACCACTCGAACGCGGAATG

GCGGTCTGATATTTCCGGCTAGGTACAATGGTTCCTCCGACCTCCAGCTCGAG

CGCTTGAACGTCTACTTCACCCATGTAAGTTGCGTGTCCAGTCAATGCCGAAA

TACTGAACTCGACTCTAATCGATGGAACTGTTGTTTCTTAGGCCAGCGGTGAC

AAGTACGTTCCCCGTGCCGTTCTGGTCGATCTGGAGCCCGGTACCATGGACG

CTGTCCGTGCCGGTCCCTTTGGCAAGCTCTTCCGTCCCGACAACTTCGTCTTC

GGTCAGTCTGGTGCTGGTAACAACTGGGCCAAGGGTCACTACACTGAGG

KP2-033B

460 bp

TAACCAAATCGGTGCTGCTTTCTGGTAAGTGCCGAGCTTTTTTTTCGCGTTGG

GTATCAATTGACAATTTACTAACTGGATTGCAGGCAAACCATCTCTGGCGAG

CACGGTCTCGATGGTGATGGACAGTAAGTTCAACGGTGATGGGTTTCTAGTA

GATCACACGTCTGATATCTTGCTAGGTACAATGGTACCTCCGACCTCCAGCTC

GAGCGTATGAACGTCTACTTCAACCATGTGAGTACACCGACTGTTTACCGAAT
AATCGTGCATCATCTGATCGGATCTTTTTCTTTGATAATCTAGGCCAGCGGTG
ACAAGTACGTTCCCCGTGCCGTTCTCGTCGATTTGGAGCCCGGTACCATGGAC
GCTGTCCGCTCCGGTCCCTTCGGCAAGCTTTTCCGCCCCGACAACTTCGTCTT
CGGTCAGTCCGGTGCTGGTAACAACTGGGCCAAGGGTC

KP2-033A

431 bp

GGTGCTGCTTTCTGGTGAGTATCGAATAACAACTCGAAAAAAATCAACACGC
TGACGTTTCTAGGCAAATCATCTCTGGCGAACACGGTCTCGACGGTGCCGGA
ATGTGAGTTTTTGATACGATCCCAATAAACCAGAAGGAACGGCACGTCTGAC
AGATACCAGTTACAATGGCTCCTCCGACCTCCAGTTGGAGCGTATGAACGTCT
ACTTCAACGAGGTGCGTCAGCAATTCATACCGAAAGAAGGACAACAGGAGCT
CACAATCGAATATAGGCTAGCGGCAACAAATATGTCCCCCGTGCCGTCCTTG
TCGACTTGGAGCCCGGTACCATGGACGCCGTCCGCGCTGGTCCCTTTGGTCAG
CTCTTCCGTCCCGACAACTTTGTTTTCGGCCAGTCTGGTGCCGGTAACAACTG
GGCCAAGGGTCA

KP2-025C

Reverse primer: bt2b

TAACCAAATCGGTGCTGCTTTCTGGTACGTGCTGCAAAACCTGAATCATCAAT
TGTTGGGTACTCGAAGCAATATACTAACCAATTTCACAGGCAAACCATTGCT
GGTGAGCACGGTCTTGACGGCGATGGACAGTGAGTTCTTTTGACAACCTTTTG
ATTTTCGAGAATGGCGGTCTGATATTTTTGGGCAGGTACAACGGTACTTCCGA

CCTCCAGCTGGAGCGCATGAACGTCTATTTCACCCACGTAAGTGATCTCGAC
ATCAATCACACTCACGATCTTCATTCTGACTGCTTTTTTCTTTCCTTTCCAATA
GGCTTCCGGTGACAAGTATGTTCCCCGTGCCGTTCTGGTCGATCTGGAGCCCG
GTACTATGGA

KP2-025B

485 bp

GTAACCAAATCGGTGCTGCTTTCTGGTACGTGCTGCAAAACCTGAATCATCAA
TTGTTGGGTACTCGAAGCAATATACTAACCAATTTCACAGGCAAACCATTGCT
GGTGAGCACGGTCTTGACGGCGATGGACAGTGAGTTCTTTTGACAACCTTTTG
ATTTTCGAGAATGGCGGTCTGATATTTTTGGGCAGGTACAACGGTACTTCCGA
CCTCCAGCTGGAGCGCATGAACGTCTATTTCACCCACGTAAGTGATCTCGAC
ATCAATCACACTCACGATCTTCATTCTGACTGCTTTTTTCTTTCCTTTCCAATA
GGCTTCCGGTGACAAGTATGTTCCCCGTGCCGTTCTGGTCGATCTGGAGCCCG
GTACTATGGACGCTGTCCGTGCCGGTCCCTTCGGCAAGCTCTTCCGTCCCGAC
AACTTCGTCTTCGGTCAGTCTGGTGCTGGTAACAACTGGGCCAAGGGTCACTA
CACTGAGG

KP2-001F

452 bp

TAACCAAATCGGTGCTGCTTTCTGGTGAGTATCGAATAACAACTCGAAAAAA
ATCAACACGCTGACGTTTCTAGGCAAATCATCTCTGGCGAACACGGTCTCGA
CGGTGCCGGAATGTGAGTTTTTGATACGATCCCAATAAACCAGAAGGAACGG
CACGTCTGACAGATACCAGTTACAATGGCTCCTCCGACCTCCAGTTGGAGCG

TATGAACGTCTACTTCAACGAGGTGCGTCAGCAATTCATACCGAAAGAAGGA

CAACAGGAGCTCACAATCGAATATAGGCTAGCGGCAACAAATATGTCCCCCG

TGCCGTCCTTGTCGACTTGGAGCCCGGTACCATGGACGCCGTCCGCGCTGGTC

CCTTTGGTCAGCTCTTCCGTCCCGACAACTTTGTTTTCGGCCAGTCTGGTGCCG

GTAACAACTGGGCCAAGGGTCACTACACTGAGG

KP1-175M

486 bp

TAACCAAATCGGTGCTGCTTTCTGGTACGTGCTGCAAAACCTGAATCATCAAT

TGTTGGGTACTCGAAGCAATATACTAACCAATTTCACAGGCAAACCWTTGCT

GGTGAGCACGGTCTTGACGGCGATGGACAGTGAGTTCTTTTGACAACCTTTTG

ATTTTCGAGAATGGCGGTCTGATATTTTTGGGCAGGTACAACGGTACTTCCGA

CCTCCAGCTGGAGCGCATGAACGTCTATTTCACCCACGTAAGTGATCTCGAC

ATCAATCACACTCACGATCTTCATTCTGACTGCTTTTTTCTTTCCTTTCCAATA

GGCTTCCGGTGACAAGTATGTTCCCCGTGCCGTTCTGGTCGATCTGGAGCCCG

GTACTATGGACGCTGTCCGTGCCGGTCCCTTCGGCAAGCTCTTCCGTCCCGAC

AACTTCGTCTTCGGTCAGTCTGGTGCTGGTAACAACTGGGCCAAGGGTCACTA

CACTGAGGGT

KP1-175G

462 bp

TAACCAAATCGGTGCTGCTTTCTGGTACGTGCAGATCTGGACACATCCATCAA

TTGAAACTTGGAGAGACCTTTTATTGACTTGATTCCAGGCAAACCATTGCTGG

TGAGCATGGCCTTGACGGCGATGGCCAGTAAGTCGCCCAAAACGCCATCCGA

CACGAACTAGCGGTCTGATGTTTTGATCTAGGTATGCTGGTGTTTCCGATCTC

CAGCGCGAGCGCATGAACGTCTACTTCAACGAGGTATGTGCCGTCTATATAA

CCTCGACTGATCGGATCTAATCTAATCATTCTTTGTTCACCTGCAGGCTAGCA

ACGACAAGTACGTTCCCCGTGCCGTTTTGGTCGACTTGGAGCCCGGTACCATG

GACGCTGTCCGTGCCGGCCCCTTCGGCAAGCTCTTCCGTCCCGACAACTTCGT

CTTCGGCCAGTCTGGTGCTGGTAACAACTGGGCCAAGGGT

KP1-131L

472 bp

GGTAACCAAATCGGTGCTGCTTTCTGGTACGTGTTGCAACCACGATTATCAAT

TGGGGATCTGTTTGACCGATAAACTGACCGACTTCTAGGCAAACCATTGCTG

GTGAGCACGGACTTGATGGCGATGGACAGTGAGTTCCAACATCGATGAGATT

GCGAGGTGGAAATGGCGGTCTGATAATTTTTTAGCGTCAACGAGGCTTCTGA

CCTCCAGTTGGAGCGCATGAACGTCTACTTCAACGAGGTATGTGTAGAATTC

AGACAAGTGTGTATGGTGGCTTCTCTAATATTGATCTTGATAGGCCAGCAGCA

ACCGTTACGTTCCCCGTGCCGTCCTTGTCGACTTGGAGCCCGGTACCATGGAC

GCCGTCCGTGCCGGTCCCTTCGGCGGTCTCTTCCGCCCCGACAACTTCGTTTT

CGGCCAGTCTGGTGCTGGTAACAACTGGGCCAAGGGTCACTACACTGAGGGT

KP1-131CC

488 bp

GTAACCAAATCGGTGCTGCTTTCTGGTACGTGCTATCCCGCCCTGACACGACT

CTGACACGACATCAATTGTCGAGGACCATCATGAATTAACCGATTCCACAGG

CAAAACATCGCTGCCGAGCACGGCCTGGACGGAGATGGACAGTAAGTTCGA

CGTTGAATCATCAACAATGGAATGGTGGTCTGATGTTTTGATTTAGCTACAAC
GGTACCTCCGACCTCCAGCTGGAGCGCATGAACGTCTACTTCACTGCTGTATG
TGCCCCCGAGTCTTCTGTGTGGCACTCAACTTTGGACTGACCGCTATGTTCTC
TATAGGCCAGCGGTGACCGCTACGTTCCCCGTGCCGTCCTTGTCGACTTGGAG
CCCGGTACCATGGATGCCATCCGTGCCGGTCCCTTCGGCAAGCTCTTCCGCCC
CGACAACTTCGTCTTCGGCCAGTCCGGTGCTGGTAACAACTGGGCCAAGGGT
CACTACACTGAGGGT

KP1-123B

525 bp

GGTAACCAAATCGGTGCTGCTTTCTGGTACGTGCCACGCCCACCAAAAAACT
GTCCAACACAACAAAGCAGCCCCTCAATTGCGAGTCTGAATGGACAAGATGT
ACTAACTCGAACTGCAGGCAGACCATTGCTGGTGAGCACGGCCTTGACGGCG
ATGGCCAGTAAGTTTCTTCGATACCCTTCGACAAAACCACTCGAACGCGGAA
TGGCGGTCTGATATTTCCGGCTAGGTACAATGGTTCCTCCGACCTCCAGCTCG
AGCGCTTGAACGTCTACTTCACCCATGTAAGTTGCGTGTCCAGTCAATGCCGA
AATACTGAACTCGACTCTAATCGATGGAACTGTTGTTTCTTAGGCCAGCGGTG
ACAAGTACGTTCCCCGTGCCGTTCTGGTCGATCTGGAGCCCGGTACCATGGA
CGCTGTCCGTGCCGGTCCCTTTGGCAAGCTCTTCCGTCCCGACAACTTCGTCT
TCGGTCAGTCTGGTGCTGGTAACAACTGGGCCAAGGGTCACTACACTGAGGG
T

KP1-123A

Reverse primer: bt2b

TAACCAAATCGGTGCTGCTTTCTGGTACGTGCCACGCCCACCAAAAAACTGT

CCAACACAACAAAGCAGCCCCTCAATTGCGAGTCTGAATGGACAAGATGTAC

TAACTCGAACTGCAGGCAGACCATTGCTGGTGAGCACGGCCTTGACGGCGAT

GGCCAGTAAGTTTCTTCGATACCCTTCGACAAAACCACTCGAACGCGGAATG

GCGGTCTGATATTTCCGGCTAGGTACAATGGTTCCTCCGACCTCCAGCTCGAG

CGCTTGAACGTCTACTTCACCCATGTAAGTTGCGTGTCCAGTCAATGCCGAAA

TACTGAACTCGACTCTAATCGATGGAACTGTTGTTTCTTAGGCCAGCGGTGAC

AAGTACGTTCCCCGTGCCGTTCTGGTCGATCTGGAGCCCGGTACCATGG

KP1-091A

522 bp

TAACCAAATCGGTGCTGCTTTCTGGTACGTGCCACGCCCACCACAMAAACTG

TCCAACACAACAAAGCAGCCCCTCAATTGCGAGTCTGAATGGACAAGATGTA

CTAACTCGAACTGCAGGCAGACCATTGCTGGTGAGCACGGCCTTGACGGCGA

TGGCCAGTAAGTTTCTTCGATACCCTTCGACAAAACCACTCGAACGCGGAAT

GGCGGTCTGATATTTCCGGCTAGGTACAATGGTTCCTCCGACCTCCAGCTCGA

GCGCTTGAACGTCTACTTCACCCATGTAAGTTGCGTGTCCAGTCAATGCCGAA

ATACTGAACTCGACTCTAATCGATGGAACTGTTGTTTCTTAGGCCAGCGGTGA

CAAGTACGTTCCCCGTGCCGTTCTGGTCGATCTGGAGCCCGGTACCATGGAC

GCTGTCCGTGCCGGTCCCTTTGGCAAGCTCTTCCGTCCCGACAACTTCGTCTT

CGGTCAGTCTGGTGCTGGTAACAACTGGGCCAAGGGTCACTACACTGAGG

KP1-075B

Forward primer: bt2a

CACAACAAAGCAGCCCCTCAATTGCGAGTCTGAATGGACAAGATGTACTAAC

TCGAACTGCAGGCAGACCATTGCTGGTGAGCACGGCCTTGACGGCGATGGCC

AGTAAGTTTCTTCGATACCCTTCGACAAAACCACTCGAACGCGGAATGGCGG

TCTGATATTTCCGGCTAGGTACAATGGTTCCTCCGACCTCCAGCTCGAGCGCT

TGAACGTCTACTTCACCCATGTAAGTTGCGTGTCCAGTCAATGCCGAAATACT

GAACTCGACTCTAATCGATGGAACTGTTGTTTCTTAGGCCAGCGGTGACAAGT

ACGTTCCCCGTGCCGTTCTGGTCGATCTGGAGCCCGGTACCATGGACGCTGTC

CGTGCCGGTCCCTTTGGCAAGCTCTTCCGTCCCGACAACTTCGTCTTCGGTCA

GTCTGGTGCTGGTAACAACTGGGCCAAGGGTCACTACACTGAGG

KP1-045A

485 bp

GTAACCAAATCGGTGCTGCTTTCTGGTACGTGCTGCAAAACCTGAATCATCAA

TTGTTGGGTACTCGAAGCAATATACTAACCAATTTCACAGGCAAACCATTGCT

GGTGAGCACGGTCTTGACGGCGATGGACAGTGAGTTCTTTTGACAACCTTTTG

ATTTTCGAGAATGGCGGTCTGATATTTTTGGGCAGGTACAACGGTACTTCCGA

CCTCCAGCTGGAGCGCATGAACGTCTATTTCACCCACGTAAGTGATCTCGAC

ATCAATCACACTCACGATCTTCATTCTGACTGCTTTTTTCTTTCCTTTCCAATA

GGCTTCCGGTGACAAGTATGTTCCCCGTGCCGTTCTGGTCGATCTGGAGCCCG

GTACTATGGACGCTGTCCGTGCCGGTCCCTTCGGCAAGCTCTTCCGTCCCGAC

AACTTCGTCTTCGGTCAGTCTGGTGCTGGTAACAACTGGGCCAAGGGTCACTA

CACTGAGG

KP1-025B

485 bp

GTAACCAAATCGGTGCTGCTTTCTGGTACGTGCTGCAAAACCTGAATCATCAA
TTGTTGGGTACTCGAAGCAATATACTAACCAATTTCACAGGCAAACCATTGCT
GGTGAGCACGGTCTTGACGGCGATGGACAGTGAGTTCTTTTGACAACCTTTTG
ATTTTCGAGAATGGCGGTCTGATATTTTTGGGCAGGTACAACGGTACTTCCGA
CCTCCAGCTGGAGCGCATGAACGTCTATTTCACCCACGTAAGTGATCTCGAC
ATCAATCACACTCACGATCTTCATTCTGACTGCTTTTTTCTTTCCTTTCCAATA
GGCTTCCGGTGACAAGTATGTTCCCCGTGCCGTTCTGGTCGATCTGGAGCCCG
GTACTATGGACGCTGTCCGTGCCGGTCCCTTCGGCAAGCTCTTCCGTCCCGAC
AACTTCGTCTTCGGTCAGTCTGGTGCTGGTAACAACTGGGCCAAGGGTCACTA
CACTGAGG

KP1-017E

501 bp

GGTGCTGCTTTCTGGTACGTGCCACGCCCACCAAAAAACTGTCCMACACAAC
AAAGCAGCCCCTCAATTGCGAGTCTGAATGGACAAGATGTACTAACTCGAAC
TGCAGGCAGACCATTGCTGGTGAGCACGGCCTTGACGGCGATGGCCAGTAAG
TTTCTTCGATACCCTTCGACAAAACCACTCGAACGCGGAATGGCGGTCTGATA
TTTCCGGCTAGGTACAATGGTTCCTCCGACCTCCAGCTCGAGCGCTTGAACGT
CTACTTCACCCATGTAAGTTGCGTGTCCAGTCAATGCCGAAATACTGAACTCG
ACTCTAATCGATGGAACTGTTGTTTCTTAGGCCAGCGGTGACAAGTACGTTCC
CCGTGCCGTTCTGGTCGATCTGGAGCCCGGTACCATGGACGCTGTCCGTGCCG
GTCCCTTTGGCAAGCTCTTCCGTCCCGACAACTTCGTCTTCGGTCAGTCTGGT
GCTGGTAACAACTGGGCCAAGGGTCAC

KP1-013B

455 bp

GGTGCTGCTTTCTGGTACGTGCAGATCTGGACACATCCATCAATTGAAACTTG

GAGAGACCTTTTATTGACTTGATTCCAGGCAAACCATTGCTGGTGAGCATGGC

CTTGACGGCGATGGCCAGTAAGTCGCCCAAAACGCCATCCGACACGAACTAG

CGGTCTGATGTTTTGATCTAGGTATGCTGGTGTTTCCGATCTCCAGCGCGAGC

GCATGAACGTCTACTTCAACGAGGTATGTGCCGTCTATATAACCTCGACTGAT

CGGATCTAATCTAATCATTCTTTGTTCACCTGCAGGCTAGCAACGACAAGTAC

GTTCCCCGTGCCGTTTTGGTCGACTTGGAGCCCGGTACCATGGACGCTGTCCG

TGCCGGCCCCTTCGGCAAGCTCTTCCGTCCCGACAACTTCGTCTTCGGCCAGT

CTGGTGCTGGTAACAACTGGGCCAAGGGTCAC

CT1-006B

445 bp

GGTGCTGCTTTCTGGTACGTGTTGCAACCACGGTCATCAATTGGTGGTCCGTT

GAACCGATTTACTGACTGAATTCTAGGCAAACCATTGCTGGTGAGCACGGAC

TTGATGGCGATGGACAGTGAGTTTCAACATCGATGAGATTGCGAGGTGGAAA

TGGCGGTCTGATAATTTTTAGCGTCAACGAGGCCTCTGACCTCCAGTTGGAGC

GCATGAACGTCTACTTCAACGAGGTACGTGTAGAATTGAAAAAGTATATATG

GAGTCTTCTCTAATGTTGATCTTGATAGGCCAGCAGCAACCGTTACGTCCCCC

GTGCCGTCCTTGTCGACTTGGAGCCCGGTACCATGGACGCTGTCCGTGCTGGT

CCCTTCGGCGGTCTCTTCCGCCCCGACAACTTCGTTTTCGGTCAGTCCGGAGC

CGGTAACAACTGGGCCAAGGGTCA

Appendix 2. Gene sequences obtained from sequencing of the calmodulin gene with primer pairs CMD5 and CMD6 of *Aspergillus* fungal isolates.

KP2-025D

501 bp

GAGTACAAGGAGGCCTTCTCTCTTTTTGTAAGTTTGGAAACTTGGTTTGTCAC
AATGTTGGCGTGGTTAGCTGACTAGCCGTTTTGATGAATAGGACAAGGATGG
AGATGGTGAGTCGCCGCGAGCAATGAAACACCTTGAACGAACATTACCGCAG
TCAACAGACATTGACCCTATCGGACAGGACAAATCACAACCAAGGAACTCGG
CACAGTCATGCGTTCTCTCGGCCAGAACCCCTCCGAATCCGAATTGCAGGAC
ATGATCAACGAAGTCGACGCTGACAACAACGGCACAATTGATTTCCCTGGTA
TGACTGACCACCAACTCGCAATATTTGATGGCAGTACTGACTGCCGCAGAAT
TCTTGACAATGATGGCCCGCAAAATGAAGGATACCGACTCCGAGGAAGAGAT
CCGTGAGGCTTTCAAGGTGTTTGACCGTGACAACAATGGATTCATTTCTGCCG
CCGAATTGCGCCACGTCATGACCTCTATCGG

KP2-001C

561 bp

GAGTACAAGGAAGCCTTCTCTCTCTTCGTAAGTGAACTGTCCAAGTCCCTGGT
CGTTGTATAGGAGGGATCTCCAGAATATTGAGGGTGTGCGCTGACACGAGAT
TTGACGTATAGGACAAGGATGGTGATGGTTAGTGACCCTTTTTCCACTCCTCG
AACTTCGGCTTCCATGCGATCATGTTCAAACGCCGACTCACAATATCCGGAA
ATGACCCGTCAGTACTGATAATATCTATGTTTGACTATCAGGCCAGATCACCA
CCAAGGAATTGGGCACTGTAATGCGCTCTCTGGGCCAGAACCCTTCCGAGTC

AGAGCTGCAAGATATGATCAACGAGGTGGATGCTGACAACAACGGCACCATC

GATTTCCCCGGTATGTGATACTTTCGGTATGAACTCGGGAGGGGAGAGAACA

ATCATTAACTTGTAATCAGAATTCCTTACCATGATGGCTCGGAAGATGAAGG

ACACCGACTCCGAAGAGGAAATTCGGGAAGCTTTCAAGGTCTTCGACCGCGA

CAACAACGGTTTCATCTCCGCTGCGGAGCTGCGCCACG

KP1-131Y

Forward primer: CMD5

TGGGATCATCTAGGCTGACGGGTTATCTTGTGATCGACAGGACAAGGATGGC

GATGGTAAGTGCGGCCCATGCTGAACATCTCGTGTCCTTTTCACGGGCGGCGT

TTCTCTCGCGATTGGGTTTCATCAAATTATCGTTGAACCTGGCTAAAACAAAT

TGCGATCTGTAGGAGAAATCACCACCAAGGAGCTTGGCACCGTCATGCGCTC

CCTCGGCCAGAACCCCTCCGAGTCTGAGCTGCAGGATATGATCAACGAGGTC

GACGCCGATAACAACGGTACCATCGATTTCCCCGGTACGCTCCCCAACTCTGT

TATTTATCCCTCTCCTCTCCTCTCCTCCGACCGTCACAAAAATATCAATATTGA

CATGCGCCCACAGAGTTCCTGACCATGATGGCTCGTAAGATGAAGGACACCG

ACTCCGAGGAGGAGATCCGTGAGGCCTTCAAGGTCTTTGACCGTGATAACAA

CGGTTTCATCTCCGCCGCTGAGCTGCGCCACG

KP1-131T

Reverse primer: CMD6

CCTTCTCTCTCTTCGTAAGTGAACTGTCCAAGTCCCTGGTCGTTGTATAGGAG

GGATCTCCAGAATATTGAGGGTGTGCGCTGACACGAGATTTGACGTATAGGA

CAAGGATGGTGATGGTTAGTGACCCTTTTTCCACTCCTCGAACTTCGGCTTCC

ATGCGATCATGTTCAAACGCCGACTCACAATATCCGGAAATGACCCGTCAGT
ACTGATAATATCTATGTTTGACTATCAGGCCAGATCACCACCAAGGAATTGG
GCACTGTAATGCGCTCTCTGGGCCAGAACCCTTCCGAGTCAGAGCTGCAAGA
TATGATCAACGAGGTGGATGCTGACAACAACGGCACCATCGATTTCCCCGGT
ATGTGATACTTTCGGTATGAACTCGGGAGGGGAGAGAACAATCATTAACTTG
TAATCAGAATTCCTTACCATGATGGCTCGGAAGATGAAGGACACCGACTCCG
AAGAGGAAATTCGGGAAGC

KP1-131Q

548 bp

CCTTCTCTCTCTTCGTAAGTGAACTGTCCAAGTCCCTGGTCGTTGTATAGGAG
GGATCTCCAGAATATTGAGGGTGTGCGCTGACACGAGATTTGACGTATAGGA
CAAGGATGGTGATGGTTAGTGACCCTTTTTCCACTCCTCGAACTTCGGCTTCC
ATGCGATCATGTTCAAACGCCGACTCACAATATCCGGAAATGACCCGTCAGT
ACTGATAATATCTATGTTTGACTATCAGGCCAGATCACCACCAAGGAATTGG
GCACTGTAATGCGCTCTCTGGGCCAGAACCCTTCCGAGTCAGAGCTGCAAGA
TATGATCAACGAGGTGGATGCTGACAACAACGGCACCATCGATTTCCCCGGT
ATGTGATACTTTCGGTATGAACTCGGGAGGGGAGAGAACAATCATTAACTTG
TAATCAGAATTCCTTACCATGATGGCTCGGAAGATGAAGGACACCGACTCCG
AAGAGGAAATTCGGGAAGCTTTCAAGGTCTTCGACCGCGACAACAACGGTTT
CATCTCCGCTGCGGAGCTGCGCCACG

KP1-131AA

548 bp

CCTTCTCTCTCTTCGTAAGTGAACTGTCCAAGTCCCTGGTCGTTGTATAGGAG
GGATCTCCAGAATATTGAGGGTGTGCGCTGACACGAGATTTGACGTATAGGA
CAAGGATGGTGATGGTTAGTGACCCTTTTTCCACTCCTCGAACTTCGGCTTCC
ATGCGATCATGTTCAAACGCCGACTCACAATATCCGGAAATGACCCGTCAGT
ACTGATAATATCTATGTTTGACTATCAGGCCAGATCACCACCAAGGAATTGG
GCACTGTAATGCGCTCTCTGGGCCAGAACCCTTCCGAGTCAGAGCTGCAAGA
TATGATCAACGAGGTGGATGCTGACAACAACGGCACCATCGATTTCCCCGGT
ATGTGATACTTTCGGTATGAACTCGGGAGGGGAGAGAACAATCATTAACTTG
TAATCAGAATTCCTTACCATGATGGCTCGGAAGATGAAGGACACCGACTCCG
AAGAGGAAATTCGGGAAGCTTTCAAGGTCTTCGACCGCGACAACAACGGTTT
CATCTCCGCTGCGGAGCTGCGCCACG

KP1-063N

548 bp

CCTTCTCTCTCTTCGTAAGTGAACTGTCCAAGTCCCTGGTCGTTGTATAGGAG
GGATCTCCAGAATATTGAGGGTGTGCGCTGACACGAGATTTGACGTATAGGA
CAAGGATGGTGATGGTTAGTGACCCTTTTTCCACTCCTCGAACTTCGGCTTCC
ATGCGATCATGTTCAAACGCCGACTCACAATATCCGGAAATGACCCGTCAGT
ACTGATAATATCTATGTTTGACTATCAGGCCAGATCACCACCAAGGAATTGG
GCACTGTAATGCGCTCTCTGGGCCAGAACCCTTCCGAGTCAGAGCTGCAAGA
TATGATCAACGAGGTGGATGCTGACAACAACGGCACCATCGATTTCCCCGGT
ATGTGATACTTTCGGTATGAACTCGGGAGGGGAGAGAACAATCATTAACTTG
TAATCAGAATTCCTTACCATGATGGCTCGGAAGATGAAGGACACCGACTCCG

AAGAGGAAATTCGGGAAGCTTTCAAGGTCTTCGACCGCGACAACAACGGTTT

CATCTCCGCTGCGGAGCTGCGCCAC

Appendix 3. Gene sequences obtained from sequencing of the ITS gene with primer pairs ITS1 and ITS4 of *Penicillium* fungal isolates.

KP2-033H

497 bp

CGTACCTTGTTGCTTCGGCGGGCCCGCCTCACGGCCGCCGGGGGGCACCTGC

CCCCGGGCCCGCGCCCGCCGAAGACACCATTGAACTCTGTCTGAAGATTGCA

GTCTGAGCGATTAACTAAATCAGTTAAAACTTTCAACAACGGATCTCTTGGTT

CCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAG

AATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCG

GGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTT

GGGCTCCGCCCCCCTCCCGGGGGGCGGGCCCGAAAGGCAGCGGCGGCACCG

CGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCG

GCGCCCGCCGGCGACCCCAATCAATCTTTCCAGGTTGACCTCGGATCAGGTA

GGGATACCCGCTGAACTTAAGCATATC

KP2-033B

472 bp

GCCGGGGGGCTCACGCCCCCGGGCCCGCGCCCGCCGAAGACACCCCCGAACT

CTGCCTGAAGATTGTCGTCTGAGTGAAAATATAAATTATTTAAAACTTTCAAC

AACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATACG

TAATGTGAATTGCAAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGC

CCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGC

CCGGCTGTGTGTTGGGCCCCGTCCTCCGATTCCGGGGGACGGGCCCGAAAGG

CAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCT

CTGTAGGCCCGGCCGGCGCTTGCCGATCAACCCAAATTTTTATCCAGGTTGAC

CTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGAG

KP2-001F

505 bp

ACCTGCGGAAGGATCATTACTGAGTGAGGGCCCCTCGGGGTCCAACCTCCCA

CCCGTGTTTAACGAACCTTGTTGCTTTGGCGGGCCCGCCTCACGGCCGCCGGG

GGGCATCTGCCCCCGGGCCCGCGCCCGCCGAAGCCACCTGTGAACTCTGTCT

GAAGTATGCAGTCTGAGACAATTATTAAATTAATTAAAACTTTCAACAACGG

ATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATG

TGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCTC

TGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCCAGCCCGG

CTGGTGTGTTGGGCCCCGCCCCCCTTCCCGGGGGGGCGGGCCCGAAAGGCAG

CGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTN

NTAGGCCCGGCCGGCGCCAGCCGACCCCCTCAAT

KP1-175M

499 bp

CGAACCTTGTTGCTTTGGCGGGCCCGCCTCACGGCCGCCGGGGGGCATCTGC

CCCCGGGCCCGCGCCCGCCGAAGCCACCTGTGAACTCTGTCTGAAGTATGCA

GTCTGAGACAATTATTAAATTAATTAAAACTTTCAACAACGGATCTCTTGGTT

CCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGA

ATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCTCTGGTATTCCGG

AGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCCAGCCCGGCTGGTGTGTTG

GGCCCCGCCCCCCTTCCCGGGGGGGCGGGCCCGAAAGGCAGCGGCGGCACC

GCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTTGTAGGCCCGGC

CGGCGCCAGCCGACCCCCTCAATCTATTTTTTCAGGTTGACCTCGGATCAGGT

AGGGATACCCGCTGAACTTAAGCATAT

KP1-175G

484 bp

ACCCCCGGTCGCCGGGGGGCACTGCGCCCCCGGGCCCGCGCCCGCCAGAGCG

CCTCTGAACCCTAATGAAGAAGGACTGTCTGAGTCTACGATATAATTATCAA

AACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAA

ATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAA

CGCACATTGCGCCCCCTGGCATTCCGGGGGGCATGCCTGTCCGAGCGTCATTT

CTGCCCTCAAGCCCGGCTTGTGTGTTGGGCGTGGTCCCCCCGGTGTCGGGGG

GACCTGCCCCAAAGGCAGCGGCGACGTTCCGCCTAGGTCCTCGAGCGTATGG

GGCTTTGTCACCCGCTCGGGAGGGGCCTACGGGCGTTGGCCACCCACCAATT

TTTTTTACGGTTGACCTCGGATCAGGTAGGAGTTACCCGCTGAACTTAAGCAT

ATCAATAAGCGGAG

KP1-131L

491 bp

TGTACCTTGTTGCTTCGGTGCGCCCGCCTCACGGCCGCCGGGGGGCTTCTGCC

CCCGGGTCCGCGCGCACCGGAGACACCATTGAACTCTGTCTGAAGATTGCAG

TCTGAGCATAAACTAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCC

GGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAAT
TCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGG
GGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGG
CTCCGTCCCCCCGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGAGTCCGGT
CCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCCAG
CCGACAACCAATCATCCTTTTTTCAGGTTGACCTCGGATCAGGTAGGGATACC
CGCTGAACTTAAGCATAT

KP1-123B

499 bp

TGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCG
TGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCTCACGGCCGCCGGGGGGC
ACCTGCCCCCGGGCCCGCGCCCGCCGAAGACACCATTGAACTCTGTCTGAAG
ATTGCAGTCTGAGCGATTAACTAAATCAGTTAAAACTTTCAACAACGGATCTC
TTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAAT
TGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTA
TTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGT
GTGTTGGGCTCCGCCCCCCTCCCGGGGGGCGGGCCCGAAAGGCAGCGGCGGC
ACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCG
GCCGGCGCCCGCCGGCGACCCCAATC

KP1-123A

422 bp

ACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCTCCCAC

CCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCTCACGGCCGCCGGGG
GGCACCTGCCCCCGGGCCCGCGCCCGCCGAAGACACCATTGAACTCTGTCTG
AAGATTGCAGTCTGAGCGATTAACTAAATCAGTTAAAACTTTCAACAACGGA
TCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGT
GAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCT
GGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGG
CTTGTGTGTTGGGCTCCGCCCCCCTCCCGGGGGGCGGGCCCGAAAGGCAGCG
GCGG

KP1-091A

472 bp

GCCGGGGGGCACCTGCCCCCGGGCCCGCGCCCGCCGAAGACACCATTGAACT
CTGTCTGAAGATTGCAGTCTGAGCGATTAACTAAATCAGTTAAAACTTTCAAC
AACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAG
TAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCG
CCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAG
CACGGCTTGTGTGTTGGGCTCCGCCCCCCTCCCGGGGGGCGGGCCCGAAAGG
CAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCT
CTGTAGGCCCGGCCGGCGCCCGCCGGCGACCCCAATCAATCTTTCCAGGTTG
ACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGCGGA
G

KP1-075B

469 bp

ACCTGCGGAAGGATCATTACTGAGTGAGGGCCCCTCGGGGTCCAACCTCCCA

CCCGTGTTTAACGAACCTTGTTGCTTTGGCGGGCCCGCCTCACGGCCGCCGGG

GGGCATCTGCCCCCGGGCCCGCGCCCGCCGAAGCCACCTGTGAACTCTGTCT

GAAGTATGCAGTCTGAGACAATTATTAAATTAATTAAAACTTTCAACAACGG

ATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATG

TGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCTC

TGGTATTCCGGAGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCCAGCCCGG

CTGGTGTGTTGGGCCCCGCCCCCCTTCCCGGGGGGGCGGGCCCGAAAGGCAG

CGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTC

KP1-045A

499 bp

CGAACCTTGTTGCTTTGGCGGGCCCGCCTCACGGCCGCCGGGGGGCATCTGC

CCCCGGGCCCGCGCCCGCCGAAGCCACCTGTGAACTCTGTCTGAAGTATGCA

GTCTGAGACAATTATTAAATTAATTAAAACTTTCAACAACGGATCTCTTGGTT

CCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGA

ATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCTCTGGTATTCCGG

AGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCCAGCCCGGCTGGTGTGTTG

GGCCCCGCCCCCCTTCCCGGGGGGGCGGGCCCGAAAGGCAGCGGCGGCACC

GCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTTGTAGGCCCGGC

CGGCGCCAGCCGACCCCCTCAATCTATTTTTTCAGGTTGACCTCGGATCAGGT

AGGGATACCCGCTGAACTTAAGCATAT

KP1-017E

509 bp

TGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCG

TGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCTCACGGCCGCCGGGGGGC

ACCTGCCCCCGGGCCCGCGCCCGCCGAAGACACCATTGAACTCTGTCTGAAG

ATTGCAGTCTGAGCGATTAACTAAATCAGTTAAAACTTTCAACAACGGATCTC

TTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAAT

TGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTA

TTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGT

GTGTTGGGCTCCGCCCCCCTCCCGGGGGGCGGGCCCGAAAGGCAGCGGCGGC

ACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCG

GCCGGCGCCCGCCGGCGACCCCAATCAATCTTCCAG

KP1-013B

488 bp

TTGCTTCGGCGGGCCCGCCTCACGGCCGCCGGGGGGCTTCTGCCCTCTGGCCC

GCGCCCGCCGAAGACACCATTGAACGCTGTCTGAAGATTGCAGTCTGAGCAA

TTAGCTAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGA

TGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAGAATTCAGTGAA

TCATCGAGTCTTTGAACGCACATTGCGCCCCTTGGTATTCCGGGGGGCATGCC

TGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCTCCGTCC

TCCTTCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCT

CGAGCGTATGGGGCTTCGTCTTCCGCTCTTGTAGGCCCGGCCGGCGCTTGCCG

ACAACAATCAATCTTTTTTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCT

GAACTTAAGCATAT

CT1-006B

Forward primer: ITS1

TGTACCTTGTTGCTTCGGTGCGCCCGCCTCACGNNNNCCGGGGGGCTTCTGCC

CCCGGGTCCGCGCGCACCGGAGACACCATTGAACTCTGTCTGAAGATTGCAG

TCTGAGATAAACTAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCG

GCATCGATGAAGACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTC

AGTGAATCATCGAGTCTTTAACGCACATTGCGCCCCCTGGTATTCCGGGGGGC

ATGCCTGTCCGAGCGTCATTGCTGCCTCAAGCACGGCTTGTGTGTTGGGCTCC

GTCCCCCCGGGGACGGGTCCGAAAGGCAGCGCGGCACCGAGTCCGGTCCTCG

AGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGCGCCAGCCGACA

ACCAATCATCCTTTTTCAGGTTGACCTCGGATCAGGTAGGGATACCGCTGAAC

TTAAGCATATCAA

Appendix 4. Gene sequences obtained from sequencing of the ITS gene with primer pairs ITS1 and ITS4 of *Aspergillus* fungal isolates.

KP2-025D

478 bp

ACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCTCCCAC
CCGTGTCTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGTTTCGACGGCCGCC
GGGGAGGCCTTGCGCCCCCGGGCCCGCGCCCGCCGAAGACCCCAACATGAAC
GCTGTTCTGAAAGTATGCAGTCTGAGTTGATTATCGTAATCAGTTAAAACTTT
CAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGA
TAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACA
TTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCC
TCAAGCACGGCTTGTGTGTTGGGCCCCCGTCCCCCTCTCCCGGGGGACGGGC
CCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGT
CACCTGC

KP2-001C

515 bp

CTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGTTTCGACGGCCGCCGGGGAG
GCCTTGCGCCCCCGGGCCCGCGCCCGCCGAAGACCCCAACATGAACGCTGTT
CTGAAAGTATGCAGTCTGAGTTGATTATCGTAATCAGTTAAAACTTTCAACAA
CGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA
ATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCC
CCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCA

CGGCTTGTGTGTTGGGCCCCCGTCCCCCTCTCCCGGGGGACGGGCCCGAAAG

GCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCTGC

TCTGTAGGCCCGGCCGGCGCCAGCCGACACCCAACTTTATTTTTCTAAGGTTG

ACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATC

KP1-131Y

488 bp

TTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGT

CCAACCTCCCACCCGTGTCTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGTT

TCGACGGCCGCCGGGGAGGCCTTGCGCCCCCGGGCCCGCGCCCGCCGAAGAC

CCCAACATGAACGCTGTTCTGAAAGTATGCAGTCTGAGTTGATTATCGTAATC

AGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGC

AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTC

TTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGC

GTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCCGTCCCCCTCTCC

CGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCG

TATGGGGCTTTGTCACCT

KP1-131T

487 bp

TTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGT

CCAACCTCCCACCCGTGTCTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGTT

TCGACGGCCGCCGGGGAGGCCTTGCGCCCCCGGGCCCGCGCCCGCCGAAGAC

CCCAACATGAACGCTGTTCTGAAAGTATGCAGTCTGAGTTGATTATCGTAATC

AGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGC

AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTC

TTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGC

GTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCCGTCCCCCTCTCC

CGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCG

TATGGGGCTTTGTCACC

KP1-131Q

513 bp

ATCGTACCTTGTTGCTTCGGCGGGCCCGCCGTTTCGACGGCCGCCGGGGAGG

CCTTGCGCCCCCGGGCCCGCGCCCGCCGAAGACCCCAACATGAACGCTGTTC

TGAAAGTATGCAGTCTGAGTTGATTATCGTAATCAGTTAAAACTTTCAACAAC

GGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA

TGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCC

CCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCAC

GGCTTGTGTGTTGGGCCCCCGTCCCCCTCTCCCGGGGGACGGGCCCGAAAGG

CAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCTGCT

CTGTAGGCCCGGCCGGCGCCAGCCGACACCCAACTTTATTTTTCTAAGGTTGA

CCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATC

KP1-131AA

514 bp

ATCGTACCTTGTTGCTTCGGCGGGCCCGCNCNNTNGACGGCCGCCGGGGAGG

CCTTGCGCCCCCGGGCCCGCGCCCGCCGAAGACCCCAACATGAACGCTGTTC

TGAAAGTATGCAGTCTGAGTTGATTATCGTAATCAGTTAAAACTTTCAACAAC

GGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAA

TGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCC

CCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCAC

GGCTTGTGTGTTGGGCCCCCGTCCCCCTCTCCCGGGGGACGGGCCCGAAAGG

CAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTTGTCACCTGCT

CTGTAGGCCCGGCCGGCGCCAGCCGACACCCAACTTTATTTTTCTAAGGTTGA

CCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCA

KP1-063N

489 bp

TTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGT

CCAACCTCCCACCCGTGTCTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGTT

TCGACGGCCGCCGGGGAGGCCTTGCGCCCCCGGGCCCGCGCCCGCCGAAGAC

CCCAACATGAACGCTGTTCTGAAAGTATGCAGTCTGAGTTGATTATCGTAATC

AGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGC

AGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTC

TTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGC

GTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCCGTCCCCCTCTCC

CGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCG

TATGGGGCTTTGTCACCTG