



Diversity of Entomopathogenic fungi among the coleopteran pests of crops

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Abstract

Occurrence of entomopathogenic fungi among the coleopteran pests of Banana, Coconut, Cardamom, Sweet Potato and Vegetables was conducted to reveal the diversity of these fungi among various pests. The isolates identified by ITS sequencing were Beauveria bassiana (Balsamo) Vuillemin from adults of Basilepta fulvicorne Jacoby and Metarhizium sp. from its grubs. Paecilomyces sp. was isolated from the grubs of Banana rhizome weevil, Cosmopolites sordidus Germ. The adult beetles of Cylas formicarius F., Henosepilachna vigintioctopunctata F. and Metriona circumdata H. were found to be infected with Beauveria sp., Fusarium moniliformae and Beauveria brongniartii (Saccardo) Petch, respectively. The infected grubs of Oryctes rhinoceros Linn. harboured Metarhizium album, Metarhizium anisopliae var majus and Metarhizium sp.

Key words: entomopathogenic, fungi, diversity, coleopteran, ITS sequencing

I. INTRODUCTION

Entomopathogenic fungi are encountered in all the agro ecosystems and found associated with a wide array of host insects. Almost all the insect orders are susceptible to fungal infections [1] and these fungi have a broad spectrum of hosts. A study on the diversity of entomopathogenic fungi among the coleopteran pests of Banana, Coconut, Cardamom, Sweet Potato and Vegetables was carried out to assess the natural occurrence of these biocontrol agents in different agro ecosystems.

II. MATERIALS AND METHODS

A. Collection sites

The collection sites include cardamom plantations of Idukki dist., banana, coconut, sweet potato and vegetable fields in Trivandrum district. Cadavers of the infected insects of the selected hosts were collected from these collection sites by sampling.

B. Isolation of natural isolates

The naturally infected test insects obtained from field were isolated from the healthy insects, surface cleaned and incubated in moist humid chamber until mycelial growth appears. Once the fungal growth was expressed, the cadaver was transferred to PDA slants and later the active growing mycelia were transferred to fresh PDA slants and further subcultured and replicated into more slants and kept under refrigeration for further studies and identification.

C. Identification through DNA Barcoding and ITS sequencing

The DNA was isolated from the mycelium of the cultured fungus. The eluted DNA was stored at 4 °C and the quality of the isolated DNA was checked by agarose gel electrophoresis. This is followed by PCR amplification reaction in a PCR thermal cycler (GeneAmp PCR System 9700, Applied

Biosystems) using the forward and reverse primers (Table 1). The PCR products were again subjected to agarose gel electrophoresis in 1.2 per cent agarose gels prepared in 0.5 X TBE buffer containing 0.5 µg ml⁻¹ ethidium bromide. The gel run PCR products thus obtained were treated with two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications. Five micro litres of PCR product is mixed with 2 µl of ExoSAP-IT and incubated at 37 °C for 15 minutes followed by enzyme inactivation at 80 °C for 15 minutes.

Table 1. Primers used for ITS sequencing of the isolates

Target	Primer Name	Direction	Sequence (5' → 3')	Reference/Remarks
ITS	ITS-1F	Forward	TCCGTAGGTGAACCTTGCGG	[2]
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC	

Sequencing reaction was done in a PCR thermal cycler using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA). The sequencing PCR temperature profile consisted of a first cycle at 96 °C for two minutes followed by 30 cycles at 96 °C for 30 sec, 50 °C for 40 sec and 60 °C for four minutes for all the primers. Then the post sequencing PCR clean-up was done and the pellet obtained was air dried. The cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems). The sequence quality was checked using Sequence Scanner Software v1 and was identified using BLAST (Basic Local Alignment Search Tool) as demonstrated by Morgulis *et al.* [3] and Zhang *et al.* [4].

III. RESULTS AND DISCUSSION

The nine new natural isolates obtained from the host insects were subjected to ITS (Internal Transcribed Sequence) sequencing and BLAST (Basic Local Alignment Search Tool) and identified the cultures (Table 2). The natural isolate obtained from the adults of *B. fulvicorne* was identified as *B. bassiana* and the fungi isolated from the grubs of *B. fulvicorne* was *Metarhizium* sp. The entomopathogenic fungi *Paecilomyces* sp. was obtained from the grubs of *C. sordidus*. The naturally infected adults of *C. formicarius*, *H. vigintioctopunctata* and *M. circumdata* were identified as the pathogenesis of *Beauveria* sp., *Fusarium moniliformae* and *B. brongniartii* respectively. The natural isolates obtained from the grubs of *O. rhinoceros* were *Metarhizium album*, *Metarhizium anisopliae* var *majus* and *Metarhizium* sp. The natural occurrence of these entomopathogenic fungi confirms their widespread distribution and diversity worldwide [5].

The results show that the soils of these particular agro ecosystems are abundant reservoirs of these diverse strains of entomopathogenic fungi and hence conservation biological control is of utmost importance in farming systems for favouring natural epizootics for pest management as observed by Pena *et al.* [6]. The details of the sequences of the isolates obtained are presented below.

Table 2. New isolates of fungi isolated from coleopteran insects collected from Thiruvananthapuram and Idukki districts

Sl.No	Host insect	Stage of the Insect	Isolates
1	<i>B. fulvicorne</i>	A	<i>B. bassiana</i>
2	<i>B. fulvicorne</i>	G	<i>Metarhizium</i> sp.
3	<i>C. sordidus</i>	G	<i>Paecilomyces</i> sp.
4	<i>C. formicarius</i>	A	<i>Beauveria</i> sp.
5	<i>H. vigintioctopunctata</i>	A	<i>Fusarium moniliformae</i>
6	<i>M. circumdata</i>	A	<i>B. brongniartii</i>
7	<i>O. rhinoceros</i>	G	<i>Metarhizium album</i>
8	<i>O. rhinoceros</i>	G	<i>Metarhizium anisopliae</i> var <i>majus</i>
9	<i>O. rhinoceros</i>	G	<i>Metarhizium</i> sp.

A : Adult G : Grub

Isolate 1

Host insect : *B. fulvicorne*

Organism identified : *B. bassiana*

Sequence:

TACATACGCTGGTACCTCCGACCTCCAGCTCGAGCGTATGAACGTGTACTTCAACGAGGTGTGTGATGACCACA
CTGAAATATTTATTATTTATCGTTCCTAATCCCATAAGCTACAGGCTTCCGGTAAGAAATACGTGCCTCGTGCC
GTCCCTCGTCGATCTCGAACCTGGTACCATGGATGCCGTCCGCGCCGGTCCCTTTCCGGACAGCTCTTCCGCCCGA
CAACTTCGTTTTTCGGACAGTCTGGTGCCGAAACAACCTGGGCCAAGGGTCACTACACTGAGGGTATGTTTACA
ATGGCACTTCTGAGCTTCAGCTCGAGCGCATGAATGTCTACTTCAACGAGGTTTGTGTGCCCTCCCAACGCGT
TGCTTGATTTTCGTTGTGGATACTGACCGCATTTTCCAAAGGCCTCCGGCAACAAGTATGTTCTCGCGCCGTC
CTCGTCGATCTTGAGCCCGGTACCATGGATGCTGTCCGTGCCGGTCCCTTCGGTCAGCTCTTCCGTCCCGACAA
CTTCGTTTTTCGGTCAGTCCGGTGCCGGCAACAACCTGGGCCAAGGGTCACTACACTGAGGGTATAGACTCCATA
ATTTGTTGTTTCTTGTGGTGGGATAGTCGGTCTGTGGCAGGACTACGCCGGCTAGTCGACATGACATAC
GCTGCAGGTGTCATGCGCGACACAACCTGGATCGGGGAAGGCTAATGGCCTACGGGCTATGCTAATCCCGA
GTGCAGTCTGGTAGAGTATCTTCCAGGACGCATGTAGAGCGCGGAAAGGTGTGGGTGACTCTTCTGGGTAC
GCCTAGAAGGTTGCTTAAGGGACGTGCCAGACCCACGGGAAACCGTGCCGGATGCGAAGGACCTGCAGTCCA
GATCATCCGGGTGGCTCCGAGGCCGGGAGGAAATGCCCGAAGAGCCTGGTATACTATACTACATGGTATTC
GAATAGGGAATACATACGCTGGTACCTCCGACCTCCAGCTCGAGCGTATGAACGTGTACTTCAACGAGGTGTG
TGATGACCACACTGAAATATTTATTATTTATCGTTCCTAATCCCATAAGCTACAGGCTTCCGGTAAGAAATACG
TGCTCGTGCCGTCCTCGTCGATCTCGAACCTGGTACCATGGATGCCGTCCGCGCCGGTCCCTTTCCGGACAGCTC
TTCCGCCCGACAACCTTCGTTTTTCGGACAGTCTGGTGCCGAAACAACCTGGGCCAAGGGTCACTACACTGAGG
GTATGTTTACAATGGCACTTCTGAGCTTCAGCTCGAGCGCATGAATGTCTACTTCAACGAGGTTTGTGTGCC
TCCCAACGCGTTGCTTGATTTGTTGTGGATACTGACCGCATTTTCCAAAGGCCTCCGGCAACAAGTATGTT
CTCGCGCCGTCCTCGTCGATCTTGAGCCCGGTACCATGGATGCTGTCCGTGCCGGTCCCTTCGGTCAGCTCTTC
GTCCCGACAACCTTCGTTTTTCGGTCAGTCCG

Isolate 2

Host insect : *B. fulvicorne*

Organism identified : *Metarhizium* sp.

Sequence:

CGGCGGGAGTAACTATGACTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCGCATGAATGGA
TTAACGAGATTTCCACTGTCCCTATCTACTATCTAGCGAAACCACAGCCAAGGGAACGGGCTTGGCAGAATCA
GCGGGGAAAGAAGACCCTGTTGAGCTTACTCTAGTTTGTGAAAAGACATAGGAGGTGTAGAATAGG
TGGGAGCTTCGGCGCCGGTGAATAACCTACTCCTATTGTTTTTTACTTATTCAATGAAGCGGGGCTGGATT

TTCGTCCAACCTTCTGGTCTTAAGGTCCTTCGCGGGCTGACCCGGGTTGAAGACATTGTCAGGTGGGGAGTTTGG
CTGGGGCGGCACATCTGTTAAACCATAACGCAGGTGTCCTAAGGGGGGCTCATGGAGAACAGAAATCTCCAGT
AGAACAAAAGGGTAAAAGTCCCCTTGATTTTTCAGTGTGAATACAAACCATGAAAGTGTGGCCTATCG
ATCCTTTAGTCCCTCGACATTTGAGGCTAGAGGTGCCAGAAAAGTTACCACAGGGATAACTGGCTTGTGGCGG
CCAAGCGTTCATAGCGACGTCGCTTTTTGATCCTTCGATGTCCGCTCTTCTATCATACCGAAGCAGAATTCGGT
AAGCGTTGGATTGTTCAACCACTAATAGGGAACGTGAGCTGGGTTTAGACCTCTCCGTTGGTGAACCAGCGGA
GGATCATTACCGAGTTATCCAACCTCCCAACCCCTGTGAATTATACCTTTAATTGTTGCTTCGGCGGGACTCG
CGCCCGCCGGGACCCAAACCTTCTGAATTTTTTAATAAGTATCTTCTGAGTGGTTAAAAAATAATGAATCAA
AACTTTCAACAACGGACTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGTCAGTATTCTGGCGGGCATGCCTGTTTCG
AGCGTCATTACGCCCTCAAGTCCCCTGCGGACTTGGTGTGGGGATCGGCGAGGCTGGTTTTCCAGCACAGCC
GTCCCTTAAATTAATTGGCGGTCTCGCCGTGGCCCTCCTCTGCGCAGTAGTAAAGCACTCGCAACAGGAGCCCC
GCGCGGTCCACTGCCGTAACCC
CCAACCTTTTTATAGTTGACCTCGAATCAGGTAGGACTACCCGCTGAACTTAA

Isolate 3

Host insect : *C. sordidus*

Organism identified : *Paecilomyces* sp.

Sequence:

AGGGTCCATCGCAATGTGTTTTTTTTTTTTTTTTTCGGTCTCCCTCTCTACAGGTAGCCTATATAAATTACGAAGA
AGTTCCCCCTCTCCCATAGTCCCTCGATCTCGTAAACTGGTGGCAGTTCCCTGCTTCTGTCCGGCCAGTCATCGC
TGCACGCAGCCAACGACACCATGAGGATCAGCGGGTGGCAGGTTACCCTGGCCGCCTTGTGAGGCGTCTATGG
CCAGGAAGCTTACTCGCCGCCGAAATACCCCTCACCATGGGCCAACGGAGAGGGTGATTGGGCGATAGCGTAT
CAGAAAGCCGTCCAATTTGTTTCGCAGCTGAACCTGGCGGAGAAGGTCAATCTGACCACGGGGACTGGCTGGC
AACTAGGGCAATGCGTTGGTGGAGACTGGCAGCGTTCCTCGGCTGAACCTTCGTGGCCTCTGCTTCAAGATGGC
CCGCTGGGCATTGCTTCGCCGATTACATCTCCGATTTCCCGCCGGTATTAACGTCGGTGCCACCTGGGACCG
AAAGTGTGCTACTCGCGCGAAAGCCATGGGCGAGGAGAGCCGCGACAAGGGTATCGATGTCTTACTGGG
CCCCCTAGCCGGGCCCTGGGCAGATTCCCCGATGGTGGCCGCAACTGGGAGGGCTATTACCCGGATCCTGAG
GGTCCATCGCAATGTGTTTTTTTTTTTTTTTTTCGGTCTCCCTCTCTACAGGTAGCCTATATAAATTACGAAGAAG
TTCCCCCTCTCCCATAGTCCCTCGATCTCGTAAACTGGTGGCAGTTCCCTGCTTCTGTCCGGCCAGTCATCGCTG
CACGCAGCCAACGACACCATGAGGATCAGCGGGTGGCAGGTTACCCTGGCCGCCTTGTGAGGCGTCTATGGCC
AGGAAGCTTACTCGCCGCCGAAATACCCCTCACCATGGGCCAACGGAGAGGGTGATTGGGCGATAGCGTATCA
GAAAGCCGTCCAATTTGTTTCGCAGCTGAACCTGGCGGAGAAGGTCAATCTGACCACGGGGACTGGCTGGCAA
CTAGGGCAATGCGTTGGTGGAGACTGGCAGCGTTCCTCGGCTGAACCTTCGTGGCCTCTGCTTGAAGATGGCC
GCTGGGCATTGCTTCGCCGATTACATCTCCGATTTCCCGCCGGTATTAACGTCGGTGCCACCTGGGACCGAA
AGCTGTGCTACTGCGCGGAAAGGCCATGGGCGAGGAGCCGCGACAAGGGTATCGATGTCTTACTGGGCC
CTCAGCCGGGCCCTGGGCAGATTCCCCGATGGTGGCCGCAACTGGGAGGGCTATTACCCGGATCCTG

Isolate 4

Host insect : *C. formicarius*

Organism identified : *Beauveria* sp.

Sequence:

AGGGATCATTACCGAGTTTTCAACTCCCTAACCCCTTCTGTGAACCTACCTATCGTTGCTTCGGCGGACTCGCCCC
AGCCCGGACGCGGACTGGACCAGCGGCCCGCCGGGGACCTCAAACCTTGTATTCCAGCATCTTCTGAATACG
CCGCAAGGCAAAAACAAATGAATCAAAAACCTTTCAACAACCGATCTCTTGGCTCTGGCATCGATGAAGAACGCAG
CGAAATGCGATAAGTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCC
AGCATTCTGGCGGGCATGCCTGTTGAGCGTCATTTCAACCCTCGACCTCCCCTGGGGGAGGTGCGCGTTGGGG
ACCGGCAGCACACCGCCGCCCTGAAATGGAGTGGCGGCCCGTCCGCGGGGACCTCTGCGTAGTAATACAGCT
CGCACCGGAACCCCGACGCGGCCACGCCGTAACCAACCCAACTTCTGAACGTGACCTCGAATAGACTCCATAA
TTTGTGTTTCTTGTGGTGGGGATAGTCCGGTCTGTTGGCAGGACTACGCCGGCTAGTGCACATGACATACG
CTGCAGGTGTCATGCGGCGACACAACCTGGATCGGGGAAGGCTAATGGCCTACGGGCCTATGCTAATCCCGAG
TGCAGTCTGGTAGAGTGATCTTCCAGGACGCATGTAGAGCGCGGAAAGGTGTGGGTGACTCTTCTGGGTACG
CCTAGAAGGTTGCTTAAGGGACGTGCCAGACCCACGGGAAACCGTGCCGGATGCGAAGGACCTGCAGTCCAG
ATCATCCGGGTGGCTCCGAGGCCGGGAGGAAATGCCCGGAAGAGCCTGGTATACTATACCTACATGGTATTTCG
AATAGGGAA

Isolate 5

Host insect : *H. vigintioctopunctata*
Organism identified : *Fusarium moniliformae*

Sequence:

AGGGATCATTACCGAGTTTACAACCTCCCAAACCCCTGTGAACATACCAATTGTTGCCTCGGCGGATCAGCCCGC
TCCCGGTA AAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACCTTCTGAGTAAAACCAT
AAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAAAATGCGATAAG
TAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGC
ATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCCCCCGGGTTTGGTGTGGGGATCGGCGAGCCCTTGGCGCAA
GCCGGCCCCGAAATCTAGTGGCGGTCTCGCTGCAGCTTCCATTGCGTAGTAGTAAAACCCCTCGCAACTGGTACG
CGGCGCGGCCAAGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACT
TAACATTTGCGACGTACCAACACCCCCATCGGACGAGATGGTAAGCTGGCCAAACCTCGTCAGCTACACAACA
CCCATTGGGGTTTGGTGTGTCCTGCAGAAACGCCTGAGGGACAGGCTTGTGGTCTGGTCAAGAACCTGTCTCTG
ATGTGTTACGTGAGTGTGGGCTCTCTGCTGATCCTCTGATTGACTTCATGATCCACAGAGGTATGGAAGTGGT
TGAGGAGTATGAGCCAACAAGATACCCACACGCTACCAAGATTTTCGTC AACGGTAGCTGGGTTGGTGTTCAC
TCTGACCCCAAGCATCTTGTGCACCAAGTTTTGTCCACCCGACGAAAGAATGTCGTTCAATTCGAAGTGTCACT
TGTTTCGTGATATTCGAGACCGAGAATCAAGATCTTCTCTGATGCAGGCAGAGTCATGAGACCGGTCTTTACAG
TACAGCAGGAGGATGACGACGAGACTGGTGTTCAGAAGGGACAGCTTATACTGACCAAGGAGCTGGTAACCA
AGCTCGCCCAAGAGCAGGCGGAGCCATCTGATGATCCATCAGAGAAGCTCGGCTGGGAGGGTCTTGTTCGCGC
TGGAGTTATCGAGTATCTCGATGCCGAGGAAGAAGAAACGGCCATGATCTGCATGACGCCGGAAGATCTTGAA
CTTTACCGCGAGCAAAAAGAATGACGAGGCGACCCCTCACAGAGGAAGAGAGGGCGGGCTAAGCAAGAGGCGGA
GAAGAGAGAACAAGAGGAGGAACGCAACAAGAGATTGAAGACAAAGGTCAATCCTACGACTCATGTGTACAC
ACATTGTGAGATTCATCCCAGTATGATTCTTGGTATCTGTGCCAGTATCATTCCCTTCCCCGATCACACCAGGT
ATGTCAGGACGCTTAA

Isolate 6

Host insect : *M. circumdata*
Organism identified : *B. brongniartii*

Sequence:

CCTGCGGAGGGATCATTACCGAGTTTTCAACTCCCTAACCCCTTCTGTGAACCTACCTATCGTTGCTTCGGCGGA
CTCGCCCCAGCCCGGACGCGGACTGGACCAGCGGCCCGCCGGGACCTCAAACCTTGTATTCCAGCATCTTCT
GAATACGCCGCAAGGCAAAAACAATGAATCAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATGAAG
AACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATCCAGTGAATCATCGAATCTTTGAACGCACATTGC
GCCCGCCAGCATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCGACCTCCCCTTGGGGAGGTTCGGC
GTTGGGGACCGGCAGCACACCGCCGGCCCTGAAATGGAGTGGCGGCCCGTCCGCGGGCAGCTCTGCGTAGTAA
TACAGCTCGCACCGGAACCCCGACGCGGCCACGCCGTA AAAACACCCAACTTCTGAACGTTGACCTCGAATCAG
GTAGGACTACCCGCTGAACTTAAGCATATCCCTGCGGAGGGATCATTACCGAGTTTTCAACTCCCTAACCCCTT
TGTGAACCTACCTATCGTTGCTTCGGCGGACTCGCCCCAGCCCGGACGCGGACTGGACCAGCGGCCCGCCGGG
GACCTCAAACCTTGTATTCCAGCATCTTCTGAATACGCCGCAAGGCAAAAACAATGAATCAAACTTTCAAC
AACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATCCA
GTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTT
CAACCCTCGACCTCCCCTTGGGGAGGTTCGGCGTTGGGGACCGGCAGCACACCGCCGGCCCTGAAATGGAGTGG
CGGCCCGTCCGCGGGCAGCTCTGCGTAGTAATACAGCTCGCACCGGAACCCCGACGCGGCACGCCGTAAAACA
CCCAACTTCTGAACGTTGACCTCGAATCAGGTAGGACTACCCGCTGAACTTAAGCATATC

Isolate 7

Host insect : *O. rhinoceros*
Organism identified : *Metarhizium album*

Sequence:

CGAGTTACTACAACCTCCCAAACCCCTTGTGAACGTATACCTTTCCAGTTGCTTCGGCGGGTATAGCCCCGGGG
TCAGGTTTCGCAAGAGCCTGTCCGGAACAGGCGCCTGCCGGGGGACCAAAAACCTTGTATTTCTGTACGATAA
GGAATGTCTGAGTGGTTTATAGAAGAAAATGAATCAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGAT
GAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACA
TTGCGCCCCGCTAGTATTCTAGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCCCCGGCGGTTTGGTG
TTGGGGGGCCGGAATGGTTGTTGGGGGGCGATCTTTCGTCCCGCGCGCGCCGCCCCCGAAATAAATTGGCGGCC
TCGCCGCGGCTCCTCTGCGTAGTAACATGTTGCCCTTCCAACAGGAGCCGGCGCGGCACTGCCGTA AACACC

ACTTTTTT CACAAGTTGACCCAATCAGGAAGAATCCCCTACTTAGATTTCGAGTTACTACAACCTCCCAAACCCCCT
TGTGAACGTATACCTTTCCAGTTGCTTCGGCGGGTATAGCCCCGGGGTCAGGTTTCGCAAGAGCCTGTCCGGAAC
CAGGCGCTGCCGGGGGACCAAACTCTTGTATTTCTGTACGATAAGGAATGTCTGAGTGGTTTATAGAAGAA
AATGAATCAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT
AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCGTAGTATTCTAGCGGGCA
TGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCCCCGGCGGTTTGGTGTGGGGGGCCGGAATGGTTGTTGGGGG
GATCTCTTCGTCCCGCGCGCCGCCCCGAAATAAATTGGCGGCCTCGCCGCGGCTCCTCTGCGTAGTAACAT
GTTGCCCTTCCAACAGGAGCCGGCGCGGCACTGCCGTAACCACCACTTTTTT CACAAGTTGACCCAATCAGGA
AGAATCCCCTACTTAGAT T

Isolate 8

Host insect : *O. rhinoceros*

Organism identified : *Metarhizium anisopliae* var *majus*

Sequence:

ATGCATCTGTCTGCTCTTCTCACTCTTCTCCCAGCCGTTCTGGCTGCCCTGCCACTATTGGCCGGCGCGCTGAG
CCAGCTCCTCTTCACTCCTCAGGCTGAGAGCATATTGCCGACAAGTATATTGTCAAGTTCAAGGATGATAT
TGCCCGTATCGCTACCGATGATACGGTGAGCGCTTTACCTCCAAAGCCGACTTCGTTTACGAGCACGCCTTCC
ATGGGTTTTGCAGGCTCCCTCACCAAGGAGGAGCTGAAGATGCTTCGTGAGCACCCCGGTGTAAGCACCCCTC
CCACTTACCTAGGTAGTCAAAGGAGACATGTAGTTGTTTGTTCCTGACCCGCTACGCCATAGGTTGATTTTATT
GAGAAGGACGCTGTGATGCGTATCAGCGGCCTCACTGAGCAGAGCGGTGCTCCCTGGGGTCTTGGGCGCATCT
CTCACCGCAATAGGGGAAGCACCACCTATCGCTACGATGATAGTGCTGGTGAGGGTACTTGCATATATATCATT
GACACTGGTATTGAGGCCTCCCACCCCGTAAGTTGTGCCGCCAAAACCTCCATAGGGCGGAGTAGGAAATTTAA
CAATATCATCCAGGAGTTTGAGGGTCGCGCCACTTTTCTTAGGAGCTTCATCAGCGGTCAAGAACTGATGGCC
ACGGCCATGGGACTCACTGCGCTGGTACCATTGGTAGCAAAAGCTACGGTGTGGCCAAAAGGCTAAGCTCTA
TGGTGTCAAGTTCTTGACAACCAGGGCAGTGGTTTCTACTCCGGTATCATCAGTGGCATGGACTACGTTGCCA
GTGACTCCAAGACCCGCGGCTGCCCAAGAGGCCCAATTGCTTCCATGAGCCTGGGAGGTGGCTACTCGGCGTC
CGTCAACCAAGGTGCTGTCTTGGTGAATTCGGGTGTCTTCCCTGCGCTCGCCGCTGGCAACGATAACCCGGG
ATGCCAGAACACCTTCCCGCTTCCGAGCCTTCTGCCTGCCTGACTGTTGGTGCCACTGATTCAAGTGACAGACGA
TCTTCTTCTCCAACCTTCGGCAGAGTTGTCGATATTTTCGCTCCTGGTACC GGTTCTTTCCACCTGGATTGGT
GGCAGCACTGTAAGTATTGTACCTACCTCGATAAGCTTAGAGACAGGCTTTTGTCTCAGAACCAGCTCAAAAG
GTTTAGAACACCATCTCTGG
TACCTCCATGGCTACTCCCCATATTGCCGGTCTGGCTGCCTACCTCAGTGCCTCCAAGGCAAGACTACCCCTG
CCGCTCTTTGCAAGAAGATCCAGGACACTGCTACCAAGAACGCGCTCACCGGTGTTCCCTCTGGCACTGTCAAC
TA CTTGCCTAC AACGGCAACG GTGCCTAA

Isolate 9

Host insect : *O. rhinoceros*

Organism identified : *Metarhizium* sp.

Sequence:

AGGGATCATTACCGAGTTATCCAACCTCCCAACCCCTGTGAATTATACCTTTAATTGTTGCTTCGGCGGGACTTC
GCGCCCGCCGGGGACCCAAACCTTCTGAATTTTTTAATAAGTATCTTCTGAGTGGTTAAAAAATAATGATCAA
AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATT
GCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCGTAGTATTCTGGCGGGCATGCCTGTTTCG
AGCGTCATTACGCCCTCAAGTCCCCTGTGGACTTGGTGTGGGGATCGGCGAGGCTGGTTTTCCAGCACAGCC
GTCCCTTAAATTAATTGGCGGTCTCGCCGTGGCCCTCCTCTGCGCAGTAGTAAAGCACTCGCAACAGGAGCCCCG
GCGCGTCCACTGCCGTAACCCCAACTTTTTATAGTTGACCTCGAATCAGGTAGGACTACCCGCTGAACT
TATCTCCGTTGGTGAACCAGCGGAGGATCATTACCGAGTTATCCAACCTCCCAACCCCTGTGAATTATACCTTT
AATTGTTGCTTCGGCGGGACTTCGCGCCCCGGGGACCCAAACCTTCTGAATTTTTTAATAAGTATCTTCTGA
GTGGTTAAAAAATAATGAATCAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC
GAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCGTCA
GTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTACGCCCTCAAGTCCCCTGCGGACTTGGTGTGGGGATCG
GCGAGGCTGGTTTTCCAGCACAGCCGTCCCTTAAATTAATTGGCGGTCTCGCCGTGGCCCTCCTCTGCGCAGTA
GTAAAGCACTCGCAACAGGAGCCCGCGCGGTCCACTGCCGTAACCCCAACTTTTTTATAGTTGACCTCG
AATCAGGTAGGACTACCCGCTGAACTTAA

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