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# Isolation and Identification of Endophytic Fungi of *Rauwolfia serpentina* (L.) Benth. Ex Kurz by DNA Barcoding

Neetu Das<sup>1</sup>, Tanushree Chatterjee<sup>2</sup>

<sup>1</sup>Department of Microbiology, Govt. V.Y.T.PG. Autonomous College, Durg, Chhattisgarh, 491001, India.

<sup>2</sup>Department of Biotechnology, Raipur Institute of Technology, Raipur, Chhattisgarh, 492010, India

Corresponding author E mail-[microdept.07@gmail.com](mailto:microdept.07@gmail.com)

## ABSTRACT

Endophyte fungi are getting importance now a days for alternative source of valuable therapeutic compounds during their growth with host plants. Reports are available for possible association of endophytic microorganisms including bacteria, actinomycetes and fungi in different known plants having medicinal properties. *Rauwolfia serpentina* is a well-known Indian medicinal plant used in Ayurveda for treatment of snakebite, feverish illness and mental illness from ancient time and known for its antimicrobial, anti-inflammatory, antioxidants, antiproliferative, anticancerous, anti-diuretics, antifibrillar, anti-arrhythmic, anti-cholinergic, anti-dysentery, anti-diarrheal and anti-hypotensive properties. In the present study, an attempt was made to isolate endophytic fungi from leaves and stem parts of healthy young plant of *Rauwolfia serpentina* under laboratory conditions. Altogether 05 isolates namely *Aureobasidium melanogenum*, *Aureobasidium pullulans*, *Aureobasidium iranianaum*, *Simplicillium obclavatum* and *Phoma herbarum* were obtained and identified through 18 S r RNA gene sequencing analysis. Isolated fungi were identified by sequence-based DNA Barcoding by using ITS region. Endophytic fungal DNA have been submitted to NCBI, Gene Bank and obtained Accession number for reference for further work and its phylogenetic tree was constructed by using MEGA 5.2 Software.

**Keywords:** *Rauwolfia serpentina*, Endophytic fungi, DNA Barcoding, ITS region, Phylogenetic tree

**Abbreviations:** 18 s rRNA, 18 S ribosomal ribonucleic acid; DNA, Deoxyribonucleic acid; ITS, Internal transcribed Spacer; NCBI, National Center for Biotechnology Information; MEGA, Molecular Evolutionary Genetics Analysis

## 1. INTRODUCTION

*Rauwolfia serpentina* is used as medicinal plant from ancient time for its medicinal properties. It belongs to family Apocynaceae. It is reported that powdered root of this plant has been used for treatment of snakebite, feverish and mental illness in Ayurvedic literature (Singh et al., 2016). This plant has immense therapeutic values against various diseases (Pathania et al., 2015) and have antimicrobial, antioxidant, anti-inflammatory, anticancerous, antidysentery, antiarrhythmic, anticholinergic, antidysentery, antidiarrheal, antihypotensive properties (Pal & Paul, 2020).

The various alkaloids identified in *Rauwolfia serpentina* include ajmaline, ajmalimine, ajmalicine, deserpidine, indobine, indobinine, reserpine, reserpiline, rescinnamine, rescinnamidine, serpentine, serpentinine and yohimbine etc. (Bunkar, 2017). Therapeutic properties are due to presence of various phytochemicals such as alkaloids, flavonoids, reserpine, serpentine etc. in the plant extracts (Gambhir et al., 2020). It is reported that 12.4 % of alkaloids and 7.35% of saponins are present in plant sample (Hussain et al.,) (Singh et al., 2016).

Various micro-organisms reside symbiotically mutualistic association with their host plants and reside in outer as well as internal soft tissues without any harmful effect to the host plant (Saikkonen et al., 1998). They may be bacteria, actinomycetes, fungi, yeasts etc. These microorganisms are known as endophytes. Endophytic fungi are one of them that protects their host from drought, unfavourable conditions and

pathogenic invaders. They produce a number of novel bioactive compounds like alkaloids, peptides, steroids, terpenoids, phenols, quinines and flavonoids which are beneficial for host growth and survival in unfavourable conditions. These compounds are useful in agriculture, industries and pharmaceutical industries for the production of medicine, drugs and natural biochemicals (Sahu et al., 2016).

It is reported that these endophytes may produce same bioactive compounds of their host. They can produce enzyme (Khan et al., 2016), plant growth hormones, alkaloids, non-ribosomal peptides, due to the presence of biosynthetic gene clustered in their genome (Li et al., 2016). For obtaining these secondary metabolites for antimicrobial, agricultural and pharmaceutical uses, isolations of endophytic fungi from leaves and stems of *Rauwolfia serpentina* was the objective of this study.

DNA Barcoding is a technique for characterizing species of organisms using a short DNA sequence from a standard and agreed- upon position in the genome. Species identification through barcoding is usually achieved by the retrieval of a short DNA sequence- the barcode- from a standard part of the genome from the specimen under investigation. The barcode sequence from each unknown specimen is then compared with library of reference barcode sequences derived from individuals of known identity. A specimen is identified if its sequence closely matches one in the barcode library.

The DNA barcode for fungi is Internal Transcribed Spacer regions (ITS 1,5.8 and ITS 2) gene sequence collectively called as ITS region for species identification (Senthilkumar et al., 2018). Some isolated fungal species in culture media is sticky, non-cottony in appearance, they have existed in anamorphic stage and we face difficulty in authentic identification of that specific fungal sample so we need 18 S ribosomal RNA sequencing. In this study we have isolated endophytic fungi from medicinal plant *Rauwolfia serpentina* to explore the unknown endophytic fungal diversity of this plant and identified by DNA barcoding and constructed phylogenetic tree by using MEGA 5.2 Software.

## **2.MATERIALS AND METHODS**

### **2.1 Selection of Plant and Sample collection**

*Rauwolfia serpentina* is a well-known medicinal plant of family Apocynaceae.

Healthy leaves and stems were collected as sample of *Rauwolfia serpentina* (L.) Benth. ex Kurz. from the Herbal Plant Nursery, Mana, Raipur, Department of Forest, Govt. of Chhattisgarh, India in July 2019.

### **2.2 Isolation of Endophytic fungi**

Stems and Leaves were washed under running tap water until the surface adherents are removed. Leafy parts of fresh healthy *Rauwolfia serpentina* plants were cut into small pieces (5 mm × 5 mm) using sterile surgical blade and washed with sterile distilled water. The samples were surface sterilized by dipping into 4% sodium hypochlorite for 5 minutes and 96% ethanol for 1 minute, rinsed with sterile water and allowed to surface dry under sterile conditions. The surface sterilized samples were placed on Potato Dextrose Agar (PDA) plates amended with 50 mg/L tetracycline antibiotic to suppress the bacterial growth and incubated at 28°C to 30 °C for 2 to 3 days. The outer bark of the stem was removed with sterile sharp blade and cut in to small pieces of inner part of the stem and inoculated on PDA media (Senthilkumar et al., 2018).

Inoculated plates were incubated at 28 °C till vegetative growth appears from inoculated tissues.

The hyphal tip of endophytic fungi growing out from the plant tissue was transferred to fresh PDA plates amended with 50 mg/L tetracycline. After incubation at 28 °C for 7 to 14 days, purity of the culture was determined by colony morphology.

### **2.3.3 Microscopic study of isolated fungal strains**

Endophytic fungi isolated from *Rauwolfia. serpentina* were coded as S1, S2, S3, S4 and S5 Sample and studied under compound microscope after staining with Lactophenol cotton blue stain.

### **2.3.4 Molecular identification**

### **Fungal Genomic DNA Extraction and 18 s ribosomal RNA sequencing**

Isolated cultures were inoculated in PDB Broth at 28 °C for 7 days. After 7 days harvested cultures were centrifuged and pellets were collected. To the pellets, 50 ml. of lysis buffer (20 ml. tris HCl 400mM, 6 ml EDTA 60Mm, 0.7 ml NaCl 150 mM, 0.5 gm SDS 1%, 22.8 ml MQ) were added, mixed and incubated at room temperature for 40 min. Chloroform and isoamyl alcohol in the ratio of 24:1 to the above mixture added, centrifuged at 10000 rpm for 5 min. Supernatant were collected and added equal volume isopropyl alcohol, mixed and centrifuged at 10000 rpm for 7 min. .Supernatant were discarded and pellets were resuspended with 75 % ethanol , again centrifuged at 10000 rpm for 7 min. . 75% ethanol were discarded and pellets were oven dried and MQ water were added. Hence fungal DNA were obtained (Tripathy et al., 2017). Extracted Fungal DNA has been sent to Bio serve Biotechnologies (India) Pvt. Limited, Mallapur, Hyderabad, Telangana for molecular identification through 18 S Ribosomal RNA sequencing. The identification was confirmed solely based on the gene sequencing methodology using 18S RNA primer and primer synthesized by using Internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence. Investigated fungi were identified by subjecting the DNA sequence to BLASTn analysis and by using MEGA 5.2 Software, phylogenetic tree was constructed.

### **RESULTS AND DISCUSSION**

Five endophytic fungi were isolated from the different parts (leaves and stems) of the *Rauwolfia. serpentina* collected from Herbal Plant Nursery, Mana, Raipur, Department of Forest, Govt. of Chhattisgarh, India in July 2019 as depicted in table 1. A total 32 segments (leaf 16, stem 8) of *Rauwolfia. serpentina* were processed for the isolation of endophytic fungi. Isolated Five endophytic fungi were identified as *Aureobasidium melanogenum*, *Aureobasidium iranianum* *Aureobasidium pullulans*, *Simplicillium obclavatum* and *Phoma herbarum*.

#### **Molecular Phylogenetic analysis by Maximum Likelihood method**

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [1]. The tree with the highest log likelihood (-3394.3534) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and Bio NJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The optimal tree with the sum of branch length = 12.25508551 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The analysis involved 5 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 491 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011)

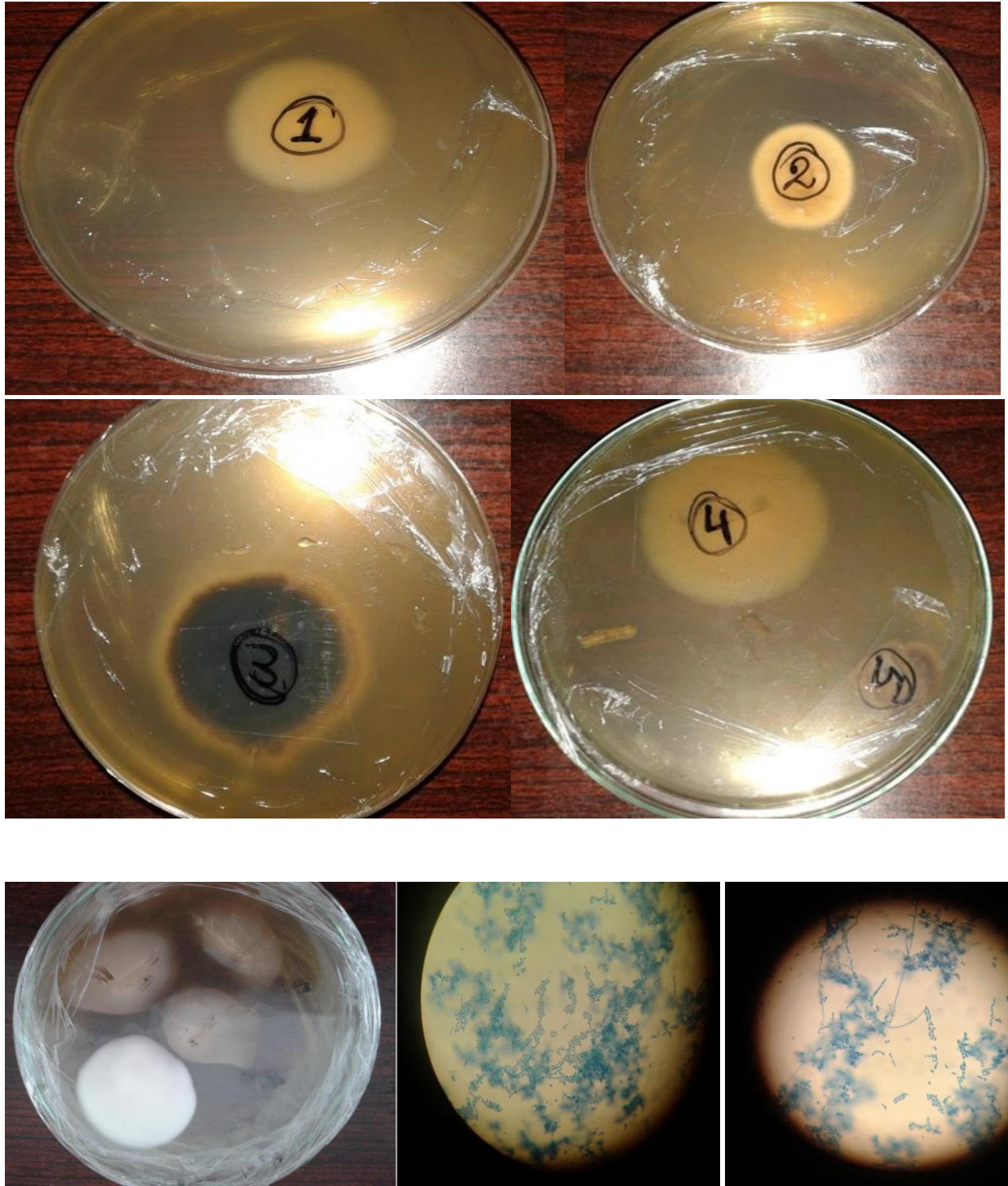


Figure 1. Fungal cultures of S1, S2,S3,S4,S5- in PDA plates and Microscopic images of Sample S2

Table 1: List of Isolated endophytic fungi

Sr.	Fungal Culture code	Identified fungi	Class
1	S1	<i>Aureobasidium melanogenum</i>	Dothideomycetes
2	S2	<i>Simplicillium obclavatum</i>	Sordariomycetes
3	S3	<i>Phoma herbarum</i>	Dothideomycetes
4	S4	<i>Aureobasidium pullulans</i>	Dothideomycetes
5	S5	<i>Aureobasidium iranianum</i>	Dothideomycetes

### Sanger Sequences of five fungal samples

#### Sample 1 *Aureobasidium melanogenum* strain,

>MK035871.1:1-530 *Aureobasidium melanogenum* clone AB1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence  
 ATACGGTGCTCAGCGCCCGACCTCCAACCCTTTGTTGTTAAACTACCTTGTTGCTTT  
 GGCGGGACCGCTCGGTCTCGAG  
 CCGCTGGGGATTCGTCCCAGGCGAGCGCCCGCCAGAGTTAAACCAAACCTCTTGTTAT  
 TAAACCGGTCGTCTGAGTTAAAA  
 TTTTGAATAAATCAAACCTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGA  
 ACGCAGCGAAATGCGATAAGTAA  
 TGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTG  
 GTATTCCGAGGGGCATGCCTGTT  
 CGAGCGTCATTACACCACTCAAGCTATGCTTGGTATTGGGTGCCGTCCTTAGTTGGG  
 CGCGCCTTAAAGACCTCGGCGAG  
 GCCTCACCGGCTTTAGGCGTAGTAGAATTTATTCGAACGTCTGTCAAAGGAGAGGAC  
 TTCTGCCGACTGAAACCTTTTAT  
 TTTTCTAGGTTGACCTCGGATCAGGTAGGGATAACCGCTGAACTTAAGCA

#### Sample 2 *Simplicillium obclavatum* strain,

>MK035872.1:1-491 *Simplicillium obclavatum* clone AB2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence

CCCTTTGTGACCTACCTTTATGTTGCTTCGGCGGTGACGCGCCGGGTTGCTCCCTCAG  
 GGAGCTCCCGGGACCACGCGCC

CGCCGGAGACCACAAACTCTTGATTTTGGCGAAAGCAGTATTCTTCTGAGTGGCCGAA  
AGGCAAAAAACAAATGAATCAA

AACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCG  
ATAAGTAATGTGAATTGCAGAAT

TCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCA  
TGCCTGTTTCGAGCGTCATTTCAA

CCCTCGAGCTCGTCTTCATTGACGAGATCGGTGTTGGGACCCGGCGATCGGGGACTT  
TAGTTCCTCTGCCGGTCCTGAAA

TTCAGTGGCGGCCCGTTGCGGCGACCTCTGCGTAGTAACTTAACCTCGCACTGGGAC  
AGCAGCGCGGCCACGCCGTAATA

CCCCCGACTTT

### **Sample 3 *Phoma herbarum* strain**

>MK035873.1:1-525 *Phoma herbarum* clone AB3 internal transcribed spacer 1, partial sequence;  
5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large  
subunit ribosomal RNA gene, partial sequence

TGAGACCGATCATCTAGAGTGCGAGCTTTGCCTGCCATCTCTTACGCCATGTCTTTTG  
AGTACCTTACGTTTCCTCGGCG

GGTCCGCCCGCCGATTGGACAATTTAAACCACTTGCAGTTGCAATCAGCGTCTGAAA  
AAACTTAATAGTTACAACCTTCA

ACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTA  
GTGTGAATTGCAGAATTCAGTGAA

TCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCATGGGGCATGCCTGTTC  
GAGCGTCATTTGTACCTTCAAG

CTTTGCTTGGTGTGGGTGTTTGTCTCGCCTTTGCGCGCAGACTCGCCTCAAAACAAT  
TGCGAGCCGGCGTATTGATTC

GGAGCGCAGTACATCTCGCGCTTTGCACTCAGAACGACGACGTCCAAAAGTACATTT  
TTACACTCTTGACCTCGGATCAG

GTAGGGATACCCGCTGAACTTAAGCATATCAAAAAGCCGGGAGGAA

### **Sample 4 *Aureobasidium pullulans* strain**

>MK035874.1:1-655 *Aureobasidium pullulans* clone AB4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GGGTGCTCAGCGCCCGACCTCCAACCCTTTGTTGTTAAAACTACCTTGTTGCTTTGGC  
GGGACCGCTCGGTCTCGAGCCG

CTGGGGATTTCGTCCCAGGCGAGCGCCCGCCAGAGTTAAACCAAACCTCTTGTTATTTA  
ACCGGTCGTCTGAGTTAAAATTT

TGAATAAATCAAACTTTCAACAACGGATCTCTTGTTTCTCGCATCGATGAAGAACG  
CAGCGAAATGCGATAAGTAATGT

GAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGA  
TTCCGAGGGGCATGCCTGTTCTGA

GCGTCATTACACCACTCAAGCTATGCTTGGTATTGGGCGTCGTCCTTAGTTGGGCGC  
GCCTTAAAGACCTCGGCGAGGCC

ACTCCGGCTTCAGGCGTAGTAGAATTTATTCGAACGTCTGTCAAAGGAGAGGAACTC  
TGCCGACTGAAACCTTTATTTTT

CTAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAAGC  
GGAGGAAAGGATCATTAAGAGT

AAGGGTGCTCAGCGCCCGACCTCCAACCCTTTGTTGTTAAAACTACCTTGTTGCTTTG  
GCGGGACCGCTCGGTCTCGAGC

GCTGGGGATTTCGTCC

**Sample 5** *Aureobasidium iranianum*

>MK035875.1:1-567 *Aureobasidium iranianum* clone AB5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

AAAGAGTAAGGGTGCTCAGCGCCCGACCTCCAACCCTCTGTTGTTAAAACTACCTTG  
TTGCTTTGGCGGGACCGCTCGGT

CCCGAGCCGCGGGGATCCGTCCCAGGCGAGCGCCCGCCAGAGTCCAACCAAACT  
CTTGTTAATCGTAACCGGTCGTCT

GAGTAAAAATTTTTGAATAAATCAAACTTTCAACAACGGATCTCTTGTTTCTCGCA  
TCGATGAAGAACGCAGCGAAATG

CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATT  
GCGCCCCTTGTTATTCCGAGGGG

CATGCCTGTTTCGAGCGTCATTACACCACTCAAGCTATGCTTGGTATTGGGTGCCGTC  
CCCTTCCACGGGGGGCGCGCTC

AAAGACCTCGGCGAGGCCTCACCGGCTTTAGGCGTAGTAGAATTTATTCGAACGTCT  
GTCAAAGGAGAGGACTTCTGCCG

ACCGAAACCTTTTATTTTTTCTAGGTTGACCTCGGATCAGGTAGGGATACCCGCTG  
 AACTTAAGCATATCAATAAGCGG  
 AGGAAAA

### After BLASTn of *Aureobasidium melanogenum*

Other reports: [Distance tree of results](#) [MSA viewer](#) [Filter](#) [Reset](#)

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments

select all 100 sequences selected

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<a href="#">Aureobasidium melanogenum clone AB1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ...</a>	<i>Aureobasidium ...</i>	979	979	100%	0.0	100.00%	530	<a href="#">MK035871.1</a>
<a href="#">Aureobasidium melanogenum strain TN1-2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RN...</a>	<i>Aureobasidium ...</i>	979	979	100%	0.0	100.00%	565	<a href="#">MF370938.1</a>
<a href="#">Fungal sp. isolate NWUA24 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and int...</a>	<i>fungal sp.</i>	974	974	99%	0.0	100.00%	546	<a href="#">ON261432.1</a>
<a href="#">anogenum strain BEL102 internal transcribed spacer 1, partial sequence; 5.8S ribosomal R...</a>	<i>Aureobasidium ...</i>	972	972	99%	0.0	100.00%	550	<a href="#">MT355631.1</a>

Windows taskbar: 33°C Sunny, 21-02-2023

### Blast Tree view tree of *Aureobasidium melanogenum*

BLAST RID: Z8W9683X016 | Query ID: MK035871.1 | Database: nt

Tree method: Fast Minimum Evolution | Max Seq Difference: 0.1 | Sequence Label: Sequence Title (if avail.)

Find: | all | + TXT | Tools | Upload | ?

Mouse over an internal node for a subtree or alignment. Click on tree label to select sequence to download

- Aureobasidium sp. isolate SQUCC\_2264 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene...
- ascomycete fungi | 13 leaves
- Aureobasidium pullulans genes for SSU rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, LSU rRNA, partial and complete sequence, strain: kz24
- ascomycete fungi | 12 leaves
- Aureobasidium pullulans genes for SSU rRNA, ITS1, 5.8S rRNA, ITS2, LSU rRNA, partial and complete sequence, strain: kz25
- Aureobasidium sp. genes for ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, strain: GY113330PS
- Aureobasidium sp. genes for ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, strain: GY1131110PS
- Aureobasidium sp. genes for ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, strain: GY113451PS
- Aureobasidium sp. genes for ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, strain: GY1131112PS
- Uncultured fungus clone ZSH201108-12 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; a...
- Aureobasidium sp. strain BEL38 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
- Aureobasidium sp. strain BEL54 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
- Aureobasidium sp. strain BEL52 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
- Multiple organisms | 65 leaves

Success | Nodes 201(0 selected) | View port at (0,0) of 1178x312 | 0.0006

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## After BLASTn of *Aureobasidium pullulans*

Sequences producing significant alignments

Download Select columns Show 100

Select all 100 sequences selected

GenBank Graphics Distance tree of results MSA Viewer

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Aureobasidium pullulans clone AB4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Aureobasidium p...	1210	1545	100%	0.0	100.00%	655	MK035874.1
Aureobasidium pullulans strain YY20 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Aureobasidium p...	1210	1653	100%	0.0	100.00%	982	KR912253.1
Aureobasidium pullulans strain YY23 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene a...	Aureobasidium p...	1138	1515	100%	0.0	98.61%	1006	KR912256.1
Aureobasidium pullulans isolate AD341 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Aureobasidium p...	1129	1399	100%	0.0	99.36%	623	MN922114.1
Yarrowia sp. 3/S/S/17/2 18S ribosomal RNA, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RN...	Yarrowia sp. 3/S/...	1122	1436	100%	0.0	98.14%	645	JX040865.1
Aureobasidium pullulans isolate AD294 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Aureobasidium p...	1099	1334	100%	0.0	99.83%	599	MN922105.1
Aureobasidium pullulans isolate 73JAN internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Aureobasidium p...	1088	1403	99%	0.0	98.54%	653	MW723769.1
Aureobasidium pullulans isolate AD331 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Aureobasidium p...	1064	1224	99%	0.0	99.66%	582	MN922111.1
Aureobasidium pullulans isolate AD275 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Aureobasidium p...	1064	1209	98%	0.0	98.51%	602	MN922099.1
Aureobasidium pullulans isolate AD291 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Aureobasidium p...	1062	1209	98%	0.0	98.99%	592	MN922102.1
Aureobasidium pullulans isolate AD279 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Aureobasidium p...	1062	1208	98%	0.0	98.83%	597	MN922101.1

## Blast Tree view tree of *Aureobasidium pullulans*

BLAST®

Blast Tree View

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This tree was produced using BLAST pairwise alignments. more...

Reset Tree

BLAST RID ZBXAVZCC013 Query ID MK035874.1 Database nt

Tree method Fast Minimum Evolution Max Seq Difference 0.75 Sequence Label Sequence Title (if avail.)

Find: all - + TXT

budding yeasts and ascomycete fungi | 2 leaves

- Aureobasidium pullulans isolate AD294 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, ...
- Aureobasidium pullulans strain XJ-96 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer ...
- Aureobasidium pullulans strain KM15 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and...
- Aureobasidium pullulans genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene, strain TSN-43
- Aureobasidium pullulans isolate SO internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, ...
- Aureobasidium pullulans genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene
- Aureobasidium pullulans strain CL-2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2...
- Aureobasidium pullulans strain ISR2\_10 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RN...
- Uncultured Aureobasidium genomic DNA sequence contains ITS1, 5.8S rRNA gene and ITS2, clone 09\_A3
- Dothideomycetes sp. BLD6 genomic DNA containing ITS1, 5.8S rRNA gene, ITS2, strain BLD6

ascomycete fungi and fungi | 80 leaves

- Aureobasidium pullulans genomic DNA containing ITS1, 5.8S rRNA gene, ITS2, strain BLE6

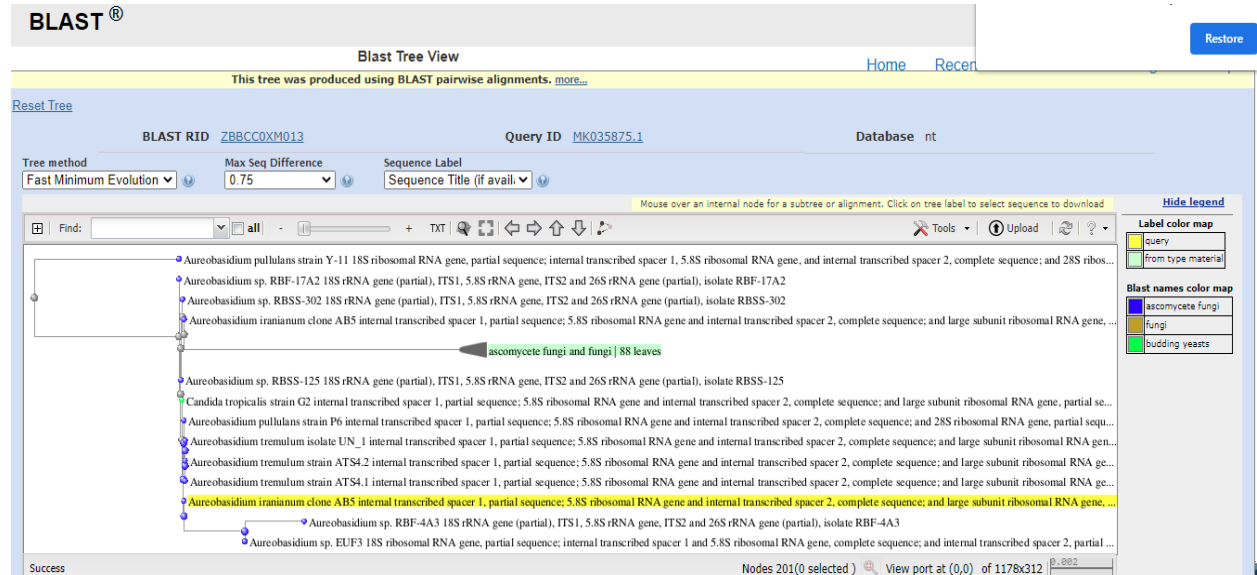
ascomycete fungi | 8 leaves

Success Nodes 201(0 selected) View port at (0,0) of 1178x312

## Blast Tree view tree of *Aureobasidium iranum*

Descriptions		Graphic Summary	Alignments	Taxonomy				
Sequences producing significant alignments								
<input checked="" type="checkbox"/> select all 100 sequences selected		<a href="#">Download</a> <a href="#">Select columns</a> Show 100						
		<a href="#">GenBank</a> <a href="#">Graphics</a> <a href="#">Distance tree of results</a> <a href="#">MSA Viewer</a>						
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/> Aureobasidium iranum clone AB5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene...	Aureobasidium i...	1048	1048	100%	0.0	100.00%	567	MK035875.1
<input checked="" type="checkbox"/> Aureobasidium sp. RBF-17A2 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 26S rRNA gene (par...	Aureobasidium s...	1048	1048	100%	0.0	100.00%	1187	FN665420.1

## BLAST TREE VIEW



## After BLASTn of *Simplicillium obclavatum*

Descriptions		Graphic Summary	Alignments	Taxonomy				
Sequences producing significant alignments								
<input checked="" type="checkbox"/> select all 100 sequences selected		<a href="#">Download</a> <a href="#">Select columns</a> Show 100						
		<a href="#">GenBank</a> <a href="#">Graphics</a> <a href="#">Distance tree of results</a> <a href="#">MSA Viewer</a>						
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/> <i>Simplicillium obclavatum</i> clone AB2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, co...	<i>Simplicillium obcl...</i>	907	907	100%	0.0	100.00%	491	MK035872.1
<input checked="" type="checkbox"/> <i>Simplicillium obclavatum</i> strain UAS_046 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gen...	<i>Simplicillium obcl...</i>	907	907	100%	0.0	100.00%	558	FJ156235.1

## BLAST TREE VIEW

**BLAST**<sup>®</sup> Chrome didn't shut down correctly. [Restore](#)

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This tree was produced using BLAST pairwise alignments. [more...](#)

**Reset Tree**

BLAST RID: ZBBXBVF5013      Query ID: MK035872.1      Database: nt

Tree method: Fast Minimum Evolution      Max Seq Difference: 0.75      Sequence Label: Sequence Title (if avail.)

Find:       all      +      TXT           Upload      Help

Mouse over an internal node for a subtree or alignment. Click on tree label to select sequence to download

- Simplicillium obclavatum strain CUPD05 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spa...
- Simplicillium obclavatum strain AQF16 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spac...
- Simplicillium obclavatum isolate UHSB-S4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed s...
- Simplicillium obclavatum isolate UHSB-S2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and i...
- Simplicillium obclavatum strain SGE30 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, a...
- Simplicillium sp. SGE13 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete s...
- Simplicillium obclavatum strain SO1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2...
- Simplicillium obclavatum genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene
- ▶ Multiple organisms | 78 leaves
- ▶ ascomycete fungi | 3 leaves
- ▶ Ustilago kamerunensis strain MUR003 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer...
- ▶ Ustilago kamerunensis strain NAK002 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer...
- ▶ Simplicillium obclavatum genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2, 28S rRNA gene
- ▶ ascomycete fungi and smut fungi | 9 leaves

Success Nodes 201(0 selected)      View port at (0,0) of 1178x312      8.881

### After BLASTn of *Phoma herbarum*

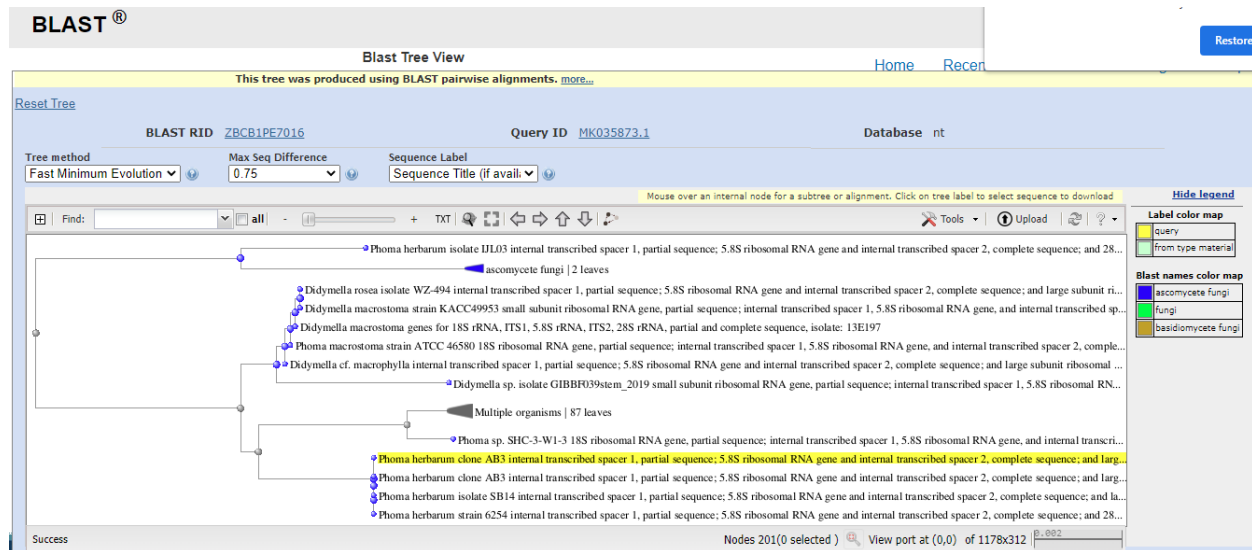
**Descriptions**      Graphic Summary      Alignments      Taxonomy

**Sequences producing significant alignments**      Download ▾      Select columns ▾      Show 100 ▾      ⓘ

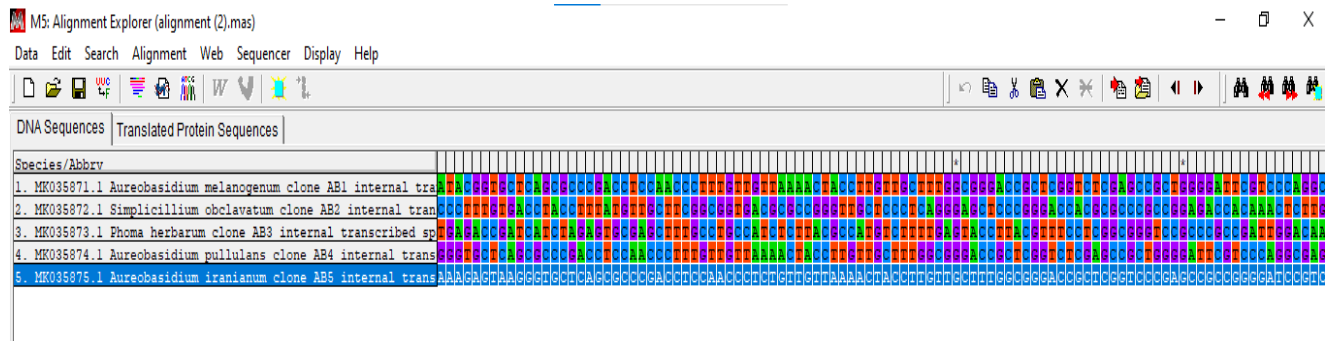
select all      100 sequences selected      [GenBank](#)      [Graphics](#)      [Distance tree of results](#)      [MSA Viewer](#)

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	<a href="#">Phoma herbarum clone AB3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and intern...</a>	<a href="#">Phoma herbarum</a>	970	970	100%	0.0	100.00%	525	<a href="#">MK035873.1</a>
<input checked="" type="checkbox"/>	<a href="#">Phoma herbarum isolate SB14 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inte...</a>	<a href="#">Phoma herbarum</a>	970	970	100%	0.0	100.00%	525	<a href="#">QM943864.1</a>
<input checked="" type="checkbox"/>	<a href="#">Phoma herbarum strain 6254 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inter...</a>	<a href="#">Phoma herbarum</a>	970	970	100%	0.0	100.00%	525	<a href="#">JN903924.1</a>

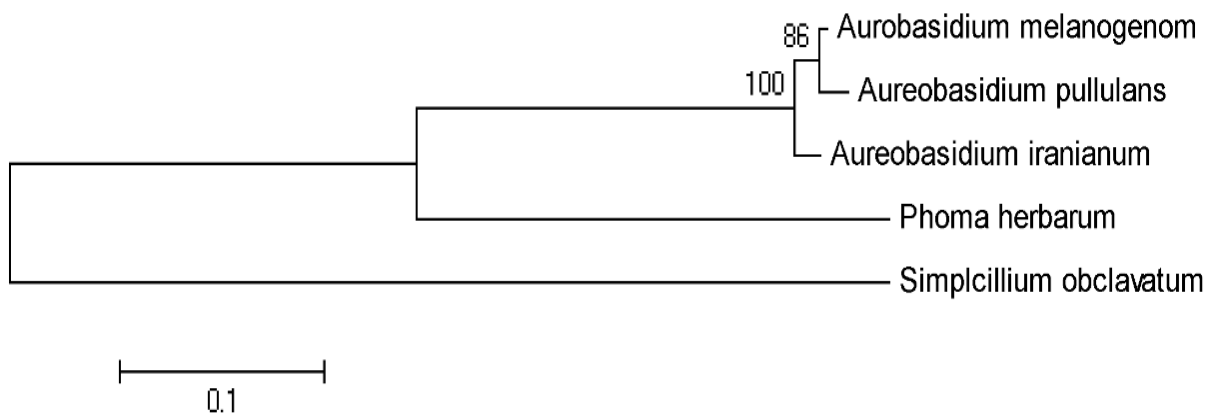
### BLAST TREE VIEW of *Phoma herbarum*



## Multiple Alignment of identified fungal strains



## Phylogenetic Tree of identified fungal strains using MEGA 5.2



## CONCLUSION

From fresh and healthy leaves of *Rauwolfia serpentina* 03 species of *Aureobasidium* were isolated of family *Aureobasidium*, 01 species of family *Simplicillium* and 01 species of family *Phoma*. These species were identified by 18 S Ribosomal RNA sequencing. In some research studies *Aureobasidium pullulans* produce secondary metabolites auxin, Exopolysaccharides pullulans which have plant growth promoting properties and bioremediation in Cadmium and Lead. New antifungal and antiviral peptides were isolated from *Simplicillium* species. These reports will help for further research to produce useful bioactive chemical substances from pure isolated fungi as well as bioremediation of heavy metals to reduce environmental pollutions.

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## CONFLICT OF INTERESTS

The authors have no potential conflict of interest regarding publication of the said manuscript.

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