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Morphological Characterization and Segregation of *penicillium pimiteouiense* from Soil Samples

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Abstract

Penicillium is the most common fungal species found in most of the environs.Replicates of *Penicillium pimiteouiense* were recovered from the soil samples of Vel Tech High Tech Dr. Rangarajan Dr. Sagunthala Engineering College campus. In the light of atomic and morphological characteristics derived by phylogenetic analysis of internal transcribed spacer region S. This is a first record of *P. pimiteouiense* in Chennai.The aim objective of this research is segregation of *penicillium sp*.from soil sample, DNA extraction,PCR amplification and phylogenetic analysis to find its economic impact on human life.

NCBI GenBank Accession no: MG515385.1

Keywords: *Penicillium pimiteouiense*, soil sample, morphological characteristics,ITS, phylogenetic analysis.

INTRODUCTION

*Penicillium*species mostly grow in environments such as agricultural soil, hilly regions and sandy soil (Attia et al, 2022). The main purpose of fungal screening is to find use in the conversion of organic matter into value added products, lipidprecursor to biofuel, animal feed formulation and also in many enzyme factories (Fatima, 2021) (Wu et al, 2022) despite of disease causing ability in human being (Jameel et al, 2021) and also in animals (Ganaie & Sharma, 2021). In early studies, identification of *Penicillium* species was based only on morphological characteristics (Glodjinon et al, 2020). Nowadays, molecular methods have been used extensively for studying phylogenetic relationships among closely related *Penicillium*species (Sharaf et al, 2022). Based on morphological and molecular characteristics, this species was identified as *P.pimiteouiense* (Teh et al, 2013). Soil samples were collected behind Vel Tech High Tech Dr. Rangarajan Dr.Sagunthala Engineering College, Avadi, Chennai, TamilNadu. Moreover, *P. pimiteouiense* was first isolated from polycystic kidney cell cultures (Peterson et al, 1999). *Penicillium pimiteouiense* has also been isolated from the low moisture agricultural fields in India.The morphological characteristics of the species were visualized and the results are discussed in this paper.

METHODS

Sample Collection

Each soil sample was air dried and stored in paper bags at 4°C until used. *Penicillium* isolates were obtained from direct isolation technique. The soil was weighed 0.3 g and evenly distributed on malt extract agar (MEA) where three replicates were prepared and the plates were incubated for 5 to 7 days at room temperature until the growth of fungal colonies was observed. Individual colonies were picked with a sterile inoculation needle andtransferred onto new MEA media, CYA media, and YES media. Three-point inoculation were done on Petri plates and incubated in the dark at RT for 7 days.

Materials and Composition:

1. Czapek Yeast Agar Media:

Czapek yeast agar is recommended for cultivation and maintenance of fungal species. Sucrose serves as the source of energy. Yeast extract provides essential amino acids, vitamins and other essential nutrients. Sodium nitrate serves as the nitrogen sources. The various salts buffer the medium in addition to supplying essential ions to the growing fungi (Pitt, 1979).

Table 1: Composition of CYA media

Ingredients	**g / L**
Sucrose	30.0
Yeast Extract	5.0
Dipotassium Hydrogen Phosphate	1.000
Sodium Nitrate	0.3
Potassium Chloride	0.05
Magnesium Sulphate	0.05
Ferrous Sulphate	0.001
Zinc Sulphate	0.001
Copper Sulphate	0.0005
Agar	15.00

2. Malt Extract Agar Media:

Malt Extract Agar is recommended for the detection, isolation and enumeration of yeasts and moulds from clinical and non-clinical samples. Malt extract provides an acidic environment and nutrients favourable for growth and metabolism of yeasts and moulds. Mycological peptone rapidly gives a luxuriant growth with typical morphology and pigmentation. Suspend 50.0 grams in 1000 ml distilled water and soak for 15 minutes (Reddish 1919).

Table 2: Composition of MEA media

Ingredients	g/L
Malt Extract	30.000
Mycological Peptone	5.000
Agar	15.000
Final pH	5.4±0.2

3. Yeast Extract Sucrose Agar Media:

YES functions as a complete medium for yeast growth and it contains yeast extract, glucose and other supplements which include adenine, histidine, leucine, and uracil and lysine hydrochloride. Dextrose serves as the carbon source. Since the medium contains 30 grams per litre dextrose, the medium should be properly dissolved in water before autoclaving to avoid caramelization of the sugar. Suspend 50.25 grams in 1000 ml distilled water (Taylor et al, 1958).

Table 3: Composition of YES media

Ingredients	g/L
Yeast extract	5.0
Dextrose	30.0
Adenine	0.05
Histidine	0.05
Leucine	0.05
Lysine	0.05
Uracil	0.05
Agar	15.0

Fungal Identification- 18 S rRNA Sequencing:

DNA Extraction:

Lysis/homogenization:

Cells grown in monolayer should be lysed by suspending colonies aseptically and mixed with 450 µl of lysis buffer in a 2 ml microcentrifuge tube and lyse the cells by repeated pipetting. Add 4 µl of RNAse A and 250 µl of neutralization buffer. Vortex the content and incubate the tubes for 30 minutes at 65˚C in water bath. To minimize shearing the DNA molecules, mix DNA solutions by inversion. Centrifuge the tubes for 20 minutes at 14,000 rpm at 10 ˚C. Following centrifugation, transfer the resulting viscous supernatant into a fresh 2mlmicro centrifuge tube without disturbing the pellet. Add 600 µl of binding buffer to the content and mix thoroughly by pipetting and incubate the content at room temperature for 5 minutes. Transfer 600 µl of the contents to a spin column placed in 2 ml collection tube. Centrifuge for 2 minutes at 14,000 rpm and discard flow-through. Reassemble the spin column and the collection tube then transfer the remaining 600 µl of the lysate. Centrifuge for 2 minutes at 14,000 rpm and discard flow-through. Add 500 µL washing buffer I to the spin column. Centrifuge at 14,000 rpm for 2 minsand discard flow-through. Reassemble the spin column and add 500 µl washing buffer II and Centrifuge at 14,000rpm for 2 mins and discard flow-through. Transfer the spin column to a sterile 1.5-ml microcentrifuge tube. Add 100 µl of Elution buffer at the middle of spin column. Care should be taken to avoid touch with the filter. Incubate the tubes for 5 minutes at room temperature and Centrifuge at 6000 rpm for 1min. Repeat the above mentioned step 14 and 15 for complete elution. The buffer in themicrocentrifuge tube contains the DNA. DNA concentrations were measured by running aliquots on 1% agarose gel. The DNA samples were stored at -20°C until further use.

PCR amplification:

Polymerase Chain Reaction (PCR) is a process that uses primers to amplify specific cloned or genomic DNA sequences with the help of a very unique enzyme. PCR uses the enzyme DNA polymerase that directs the synthesis of DNA from deoxynucleotide substrates on a single-stranded DNA template. DNA polymerase adds nucleotides to the 3` end of a custom-designed oligonucleotide when it is annealed to a longer template DNA. Thus, if a synthetic oligonucleotide is annealed to a single-stranded template that contains a region complementary to the oligonucleotide, DNA polymerase can use the oligonucleotide as a primer and elongate its 3` end to generate an extended region of double stranded DNA.

Composition of the Taq Master Mix:

- Taq DNA polymerase is supplied in 2X Taq buffer
- 0.4mM dNTPs,
- 3.2mM MgCl2 and
- 0.02% bromophenol blue.

PRIMER DETAILS:

Table 4: Reverse and forward primer used for amplification

PRIMER NAME	SEQUENCE DETAILS	NUMBER OF BASE
ITS1	5' TCCGTAGGTGAACCTGCGG 3'	19
ITS4	5' TCCTCCGCTTATTGATATGC 3	20

Add 5 µL of isolated DNA in 25 µL of PCR reaction solution (1.5 µL of Forward Primer and Reverse Primer, 5 µL of deionized water, and 12 µL of Taq Master Mix). Perform PCR using the following thermal cycling conditions.

1. Denaturation

The DNA template is heated to 94°C. This breaks the weak hydrogen bonds that hold DNA strands together in a helix, allowing the strands to separate creating single stranded DNA.

2. Annealing

The mixture is cooled to anywhere from 60℃. This allows the primers to bind (anneal) to their complementary sequence in the template DNA.

3. Extension

The reaction is then heated to 72° C, the optimal temperature for DNA polymerase to act. DNA polymerase extends the primers, adding nucleotides onto the primer in a sequential manner, using the target DNA as a template.

Purification of PCR Production:
Removed unincorporated PCR primers and dNTPs from PCR products by using Montage PCR Clean up kit (Millipore).The PCR product was sequenced using the primers.Sequencing reactions were performed using a ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems). Sequencing protocol Single-pass sequencing was performed on each template using below 18s rRNA universal primers (ITS1/ITS4). The fluorescent-labelled fragments were purified from the unincorporated terminators with an ethanol precipitation protocol. The samples were resuspended in distilled water and subjected to electrophoresis in an ABI 3730xl sequencer (Applied Biosystems). These stages were run for thirty (30) cycles.

Table 5: PCR conditions for amplification

STAGES	TEMPERATURE (°C)	TIME (minutes)
Initial Denaturation	94	3
Denaturation	94	0.5
Annealing	55	0.5
Extension	72	1
Final Extension	72	10
Hold	4	∞

Phylogenetic Analysis:
The 18s rRNA sequence was blast using NCBI blast similarity search tool. The phylogeny analysis of query sequence with the closely related sequence of blast results was performed followed by multiple sequence alignment.The program MUSCLE 3.7 was used for multiple alignments of sequences (Edgar 2004). The resulting aligned sequences were cured using the program Gblocks 0.91b.This Gblocks eliminates poorly aligned positions and divergent regions (removes alignment noise) (Talavera & Castresana, 2007) (Castresana, 2000). Finally, the program PhyML 3.0 aLRT was used for phylogeny analysis and HKY85 as Substitution model.3.PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data, while being one order of magnitude faster. PhyML was shown to be at least as accurate as other existing phylogeny programs using simulated data, while being one order of magnitude faster. The program Tree Dyn 198.3 was used for tree rendering (Dereeper et al, 2008).

RESULTS AND DISCUSSION
The results confirmed that the three replicates were *P.pimiteouiense.* Morphological characteristics of the isolates were agreed.*Penicillium pimiteouiense*isolates were identified based on the production of yellowto red exudates on the upper surface of the colonies onMEA, CYA and YES as well as the yellow to orange colour on the lower surface. Colony diameters of*P.pimiteouiense*isolates were 18-22 mm on CYA, 20-27 mm on MEA and 25-30 mm on YES (Taha et al, 2019). Colonies on CYA was radially sulcate,yellow exudates on the upper surface and yellow to orangecolour on the lower surface. On MEA, colonies were thick, cottony and radially sulcate with whitemycelium, conidial areas were light green and yellow tored exudates were present. The colonieson YES were wrinkled, white and the lower surface wasorange to brown (Paul et al, 2014) (Yee & Zakaria, 2014).

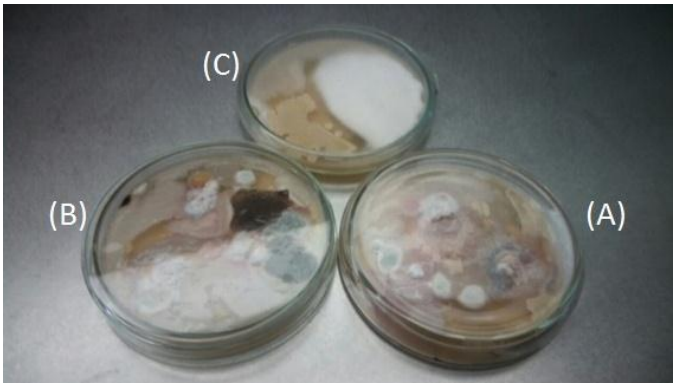


Figure 1: Overview of fungal species grown in (A) CYA Media (B) MEA Media (C) YES Media

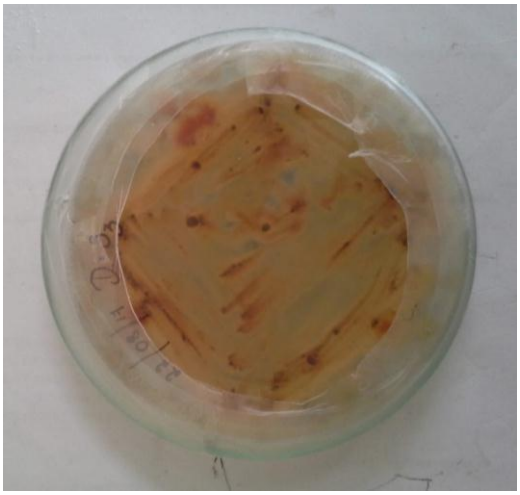


Figure 2: Yellow to Orange extrudes observed in CYA medi

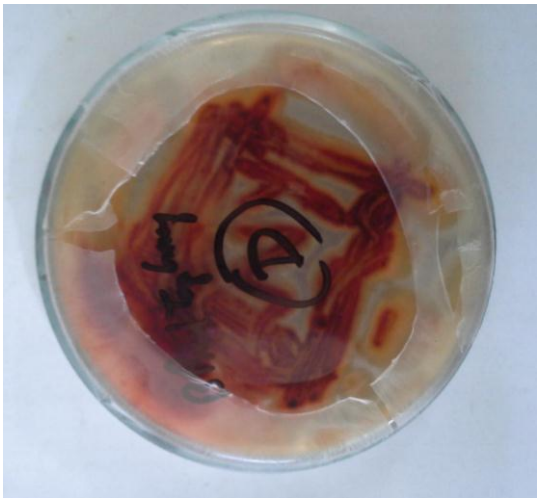


Figure 3: Yellow to Red extrudes observed in MEA media

For confirming the morphological results, molecular identification of the replicates was done using phylogenetic analysis and ITS region sequencing methods. For amplification of internal transcribed spacer (ITS) regions, ITSl and ITS4 primers were used. The DNA sequences were analysed for phylogenetic relationship. The sequences of *P. pimiteouiense*isolates were compared with sequences in the GenBank by using Basic Local Alignment Search Tool (BLAST). Thecombined datasets of both ITS regionswere used to generate phylogenetic tree. Maximum likelihood (ML) tree was constructed. Tree was inferred using the MLheuristics search option with nearest-neighbour-interchange.

>Contig P.Vina
CTCTGGGTCACCTCCCACCCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGCAAGGCCGCCGGGG
GGCTTCCGTCCCCGGGCCCGCGCCCGCCGAAGACACCTGTGAACGCTGTATGAAGATTGCAGTCTGAGCG
AAAAGCTAAATTTATTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAA
TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGT
ATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCTCGTCC
CCCGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCCTCACCCG
CTCTGTAGGCCCGGCCGGCGCCTGCCGACACCATCAATCTTTTTTCCAGGTTGACCACGCATCAGGTAGGC
ATACCCGCTGAACTTAAGCATATCAATAAGCGGAGAAA

Figure 4: 18s rRna Sequrnce of *Penicillium pimiteouiense*

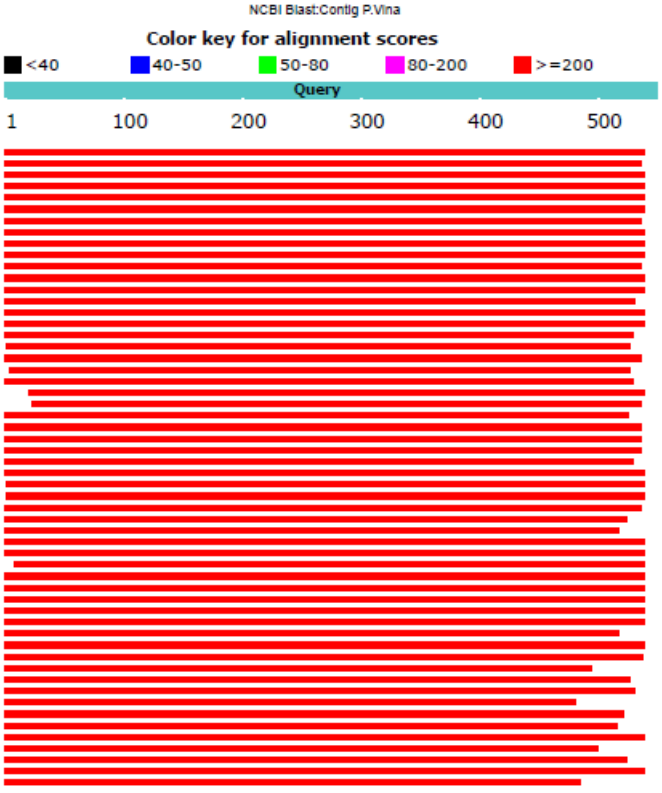
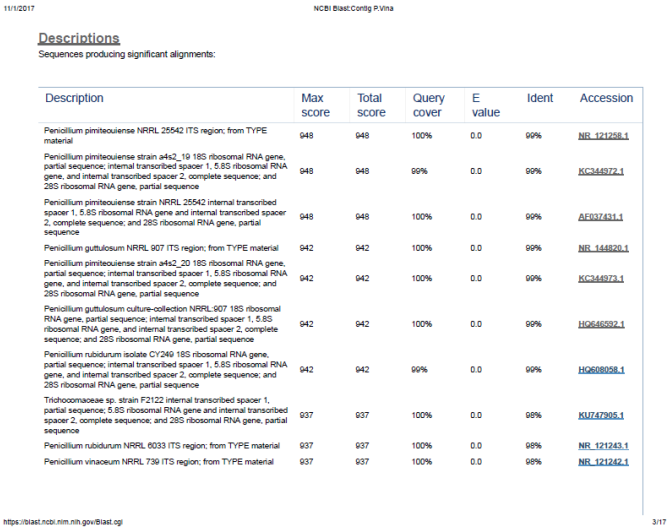


Figure 5: Blast Alignment score.


11/1/2017 NCBI Blast:Contig P.Vina

Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Penicillium pimiteouiense NRRL 25542 ITS region; from TYPE material	948	948	100%	0.0	99%	NR_121258.1
Penicillium pimiteouiense strain a4s2_19 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	948	948	99%	0.0	99%	KC344972.1
Penicillium pimiteouiense strain NRRL 25542 internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	948	948	100%	0.0	99%	AF037431.1
Penicillium guttulosum NRRL 907 ITS region; from TYPE material	942	942	100%	0.0	99%	NR_144820.1
Penicillium pimiteouiense strain a4s2_20 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	942	942	100%	0.0	99%	KC344973.1
Penicillium guttulosum culture-collection NRRL:907 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	942	942	100%	0.0	99%	HQ646592.1
Penicillium rubidurum isolate CY249 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	942	942	99%	0.0	99%	HQ608058.1
Trichocomaceae sp. strain F2122 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	937	937	100%	0.0	98%	KU747905.1
Penicillium rubidurum NRRL 6033 ITS region; from TYPE material	937	937	100%	0.0	98%	NR_121243.1
Penicillium vinaceum NRRL 739 ITS region; from TYPE material	937	937	100%	0.0	98%	NR_121242.1

https://blast.ncbi.nlm.nih.gov/Blast.cgi 3/17

Figure 6: Best Significant alignment report.

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CLUSTAL O(1.2.4) multiple sequence alignment

KC344972.1      CTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCT   60
KC344973.1      CTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCT   60
MG515385.1      ----------------------------------------------CTCTGGGTC--ACCT   13
NR_121258.1     ----------------------AAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCT   39
AF037431.1      ----------------------AAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCT   39
NR_144820.1     ----------------------AAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCT   39
HQ646592.1      ----------------------AAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCT   39
                                                        ********  ****

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KC344973.1      CCCACCCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGCAAGGCCGCCGGGGGG   120
MG515385.1      CCCACCCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGCAAGGCCGCCGGGGGG   73
NR_121258.1     CCCACCCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGCAAGGCCGCCGGGGGG   99
AF037431.1      CCCACCCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGCAAGGCCGCCGGGGGG   99
NR_144820.1     CCCACCCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGCAAGGCCGCCGGGGGG   99
HQ646592.1      CCCACCCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGCAAGGCCGCCGGGGGG   99
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KC344973.1      CTTCCGTCCCCGGGCCCGCGCCCGCCGAAGACACCTGTGAACGCTGTATGAAGATTGCAG   180
MG515385.1      CTTCCGTCCCCGGGCCCGCGCCCGCCGAAGACACCTGTGAACGCTGTATGAAGATTGCAG   133
NR_121258.1     CTTCCGTCCCCGGGCCCGCGCCCGCCGAAGACACCTGTGAACGCTGTATGAAGATTGCAG   159
AF037431.1      CTTCCGTCCCCGGGCCCGCGCCCGCCGAAGACACCTGTGAACGCTGTATGAAGATTGCAG   159
NR_144820.1     CTTCCGTCCCCGGGCCCGCGCCCGCCGAAGACACCTGTGAACGCTGTATGAAGATTGCAG   159
HQ646592.1      CTTCCGTCCCCGGGCCCGCGCCCGCCGAAGACACCTGTGAACGCTGTATGAAGATTGCAG   159
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KC344973.1      TCTGAGCGAAAAGCTAAATTTATTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATC   240
MG515385.1      TCTGAGCGAAAAGCTAAATTTATTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATC   193
NR_121258.1     TCTGAGCGAAAAGCTAAATTTATTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATC   219
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NR_144820.1     TCTGAGCGAAAAGCTAAATTTATTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATC   219
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MG515385.1      GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG   253
NR_121258.1     GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG   279
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AF037431.1      GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG   279
NR_144820.1     GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG   279
HQ646592.1      GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCG   279
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KC344973.1      AGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCA   360
MG515385.1      AGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCA   313
NR_121258.1     AGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCA   339
AF037431.1      AGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCA   339
NR_144820.1     AGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCA   339
HQ646592.1      AGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCA   339
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KC344973.1      TTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCTCGTCCCCCGGGACGGGCCCGAAAG   420
MG515385.1      TTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCTCGTCCCCCGGGACGGGCCCGAAAG   373
NR_121258.1     TTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCTCGTCCCCCGGGACGGGCCCGAAAG   399
AF037431.1      TTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCTCGTCCCCCGGGACGGGCCCGAAAG   399
NR_144820.1     TTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCTCGTCCTCCGGGACGGGCCCGAAAG   399
HQ646592.1      TTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCTCGTCCTCCGGGACGGGCCCGAAAG   399
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KC344973.1      GCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCGTCACCCGCTCTGTAGG   480
MG515385.1      GCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCCTCACCCGCTCTGTAGG   433
NR_121258.1     GCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCGTCACCCGCTCTGTAGG   459
AF037431.1      GCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCGTCACCCGCTCTGTAGG   459
NR_144820.1     GCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCGTCACCCGCTCTGTAGG   459
HQ646592.1      GCAGCGGCGGCACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCGTCACCCGCTCTGTAGG   459
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KC344972.1      CCCGGCCGGCGCCTGCCGACACCATCAATCTTTTTTCCAGGTTGACCTCGGATCAGGTAG   540
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MG515385.1      CCCGGCCGGCGCCTGCCGACACCATCAATCTTTTTTCCAGGTTGACCACGCATCAGGTAG   493
NR_121258.1     CCCGGCCGGCGCCTGCCGACACCATCAATCTTTTTTCCAGGTTGACCTCGGATCAGGTAG   519
AF037431.1      CCCGGCCGGCGCCTGCCGACACCATCAATCTTTTTTCCAGGTTGACCTCGGATCAGGTAG   519
NR_144820.1     CCCGGCCGGCGCCTGCCGACACCATCAATCTTTTTTCCAGGTTGACCTCGGATCAGGTAG   519
HQ646592.1      CCCGGCCGGCGCCTGCCGACACCATCAATCTTTTTTCCAGGTTGACCTCGGATCAGGTAG   519
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KC344972.1      GGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA--------------------   580
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MG515385.1      GCATACCCGCTGAACTTAAGCATATCAATAAGCGGAGAAA--------------------   533
NR_121258.1     GGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGC   579
AF037431.1      GGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGC   579
NR_144820.1     GGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGC   579
HQ646592.1      GGATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGC   579
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Figure 7: ClustalW report for the best significant alignment.

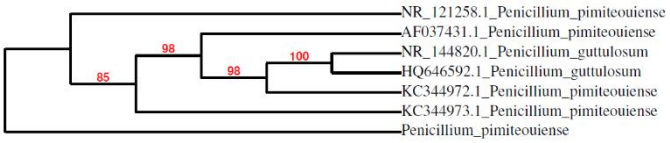

Figure 8: Phylogenetic tree diagram.

CONCLUSION

It is well known that fungal species do not require a specific environmental condition for its growth. *Penicilium pimiteouiense* is also found to grow in low moisture condition (Wadud et al, 2012). *Penicillium* lives as saprophytes on foods and fruits. It appears on different substances including buildings, ceilings and machineries. The importance of *Penicillium* is due to its wide application in biotechnology to benefit mankind. The low cost production of valuable chemicals, enzymes, vitamins, proteins and organic molecule has become possible due to *Penicillium.*Penicillium sp. is consider as the most useful fungi in the industry because they produce most valuble industrial enzymes like amylase, cellulose and protease. The maximal amylase and protease activities for P. camemberti were 2.77U/mL and 0.41U/mL, respectively. The highest cellulase activity of Penicillium brevicompactum is 2.38U/mL (Thippeswamy et al, 2020). Industrial activities are causing various environmental pollution which is responsible for increased organic and inorganic xenobiotics in the ecosystem (Ali et al, 2016). In the current years obligate and extreme halophilic, thermophile, acidophilic and basophilic *Penicillium* are identified from the extreme environments of the world which can come with huge achievement after research. Furthermore, metabollics study of our isolates by the means of GC-MS will be studied for the determination of active compounds by the means of chromatographic techniques (Sangappa & Thiagarajan, 2013).

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