

# Novel Pravastatin-Producing *Penicillium janthinellum* Strain Isolated from Soil

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Manuscript submitted February 6, 2015; accepted March 31, 2015.

doi: 10.17706/ijbbb.2015.5.2.80-90

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**Abstract:** Hyperlipidemia is a main risk factor for coronary heart disease and stroke. The lipid-lowering drug, pravastatin, is currently obtained by the microbial biotransformation of compactin (mevastatin) to pravastatin. It is clear that pharmaceutical industry has to date utilized only a very minor portion of nature's microbial arsenal for the discovery of pravastatin. Therefore, the search for microfungi which is able to produce this statin directly has gained increasing importance. In this study, a total of 33 soil fungal cultures were isolated from nine sampling sites in oil palm plantations in Malaysia and tested for their potential to produce pravastatin. The cultures were cultivated in submerged fermentation followed by screening for statin production using high-performance liquid chromatography. The best pravastatin producer was identified to species level using morphological characteristics and molecular biological approaches. From the tested cultures, 18 fungal isolates were able to produce pravastatin directly by fermentation. Among these, the isolate ESF20P was the best producer, with a yield of 15.8 mg/l pravastatin. Molecular identification of this strain showed the highest homology (98%) with *Penicillium janthinellum*.

**Key words:** Microfungi, pravastatin, screening, soil.

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## 1. Introduction

Cholesterol is an essential lipid component of the human cell membrane and a precursor for steroid hormones and bile acids. However, any biomolecule in excess can be harmful for human health. Because lipids are carried in the bloodstream, hyperlipidemia is always a threat to coronary arteries and the most important risk factor for coronary heart disease and stroke [1]. To fight these problems, medical science has increasingly relied on several drugs, commonly known as lipid-lowering drugs. One group of these drugs known as statins lowers cholesterol by interfering with the cholesterol biosynthetic pathway [1], [2]. Only one-third of total body cholesterol is diet-derived; while two-thirds are synthesized by the liver and, to a lesser extent, by other organs [3], [4]. For this reason, control of cholesterol by inhibiting its biosynthesis provides an important strategy to lower cholesterol levels in the blood.

Statins selectively inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the regulatory and rate-limiting enzyme in cholesterol biosynthesis. The remarkable safety of these drugs derives from their unique mechanisms of action. The inhibition of HMG-CoA reductase results in accumulation of HMG-CoA, which can be metabolized to simpler compounds, without any buildup of lipophilic intermediates with a sterol ring [5], [6]. In this way, statins lower cholesterol, thus, preventing plaque

buildup within the arteries.

There are currently several statins commercially available in pharmaceutical form [1], [7]. In comparison with other statins, pravastatin is a highly selective natural inhibitor of hepatic cholesterol synthesis. It is taken specifically into hepatic cells via a carrier-mediated active transport system and inhibits cholesterol synthesis selectively in the liver [8]. This lipid-lowering agent has been studied with reference to its long-term lipid-lowering effect, patient tolerance and clinical safety [9], [10]. In both studies, no serious clinical or laboratory abnormalities were observed, supporting the fact that pravastatin seems to be the HMG-CoA reductase inhibitor that is best tolerated in comparison with other natural, semi-synthetic and synthetic statins.

Pravastatin currently can be obtained on an industrial scale only by the biotransformation of compactin (mevastatin) produced by *Penicillium* strains [7]. However, the conversion of compactin to pravastatin is itself inhibited by its own high concentration. This apparently is an unavoidable limitation of the microbial biotransformation process. The development of a one-step fermentation process for production of pravastatin based on an active microbial producer could therefore provide a novel and effective alternative. Therefore, in current study, the isolation of soil microfungi and assessment of their ability to produce pravastatin directly were required.

## **2. Materials and Methods**

### **2.1. Soil Sampling**

Soil samples for microfungal isolation were obtained from oil palm plantations of different ages located in Gambang (Yayasan Pahang Plantation Holding Sdn Bhd, Gambang, Pahang State, Malaysia). Thirty soil samples were randomly collected from 10 sampling sites. Samples were returned to the laboratory rapidly for processing. These soil samples were used to determine soil pH in the laboratory [11].

### **2.2. Isolation of Microfungi**

Microfungi were isolated using the soil plate method of Warcup [12]. A small amount of soil (5-15 mg) was dispersed in liquid (40°C) PDA medium in a sterile Petri dish without prior suspension in water. Control and experimental plates were prepared in triplicate. Inoculated Petri dishes were incubated at 25°C until the colonies were fully formed. No growth was observed on control plates containing PDA medium alone. Individual fungal colonies were picked and purified by streaking onto fresh PDA medium. After several cycles of picking and replating, separate colonies were transferred to PDA slants.

### **2.3. Fermentation Procedure**

The fungal strains were maintained on PDA slants and incubated at 25°C for 7 days. A spore suspension was prepared by suspending spores from the appropriate slant in 10 ml of sterilized distilled water containing 0.01% (v/v) Tween-80. The number of spores was counted using a haemocytometer after vortexing and appropriate dilution. Ten percent (v/v) of the medium volume of the spore suspension ( $10^8$  spores/ml) prepared from the slant was used to inoculate sterile screening medium containing: 3% (w/v) glucose, 3% (w/v) glycerol, 0.4% (w/v) peptone, 0.2% (w/v)  $\text{NaNO}_3$ , 0.1% (w/v)  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ . The pH value of the medium was adjusted to 6.5 with either 10% HCl or 10% NaOH before sterilization [13]. Shake flask fermentation was carried out in a 100-ml flask at 25°C, 180 rpm, for 7 days in an incubator shaker (Multitron Version 2; Infors AG, Switzerland).

### **2.4. Analytical Determination of Statins**

Thirty-three fungal isolates obtained from the soil samples were tested for their ability to produce mevastatin (precursor of pravastatin) and pravastatin. Ethyl acetate extracts from the whole cell broths

obtained according to the slightly modified extraction procedure of Manzoni *et al.* [14] were used for HPLC analysis (Agilent 1200; Agilent Technologies, USA) of mevastatin and pravastatin. The pH value of the whole cell broth was adjusted to  $3\pm 0.2$  with 1 M trifluoroacetic acid, and then an equal volume of ethyl acetate was added. Extraction was performed in an Ecotron incubator shaker (Infors HT, Switzerland) at 200 rpm and 30°C for 1 h. The fermentation samples were subsequently filtered through a Whatman filter paper no. 41 and the organic phase of each sample was collected using a nonsterile hydrophobic PTFE syringe filter (pore size: 0.22  $\mu\text{m}$ ). The ethyl acetate filtrates were then dried over anhydrous  $\text{Na}_2\text{SO}_4$  followed again by filtration from the drying agent through a nonsterile hydrophobic PTFE syringe filter (pore size: 0.22  $\mu\text{m}$ ), and concentrated using a rotary evaporator to a final volume of 4 ml. Five microliters from the organic phase were then injected for HPLC analysis on a 250  $\times$  4.6 mm ID Zorbax Eclipse Plus  $\text{C}_{18}$  column, 5 $\mu\text{m}$  particle size (Agilent Technologies, USA). The mobile phase consisted of acetonitrile and water (60:40, v/v) with a pH value adjusted to pH  $3\pm 0.2$  by addition of 1 M  $\text{H}_3\text{PO}_4$  [13], [14]. The flow rate was maintained at 0.8 ml/min and detection was measured at 238 nm. HPLC grade mevastatin ( $\geq 95\%$  purity) and pravastatin ( $\geq 98\%$  purity) from Sigma-Aldrich (USA) were used as standards.

## **2.5. Dry Cell Weight Estimation**

Dry cell weight (DCW) was measured by filtering the contents of each flask through a pre-weighed Whatman filter paper no. 41. The collected biomass was washed with distilled water and dried at 105°C in a UT6 model laboratory oven (Heraeus Instruments, Germany) until constant weight.

## **2.6. Cultural and Morphological Studies of the Selected Strain**

The fungal strain selected in this study was transferred to appropriate diagnostic agar media for identification [15], [16]: Czapek yeast autolysate (CYA) agar and malt extract agar (MEA). Culture was incubated in the dark at 25°C for 7 days. Fungal isolate was identified morphologically following Matsukuma *et al.* [16]. The macroscopic features examined included colony diameter, obverse and reverse colony colors. The standard medium used for microscopic observations was MEA. Conidiophore branching patterns were examined using an optical microscope (Primo Star Carl Zeiss, Germany). These techniques allowed the fungal isolate to be identified at the species level.

## **2.7. Molecular Identification of the Selected Strain**

The strain was maintained on a PDA slant. The pure isolate was cultured in a 100-ml conical flask containing 20 ml of CYA liquid medium. The culture was inoculated with a spore suspension and incubated at 25°C with shaking (150 rpm) in an Ecotron microbiological incubator (Infors HT, Switzerland). Mycelial biomass from 2-day-old culture was harvested by filtration through Whatman filter paper no. 1 and used for DNA isolation.

Genomic DNA was obtained using the Fungi/Yeast Genomic DNA Isolation Kit (Norgen Biotek Corporation, Canada), according to the slightly modified manufacturer's instructions. Fifty milligrams of fungus (wet mass) were transferred to 1 ml of 0.85% NaCl in a microcentrifuge tube and centrifuged at 13,000 rpm for 1 min in a Biofuge Pico microcentrifuge (Heraeus Instruments, Germany) to pellet the cells. The supernatant was poured off carefully and 500  $\mu\text{l}$  of lysis solution was added to the cell pellet, and the cells were re-suspended by gentle vortexing using a Vortex Mixer Classic (VELP Scientifica, Italy). The mixture was transferred to the provided bead tube and vortexed for 5 min at 400 rpm in an Ecotron microbiological incubator (Infors HT, Switzerland). The bead tube with lysate was then incubated at 65°C for 10 min in a water bath (Mettler, Germany). All of the lysate, including cell debris but not beads, was transferred to a DNA-free microcentrifuge tube by pipetting and was centrifuged for 2 min at 13,000 rpm. Clean supernatant was carefully transferred to a new DNA-free microcentrifuge tube, and the volume was

noted. An equal volume of 70% ethanol was added to the collected lysate and vortexed to mix, after which 600 µl of the lysate with ethanol was applied onto the spin column with collection tube and centrifuged for 1 min at 13,000 rpm. Then, 500 µl of wash solution was applied to the column and centrifuged for 1 min. This column washing step was repeated once more. The column was placed into a fresh 1.7-ml elution tube provided with the kit and 100 µl of elution buffer was added to the column. The mixture was centrifuged for 2 min at 2,000 rpm, followed by 1 min at 13,000 rpm. The concentration of DNA diluted 25 times by mixing 1 µl of sample with 24 µl of molecular grade water was determined at 260 nm using Eppendorf Biophotometer Plus (Eppendorf AG, Germany). Then, the final concentration of DNA was adjusted to 2.5 µg/ml by preparation of an appropriate dilution of a stock DNA solution. The DNA purity was checked using Eppendorf BioPhotometer Plus (Eppendorf AG, Germany) (OD 260/280 nm), and the DNA integrity was checked by electrophoresis at 70V for 30 min on 1% agarose gel in a Sub-Cell GT agarose gel electrophoresis system (Bio-Rad Laboratories, USA). The purified genomic DNA was stored at -20°C in a laboratory freezer (Revco, USA) until required.

The ITS1-5.8S-ITS2 region of the nuclear rDNA from the isolate was amplified using forward primer ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATG-3') [17], obtained from Vivantis Technologies, USA. These primers are considered as universal fungal primers. The amplification reaction was carried out in volume of 25 µl containing 4 µl (10 ng) of template DNA, 1.5 µl of each primer (20 µM), 2.5 µl of 10×PCR buffer, 1 µl of MgCl<sub>2</sub> (50 mM), 0.25 µl of dNTPs (100 mM) and 0.25 µl of **Taq DNA polymerase (5 U/µl) supplied** by Vivantis Technologies, USA [18]. A negative control, containing all reagents except for genomic DNA, was also prepared. The PCR reaction was performed in an Eppendorf Mastercycler Vapoprotect (Eppendorf AG, Germany) using 10 ng of genomic DNA measured with Eppendorf Biophotometer Plus (Eppendorf AG, Germany). The amplification program used was described by Henry *et al.* [19] and included one cycle of 4 min 30 s at 95°C (predenaturing), 40 cycles of 30 s at 95°C (denaturation), 30 s at 50°C (annealing), 60 s at 72°C (extension) and finally one cycle of 3 min at 72°C. For PCR, 5 µl of PCR product was examined by electrophoresis at 90V for 1 h 50 min in a 1% (w/v) agarose gel stained with GoodView nucleic acid stain (Beijing SBS Genetech Co. Ltd., China) in 1×TAE buffer in a Sub-Cell GT agarose gel electrophoresis system (Bio-Rad Laboratories, USA). The molecular mass of the amplified DNA was estimated by comparison with a ready-to-use **VC 100-bp Plus DNA ladder (Vivantis Technologies, USA)** as a molecular size marker. The agarose gel was visualized under UV light using an Alpha Ease FC Imaging system (Alpha Innotech, Germany).

The obtained PCR product was sequenced by First Base Laboratories Sdn Bhd, Malaysia. The PCR fragment was extracted from the agarose gel and purified with **GF-1 AmbiClean Kit** (Vivantis Technologies, USA) following the manufacturer's instructions. The purified PCR fragment was sequenced on both strands using forward (ITS1) or reverse (ITS4) primers. Genetic sequencing of the amplified ribosomal sequences was carried out utilizing the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Following removal of dye terminators using BigDye XTerminator Purification Kit (Applied Biosystems, USA), samples were analyzed on an ABI PRISM 3730xL Genetic Analyzer (Applied Biosystems, USA), utilizing Sequence Scanner software version 1.0.

The sequences obtained were pairwise aligned using the SDSC Biology Workbench 3.2 software [20] with default settings. For the identification of the fungal isolate, percentage of sequence identity and coverage were compared with available sequences in GenBank using the Basic Local Alignment Tool, BLASTN program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

## **2.8. Phylogenetic Analysis**

Phylogenetic analysis was performed from aligned sequences of the rDNA ITS region containing ITS1 and ITS2 and the intervening 5.8S rRNA gene. The sequences were aligned using ClustalW with all multiple

alignment parameters used at default settings. The sequence alignment included *Penicillium janthinellum* ESF20P (JX456373), *Penicillium janthinellum* CBS 340.48 (GU981585), *Penicillium citrinum* CBS 122452 (GU944576), *Penicillium janczewskii* CBS 354.48 (KC411755), *Penicillium brevicompactum* CBS 257.29 (KF465776), *Penicillium bialowiezense* CBS 227.28 (EU587315), *Penicillium chrysogenum* CBS 306.48 (JX997093), *Penicillium flavigenum* CBS 419.89 (JX997105), *Penicillium viridicatum* CBS 390.48 (JN942697), *Penicillium cyclopium* CBS 144.45 (JN942742), *Penicillium aurantiogriseum* CBS 324.89 (JN942751), *Penicillium roqueforti* CBS 221.30 (KF465778) and *Penicillium paneum* CBS 465.95 (HQ442349) species available from GenBank. *Talaromyces bacillisporus* CBS 296.48 (JN899329) was the outgroup species. Cladistic analysis using neighbour-joining method was performed with the MEGA 4.0 computer program [21]. Phylogenetic distances were calculated using Jukes-Cantor model. Confidence values for individual branches were determined by 1000 bootstrap replications.

## 2.9. Statistical Analysis

Each experiment was conducted in three replicates. Mean values with standard deviation were presented.

## 3. Results and Discussion

### 3.1. Fungal Isolates Recovered from Oil Palm Plantation Soils

Microfungi are one of the most common microorganisms in both cultivated and uncultivated soils, and have been isolated from numerous soils worldwide. Some fungal genera have representative species in most soils. For example, the genera *Aspergillus*, *Penicillium*, *Fusarium* and *Trichoderma* occur very frequently [22]. Although microfungi are commonly distributed in soils, our knowledge of fungal diversity in tropical ecosystems is limited [23]. The present study was carried out using cultivated soils from oil palm plantations. As soils from these habitats contain significant amounts of lipid compounds [11], microfungi isolated from these soils may produce antihypercholesterolemic agents such as pravastatin. In this study, 30 soil samples from 10 sampling sites in oil palm plantations were examined (Table 1).

Table 1. Soil Samples Obtained from Oil Palm Plantations in Gambang (3.72°-N 103.12°-E)

Sampling site	Age of oil palm plantation	Sampling depth (cm)
A	10-year-old	10
B	10-year-old	20
C	10-year-old	30
D	10-year-old	40
E	10-year-old	50
F	15-year-old	10
G	15-year-old	20
H	15-year-old	30
I	20-year-old	10
J	20-year-old	20

A total of 33 fungal isolates were recovered from nine sampling sites, with all of the source soils showing acidic conditions (pH ranging from 3.15 to 5.29) favourable for the growth of microfungi. No fungal isolates were recovered from site F. The majority of the isolates (22 out of 33) were recovered from the sandy soils of a 10-year-old oil palm plantation characterized by moderate moisture content and pH ranging from 4.40 to 5.29. In total nine isolates were recovered from the peat soils of a 15-year-old plantation, while only two isolates were recovered from the deep peat soils of a 20-year-old plantation.

### 3.2. Statin-Producing Ability of Fungal Isolates

Previous studies have utilized different methods for the screening of natural statins by microfungi. Thin-layer chromatography (TLC) has been used to detect mevastatin (lovastatin) production [24]. Kumar *et al.* [25] developed a more economical and less labor-intensive 'agar plug' method, utilizing the anti-fungal property of lovastatin to produce a zone of inhibition against *Neurospora crassa*. Chakravarti and Sahai [26] described a spectrophotometric method for the determination of mevastatin (compactin), the pravastatin precursor. In the present study, to investigate the ability of the fungal isolates obtained to produce mevastatin and pravastatin, samples were examined using HPLC. To increase the sensitivity of the analytical method, ethyl acetate was used to extract these statins from the whole cell broths.

Several fungal genera have been reported to be able to produce mevastatin (lovastatin) directly by fermentation [14], [24], [27]. Among the few microbial strains for mevastatin production are *Penicillium citrinum* [26], *Penicillium cyclopium* [28], and *Penicillium brevicompactum* [29]. Although mevastatin is not used directly in treatment, it is an important intermediate compound in industrial pravastatin production [7].

In current study, eighteen of the 33 cultures obtained produced pravastatin at different levels (Table 2). Of these, the isolate ESF20P was the best producer, achieving a concentration of 15.80 mg/l pravastatin, equating to 1096.72 µg/g dry cell weight. The standard pravastatin (≥ 98% purity) used showed a peak with  $R_t$  of 3.216 min (Fig. 1(a)) in current study, while that extracted from the isolate ESF20P showed a peak of 3.205 min (Fig. 1(b)), both also very similar to the  $R_t$  (3.19 min) reported by Manzoni *et al.* [14]. The next most active pravastatin producers were the isolates ESF26P and ESF32P, with 13.89 mg/l and 10.85 mg/l pravastatin, respectively.

Table 2. Pravastatin-Producing Fungal Isolates

Isolate	Pravastatin (mg/l)	Pravastatin (µg/g DCW)
ESF6P	1.07±0.11	67.74±7.12
ESF11P	1.23±0.06	103.96±11.95
ESF16P	1.02±0.17	36.91±2.95
ESF18P	1.27±0.12	105.53±9.95
ESF19P	1.39±0.06	74.64±7.24
ESF20P	15.80±1.90	1096.72±84.36
ESF23P	4.70±0.57	347.21±21.87
ESF24P	3.85±0.65	351.41±24.93
ESF25P	0.87±0.18	81.74±8.80
ESF26P	13.89±1.54	959.27±70.48
ESF27P	6.44±0.87	594.58±49.02
ESF28P	9.24±1.12	927.11±88.81
ESF29P	2.36±0.36	356.47±33.32
ESF30P	7.27±0.86	554.58±32.89
ESF31P	7.96±0.69	1149.28±83.39
ESF32P	10.85±1.23	738.41±75.09
ESF33P	4.13±0.49	322.03±31.20
ESF34P	3.82±0.61	374.20±32.04

<sup>a</sup>Results are presented as mean ± standard deviation from three replicates.

<sup>b</sup>DCW=dry cell weight.

All 33 isolates were also screened for mevastatin production ( $R_t$  of 14.602 min), with no traces of this compound being found. The results obtained here suggest that pravastatin-producing strains immediately transformed any mevastatin produced into pravastatin.

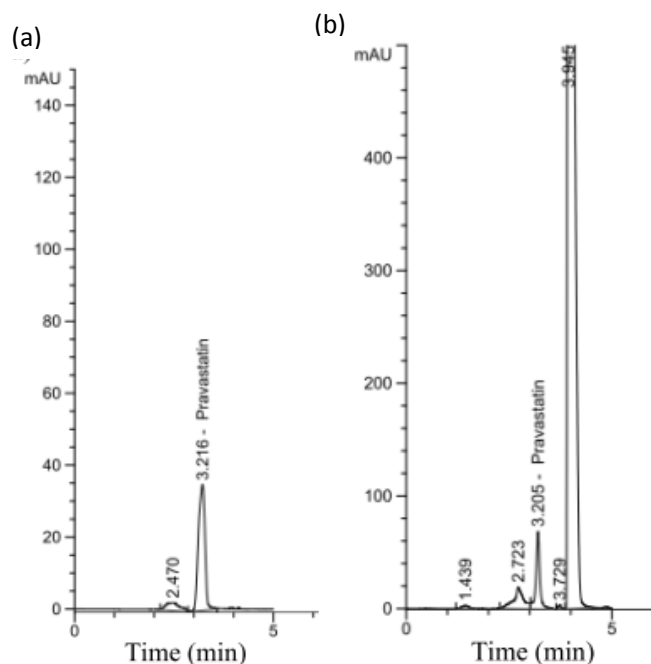


Fig. 1. High-performance liquid chromatograms: (a) Standard pravastatin; (b) Ethyl acetate extract of isolate ESF20P.

### 3.3. Identification of the Selected Strain to Species Level

Microfungi have historically been identified based on macroscopic and microscopic properties. Examination of the features of the colonies and microscopic characteristics of the conidiophores are commonly used methods in fungal identification [15], [16]. Thirty-three fungal isolates examined in this study were characterized by filamentous growth on agar media. Table 3 shows the cultural and morphological properties of the best pravastatin producer selected in this study. Obtained results were clearly similar to the literature description of *Penicillium janthinellum* [16].

Table 3. Cultural and Morphological Characteristics of the Examined Isolate in Comparison with Those Reported in the Literature for *Penicillium janthinellum*

Character	Isolate ESF20P <sup>a</sup>	<i>P. janthinellum</i> <sup>b</sup>
Cultural properties		
CD on CYA, mm	26-30	18-43
Conidial mass color	grayish green	grayish green
CRC	yellowish brown	yellowish brown
CD on MEA, mm	45-50	27-62
Conidial mass color	greenish gray	greenish gray
CRC	yellowish brown	dull yellow
Morphological properties		
Conidiophore	monoverticillate	monoverticillate
Phialide length, $\mu\text{m}$	9.66-11.92	6.5-12.0
Phialide shape	ampulliform	ampulliform

<sup>a</sup>Observations were recorded after incubation at 25°C for 7 days. CD=colony diameter; CRC=colony reverse color.

<sup>b</sup>Literature data were primarily adapted from Matsukuma *et al.* [16].

<sup>c</sup>All measurements are presented as extremes.

The strategy used here for molecular identification of the selected pravastatin-producing strain was to identify this fungal isolate by means of ribosomal DNA (rDNA) sequence comparison and assessment of the percentage of nucleotide similarity with reference sequence. Genomic DNA from the best pravastatin-producing ESF20P strain was extracted. The ITS1-5.8S-ITS2 region of the rDNA was amplified successfully producing a single PCR product of the desired length, approximately 550-600 base pairs [30]. The purified amplicon was double-stranded sequenced. The pairwise aligned sequence data from the examined fungal isolate were then compared with available sequences in the GenBank database using the BLASTN program. The section of rDNA sequenced from the selected isolate included 533 base pairs. The complete ITS1 region occupied nucleotides 8 to 180, the 5.8S rDNA gene from nucleotides 181 to 337 and ITS2 from nucleotides 338 to 507. The sequence of the obtained amplicon showed 98% identity to *Penicillium janthinellum* CBS 340.48 (accession number: GU981585).

Thus, based on the morphological characteristics described, and the ITS1-5.8S-ITS2 nucleotide sequence obtained, the best pravastatin-producing strain was identified as *Penicillium janthinellum* ESF20P. The sequence data from this study were deposited in the GenBank database under the accession number: JX456373. This fungal culture has been deposited at the Microbial Culture Collection of the Universiti Malaysia Pahang (UMPCC/F/040).

### 3.4. Phylogeny

A phylogenetic tree shows the evolutionary relationships among the strain selected in this study and a range of species whose sequence information was obtained from GenBank (Fig. 2). Bootstrap values calculated from 1000 replicates are given at the branches. Through the alignment and cladistic analysis of homologous nucleotide sequences, it was shown that the sequence of the *P. janthinellum* ESF20P strain fell in the same sub-clade as of the known *P. janthinellum* CBS 340.48 species with bootstrap value of 100%. Of the species included in the phylogenetic analysis, *P. citrinum* CBS 122452 and *P. janczewskii* CBS 354.48 appeared to be closely related to *P. janthinellum* ESF20P strain. Moreover, Fig. 2 illustrates that *P. brevicompactum* CBS 257.29 and *P. bialowiezense* CBS 227.28 species formed a well-supported clade (99% bootstrap support). *P. chrysogenum* CBS 306.48 and *P. flavigenum* CBS 419.89 formed separate sub-clade (82%); whereas *P. viridicatum* CBS 390.48 and *P. aurantiogriseum* CBS 324.89 grouped together with 97% bootstrap support.

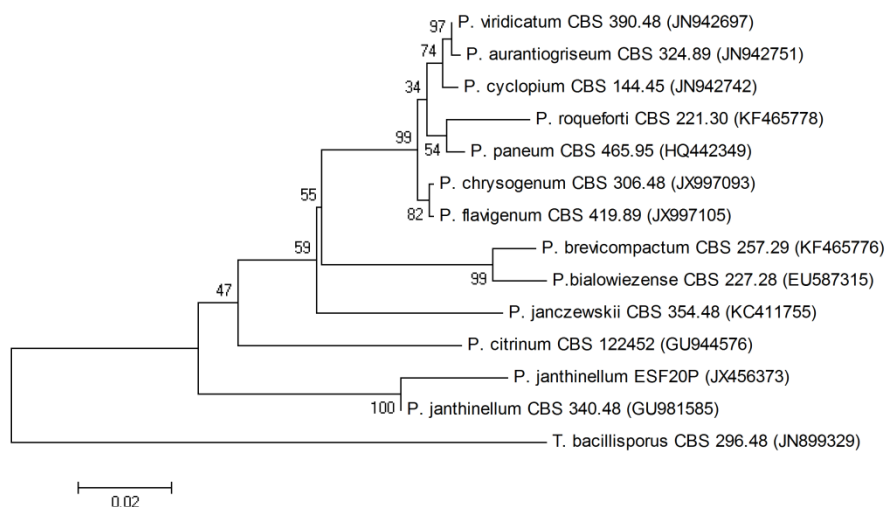


Fig. 2. Phylogenetic tree using the neighbor-joining method of representatives of *Penicillium* spp. inferred from analysis of the ITS region.

#### 4. Conclusion

The present study has identified a number of pravastatin-producing fungal strains obtained from oil palm plantation soils. All of them were able to synthesize pravastatin directly, although only isolate ESF20P did so in relatively high concentration. The most active pravastatin producer was identified to species level using standard morphological and molecular techniques, showing the highest sequence homology (98%) with *P. janthinellum* species. In comparison with previous studies, this strain can already be considered as a novel fungal pravastatin producer of scientific interest. A further strain improvement program and optimization of the significant fermentation parameters, which are under investigation, may considerably enhance production of this fungal metabolite.

#### Acknowledgment

This work was supported by the Research and Management Center of the Universiti Malaysia Pahang under short-term Grants GRS090334 and RDU100319.

The author acknowledges Mr. Mohd Hafizi and Mr. Kelvin Tan Boon Seng from Yayasan Pahang Plantation Holding Sdn Bhd for providing soil samples.

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