

## Microbiota of Palm Oil Mill Wastewater in Malaysia

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**Abstrak:** Kajian ini bertujuan untuk mengenal pasti mikroorganisma asli daripada efluen kilang minyak sawit (POME) dan untuk menentukan beban mikrob. Pengasingan dan pengenalpastian mikroorganisma asli tertakluk kepada kaedah piawai mikrobiologi dan penjujukan gen 16S rRNA dan 18S rRNA. Penjujukan daripada gen 16S rRNA dan 18S rRNA untuk strain mikrob menunjukkan bahawa mereka dikenali sebagai *Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, *Providencia vermicola* 104PB, *Klebsiella pneumonia* 105PB, *Bacillus subtilis* 106PB, *Aspergillus fumigates* 107PF, *Aspergillus nomius* 108PF, *Aspergillus niger* 109PF dan *Meyerozyma guilliermondii* 110PF. Keputusan menunjukkan bahawa jumlah populasi bakteria heterotropik (THB) berkisar antara  $9.5 \times 10^5 - 7.9 \times 10^6$  cfu/mL. Jumlah heterotopik fungi (THF) berkisar antara  $2.1 \times 10^4 - 6.4 \times 10^4$  cfu/mL. Jumlah populasi mikrob asli heterotropik pada medium agar CMC masing-masing berkisar antara  $8.2 \times 10^5 - 9.1 \times 10^6$  cfu/mL (bakteria) dan  $1.4 \times 10^3 - 3.4 \times 10^3$  cfu/mL (fungi). Penemuan mendapati bahawa mikroorganisma berkembang dengan baik di dalam efluen kilang minyak kelapa sawit (POME). Oleh itu, ini menunjukkan bahawa pengasingan mikroorganisma asli dari efluen kilang minyak sawit (POME) adalah penting untuk bioremediasi, rawatan bio dan biodegradasi sisa industri yang berkesan.

**Kata kunci:** Biodegradasi, Industri, Malaysia, MALPOM Sdn. Bhd., Microbiota, Efluen Kilang Minyak Sawit (POME), Sisa Air

**Abstract:** This study was aimed at identifying indigenous microorganisms from palm oil mill effluent (POME) and to ascertain the microbial load. Isolation and identification of indigenous microorganisms was subjected to standard microbiological methods and sequencing of the 16S rRNA and 18S rRNA genes. Sequencing of the 16S rRNA and 18S rRNA genes for the microbial strains signifies that they were known as *Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, *Providencia*

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*vermicola* 104PB, *Klebsiella pneumoniae* 105PB, *Bacillus subtilis* 106PB, *Aspergillus fumigatus* 107PF, *Aspergillus nomius* 108PF, *Aspergillus niger* 109PF and *Meyerozyma guilliermondii* 110PF. Results revealed that the population of total heterotrophic bacteria (THB) ranged from  $9.5 \times 10^5 - 7.9 \times 10^6$  cfu/mL. The total heterotrophic fungi (THF) ranged from  $2.1 \times 10^4 - 6.4 \times 10^4$  cfu/mL. Total viable heterotrophic indigenous microbial population on CMC agar ranged from  $8.2 \times 10^5 - 9.1 \times 10^6$  cfu/mL and  $1.4 \times 10^3 - 3.4 \times 10^3$  cfu/mL for bacteria and fungi respectively. The microbial population of oil degrading bacteria (ODB) ranged from  $6.4 \times 10^5 - 4.8 \times 10^6$  cfu/mL and the oil degrading fungi (ODF) ranged from  $2.8 \times 10^3 - 4.7 \times 10^4$  cfu/mL. The findings revealed that microorganisms flourish well in POME. Therefore, this denotes that isolating native microorganisms from POME is imperative for effectual bioremediation, biotreatment and biodegradation of industrial wastewaters.

**Keywords:** Biodegradation, Industry, Malaysia, MALPOM Sdn. Bhd., Microbiota, Palm Oil Mill Effluent (POME), Wastewater

## INTRODUCTION

Industrial wastewaters are essential habitat for diverse microbes. Generally, some of the microorganisms have been used for biotreatment of wastewaters (Abdel-Raouf *et al.* 2012; Bala *et al.* 2014a, 2014b, 2014c; Bala *et al.* 2015a, 2015b; Bala 2016). Microorganisms domiciled in diverse wastewaters can also cause diseases such as tuberculosis, cholera, typhoid, dermatomycosis, hepatitis and dysentery (Shaaban *et al.* 2004).

Palm oil industry has become one of the most important agricultural based industries in Malaysia that produce colossal amount of oily liquid wastewater universally named as palm oil mill effluent (POME) (Ahmad *et al.* 2005; Rupani *et al.* 2010; Mohammed *et al.* 2014). Palm oil mill wastewater is produced during oil extraction processes in palm oil mill industries. POME is an extremely polluting wastewater that contaminates the environment when released directly into rivers, streams or lakes devoid of treatment.

POME; in addition include large amounts of solids, both suspended solids and total dissolved solids in the range of 18,000 mg/L and 40,500 mg/L correspondingly. These solids are usually named palm oil mill sludges (POMS). The solid waste that are formed in the process of extraction are the leaves, trunk, decanter cake, empty fruit bunches, seed shells and fibre from the mesocarp (Rupani *et al.* 2010).

Raw POME is a warm, acidic (pH between 4 and 5), brownish colloidal suspension having lofty concentrations of organic matter, elevated amounts of total solids (40,500 mg/L), oil and grease (4,000 mg/L), chemical oxygen demand (COD) (50,000 mg/L) and biochemical oxygen demand (BOD) (25,000 mg/L) (Ma 2000). The wastewater from palm oil mill can cause significant ecological problems, if released untreated (Singh *et al.* 2010). The chemical oxygen demand (COD) and biochemical oxygen demand (BOD) values of palm oil mill wastewater are high enough to cause serious pollution and environmental problem to the rivers. Chemical oxygen demand and biochemical oxygen demand of palm oil mill

wastewater are very high and COD values greater than 60,000 mg/L are often reported (Bala *et al.* 2015a; Bala 2016). Accordingly, the adverse environmental impact from the palm oil industry cannot be overlooked. Consequently, the challenge of converting POME into an environmental friendly waste necessitates a well-organised treatment and effectual removal method.

The physicochemical properties of POME are well documented. Conversely, the microbiological aspect is overlooked; as such there seem to be dearth of information on the microbiota been documented proving that a well-developed understanding of these is needed. Therefore, this study represents one of the few studies in Malaysia. The diverse microbiota communities are known to participate effectively in the biodegradation and bioremediation of POME. Consequently, the study on the microbiological characteristics of POME lays a basis to promote better understanding of the types and nature of microorganisms domicile in POME. This will provide evidence of the microbiota characteristics of POME. Their involvement in biodegradation and biotreatment of POME may possibly abet in achieving higher reduction of organic load present in POME. This study was designed to explore the microorganisms associated with palm oil mill wastewater and to establish the microbial load from MALPOM Sdn. Bhd. in Pulau Pinang, Malaysia.

## **MATERIALS AND METHODS**

### **Sample Collection and Preservation**

Raw POME was collected aseptically from MALPOM Sdn. Bhd., Pulau Pinang, palm oil mill industry in a sterile microbiological container (20 L) and brought back to the laboratory. In collecting raw POME sample from the POME holding tank, the mouth of the tap connected to the holding tank was swabbed with cotton wool soaked in ethanol. This was done in order to disinfect the mouth of the tap. The tap was allowed to run for few minutes and the container was used to collect the POME sample and quickly corked. Prior to sample collection, the POME sample inside the container was inverted a few times in ordered to rinse the inside wall of the container. The sample was later poured out into the surrounding. This step was done three times and the container was finally placed to collect the POME sample. The POME sample was kept in an ice box while transporting to School of Industrial Technology laboratory, Universiti Sains Malaysia and preserved at 4°C until further experiment in order to stop the wastewater from undergoing biodegradation due to microbial action (American Public Health Association [APHA] 2005). Sample was brought out from the refrigerator and left at room temperature before use.

## **Isolation and Enumeration of Total Heterotrophic Indigenous Palm Oil-Utilising and Cellulose Utilising Bacteria From POME**

The populations of microorganisms in the raw POME sample was enumerated using standard spread plate method (APHA 2005; Bala *et al.* 2015a; Bala 2016). The POME was well shaken to homogenised suspension and thereafter, ten-fold (10-fold) serial dilution was made by aseptically transferring one milliliter (1 mL) of the homogenised suspension into a sterile test tubes containing nine milliliter (9 mL) of sterile, distilled water. Then, using a sterile pipette, 0.1 mL aliquots of the dilutions were aseptically removed with a sterile pipette and separately spread plated with flamed-sterilised glass spreader (bent glass rod) on well-dried Nutrient Agar (NA), oil agar (Palm Oil Agar [POA]) Mineral Salts Medium (MSM) for bacteria and Carboxymethyl cellulose (CMC) agar plates for bacteria in triplicates for the enumeration of viable heterotrophic bacteria, palm oil utilising and cellulose utilising bacteria respectively. The plates were inoculated using spread plate technique (APHA 2005; Bala *et al.* 2015a; Bala 2016). The culture plates were incubated at 37°C for 24–48 h. Three uninoculated plates were used as control. After incubation, plates that contained 30–300 colony forming units (cfu) were selected and counted with the aid of a colony counter. Viable numbers of colonies on each plate were enumerated and expressed or recorded as colony forming units per milliliter (cfu/mL) of the sample. Colonies were purified by repeatedly subcultured aseptically on to fresh NA, oil agar and CMC agar and incubated at 37°C for 48 h to obtain discrete pure colonies. Pure colonies were then stored on NA, oil agar and CMC agar slants at 8°C to maintain viability for subsequent analysis and identification. Gram staining was performed for all the isolates. The medium was incorporated with Ketoconazole antifungal (known as funginox) to inhibit fungal growth.

### **Preparation and Composition of Mineral Salt Medium (MSM) for Palm Oil Utilising Bacteria**

The MSM (oil agar medium) for palm oil utilising bacteria was prepared according to the MSM composition of Zajic and Supplisson (1972). The composition of the medium was  $\text{NH}_4\text{Cl}$  (4.0 g),  $\text{K}_2\text{HPO}_4$  (1.8 g),  $\text{KH}_2\text{PO}_4$  (1.2 g),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.2 g),  $\text{NaCl}$  (0.1 g),  $\text{FeSO}_4$  (0.01 g), 15 g agar and distilled water, 1 L). The medium was used for isolation, enumeration and identification of palm oil-utilising bacteria (oil degraders). The medium was prepared by the addition of 1% (v/v) palm oil as sole source of carbon and energy, sterilized with 0.45  $\mu\text{m}$  pore size Millipore filter paper to sterile MSM, which has been cooled to 45°C under aseptic condition. 200 mg ketoconazole antifungal (known as funginox) was added to prevent fungal growth. The MSM and palm oil were then mixed thoroughly and dispensed into sterile Petri dishes to solidify.

## Isolation and Enumeration of Total Heterotrophic Indigenous Palm Oil-Utilising and Cellulose Utilising Fungi from POME

The standard procedures for serial dilution aforementioned for bacterial isolation were followed for fungal isolation. Thereafter, using a sterile pipette, 0.1 mL aliquots of the dilutions were aseptically removed with a sterile pipette and separately spread plated with flamed-sterilised glass spreader (bent glass rod) on well-dried Potato Dextrose Agar (PDA), oil agar (POA) MSM for fungi and Carboxymethyl cellulose (CMC) agar plates for fungi in triplicates for the enumeration of viable heterotrophic fungi, palm oil utilising and cellulose utilising fungi respectively. The plates were inoculated on the surface using the standard spread plate technique (APHA 2005). The plates were allowed to remain undisturbed for 25 min in the laminar flow before being inverted and incubated.

The culture plates were incubated at 28°C for 5–7 days (APHA 2005). Three uninoculated plates were used as control. After incubation, viable numbers of colonies on each plate were enumerated and expressed or recorded as colony forming unit per milliliter (cfu/mL). Colonies were purified by repeatedly sub culturing aseptically on to fresh PDA, oil agar and CMC agar and incubated at 28°C for 5–7 days to obtain discrete pure colonies. Pure colonies were then stored on PDA, oil agar and CMC agar slants at 8°C to maintain viability for subsequent analysis and identification. Staining was also performed for all the isolates using lacto phenol cotton blue solution. The medium was incorporated with Altacef antibiotic to inhibit bacterial growth.

## Preparation and Composition of Mineral Salt Medium (MSM) for Palm Oil Utilising Fungi

The MSM (oil agar medium) for palm oil utilising fungi was prepared according to the MSM composition of Mills *et al.* (1978) as modified by Okpokwasili and Okorie (1988). The composition of the medium was NaCl, 10.0g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.42g; KCl, 0.29 g; KH<sub>2</sub>PO<sub>4</sub>, 0.83 g; Na<sub>2</sub>HPO<sub>4</sub>, 1.25 g; NaNO<sub>3</sub>, 0.42 g; agar, 20 g; distilled water, 1 L and pH of 7.2. The medium was used for isolation, enumeration and identification of palm oil-utilising fungi (oil degraders). The medium was prepared by the addition of 1% (v/v) palm oil as sole source of carbon and energy, sterilised with 0.45 µm pore size Millipore filter paper to sterile MSM, which has been cooled to 45°C under aseptic condition. 250 mg Altacef antibiotic, was added to prevent bacterial growth. The MSM and palm oil were then mixed thoroughly and dispensed into sterile Petri dishes to solidify.

## Identification of Bacteria Isolates by Sequencing of 16S rRNA Gene

Initial identification of individual bacterial isolates was achieved by standard tests (Bergey *et al.* 1994). Such identification included the shape of cells, Gram's reaction and colony morphology on solid nutrient media. Genetic identification of bacterial

isolates was performed by determining nucleotide sequences of 16S rRNA genes using commonly used primers (Table 1) for amplifying the DNA between positions 27 and 1492 of bacterial 16S rRNA genes. Genetic identification of the pure cultures of bacterial isolated from POME were sent to Centre for Chemical Biology (CCB), Universiti Sains Malaysia for sequencing of the 16S rRNA gene. Inoculum preparation was carried out by inoculating bacteria strains in nutrient broth, fungi in potato dextrose broth, incubated for 24 h (bacteria), 2–3 days (fungi) at 37°C and 28°C respectively.

**Table 1:** Genetic Identification of bacterial isolates in POME

	Bacteria
Nucleotide Sequences	16S rRNA gene
Sequences of Primers	<b>27F: 5'-AGAGTTTGATCMTGGCTCAG-3'</b> <b>1492R: 5'-GGGTACCTTGTTACGACTT-3'</b>
Strains	<i>Micrococcus luteus</i> 101PB ( <b>Accession No. AB539843.1</b> ) <i>Stenotrophomonas maltophilia</i> 102PB ( <b>Accession No. JQ 619623.1</b> ) <i>Bacillus cereus</i> 103PB ( <b>Accession No. JF 432000.1</b> ) <i>Providencia vermicola</i> 104PB ( <b>Accession No. KC775772.1</b> ) <i>Klebsiella pneumoniae</i> 105PB ( <b>Accession No. GU128173.1</b> ) <i>Bacillus subtilis</i> 106PB ( <b>Accession No. KF624694.1</b> )

### Identification of Fungal Isolates by Sequencing of 18S rRNA Gene

Initial identification of individual fungal isolates was based on microscopic staining of fungi using lactophenol blue solution (Lactophenol cotton blue solution) and macroscopic appearance which comprise pigmentation/colour, identified on the basis of cultural (colour and colonial appearance of fungal colony) and morphological characteristics in lacto-phenol blue solution wet mount by compound microscope. Genetic identification of fungal isolates was performed by determining nucleotide sequences of 18S rRNA genes using commonly used primers (Table 2) for amplifying the DNA. Genetic identification of the pure cultures of fungal isolated from POME were sent to CCB, Universiti Sains Malaysia for sequencing of the 18S rRNA gene.

**Table 2:** Genetic Identification of fungal isolates in POME

	Fungi
Nucleotide Sequences	18S rRNA genes
Sequences of Primers	<b>ITS1 F: 5'-TCCGTAGGTGAACCTGCGG -3'</b> <b>ITS4 R: 5'-TCCTCCGCTTATTGATATGC-3'</b>
Strains	<i>Aspergillus fumigatus</i> 107PF ( <b>Accession No. EU664467.1</b> ) <i>Aspergillus nomius</i> 108PF ( <b>Accession No. DQ467991.1</b> ) <i>Aspergillus niger</i> 109PF ( <b>Accession No. KC119204.1</b> ) <i>Meyerozyma guilliermondii</i> 110PF ( <b>Accession No. JN183444.1</b> )

## RESULTS AND DISCUSSION

### Microbial Populations of POME Sample

The microbial population, total heterotrophic bacteria (THB) and total heterotrophic fungi (THF) of POME are presented in Table 3 and oil degrading bacteria (ODB) and oil degrading fungi (ODF) are presented in Table 4.

**Table 3:** Microbial populations of POME.

Media	Isolates	Total heterotrophic counts (THC)
Nutrient agar (NA)	Bacteria	$9.5 \times 10^5 - 7.9 \times 10^6$ cfu/mL
Potato Dextrose agar (PDA)	Fungi	$2.1 \times 10^4 - 6.4 \times 10^4$ cfu/mL
Carboxymethyl cellulose (CMC) agar	Bacteria	$8.2 \times 10^5 - 9.1 \times 10^6$ cfu/mL
Carboxymethyl cellulose (CMC) agar	Fungi	$1.4 \times 10^3 - 3.4 \times 10^3$ cfu/mL

**Table 4:** Oil degrading microbes of POME.

Media	Isolates	Counts (cfu/mL)
Oil agar (MSM) Palm oil agar (POA)	Bacteria	$6.4 \times 10^5 - 4.8 \times 10^6$
Oil agar (MSM) Palm oil agar (POA)	Fungi	$2.8 \times 10^3 - 4.7 \times 10^4$

The population of total heterotrophic bacteria (THB) ranged from  $9.5 \times 10^5 - 7.9 \times 10^6$  cfu/mL. The total heterotrophic fungi (THF) ranged from  $2.1 \times 10^4 - 6.4 \times 10^4$  cfu/mL. Total viable heterotrophic indigenous (autochthonous) microbial population on CMC agar ranged from  $8.2 \times 10^5 - 9.1 \times 10^6$  cfu/mL and  $1.4 \times 10^3 - 3.4 \times 10^3$  cfu/mL for bacteria and fungi respectively. The microbial population of oil degrading bacteria (ODB) ranged from  $6.4 \times 10^5 - 4.8 \times 10^6$  cfu/mL and the oil degrading fungi (ODF) ranged from  $2.8 \times 10^3 - 4.7 \times 10^4$  cfu/mL (Tables 3 and 4). The findings revealed that ODB and ODF flourish well in oily waste water. Awotoye *et al.* (2011) reported THB, THF, ODF and ODB population of  $1.8 \times 10^6$  cfu/g,  $9.5 \times 10^2$  cfu/g,  $1.2 \times 10^2$  cfu/g and  $4.0 \times 10^2$  cfu/g in that order at the point of POME release from oil palm milling machine. Ugoji (1997) specified that THB and THF are  $1.3 \times 10^6$  cfu/mL and  $1.0 \times 10^3$  cfu/mL correspondingly in POME.

In a related study, Okwute and Isu (2007a; 2007b) have reported total aerobic bacterial populations of  $9.6 \times 10^8$  cfu/mL,  $1.64 \times 10^9$  cfu/mL and  $1.07 \times 10^9$  cfu/mL in POME samples. In addition, Okwute (2013) has also confirmed the population of THB, THF and ODB as  $4.0 \times 10^9$  cfu/mL,  $2.6 \times 10^3$  cfu/mL and  $2.6 \times 10^3$  cfu/mL in that order. The counts were also comparable to those described by Serikovna *et al.* (2013) with the index of  $10^8$  cfu/mL,  $10^7$  cfu/mL and  $2 \times 10^8$  cfu/mL as well as Wu *et al.* (2009) who revealed in their study the count of  $6.65 \times 10^6$  cfu/mL from oily wastewaters. Ohimain *et al.* (2012a) has also stated that the population of total heterotrophic bacteria (THB) ranged from

$7.4 \times 10^5 - 2.0 \times 10^6$  cfu/mL and total heterotrophic fungi (THF) ranged from  $3.1 - 5.7 \times 10^4$  cfu/mL while the oil degrading bacteria (ODB) ranged from  $6.5 \times 10^5 - 2.0 \times 10^6$  cfu/mL and the oil degrading fungi (ODF) ranged from  $3.1 - 5.6 \times 10^4$  cfu/mL in POME sample. Bala et al. (2012) has also reported similar counts from pharmaceutical wastewater. These corroborate the presence of diverse microorganisms in wastewaters (Bala 2016).

Results from the present study aforementioned confirmed some disparity in the microbial counts. The variations in the range of microbial populations are an indication of several reasons such as nutrient, minerals, temperature, oxygen level, acidity, volume of wastewater (Okereke et al. 2007), concentration of oil and grease and sugars in the POME. High population of bacteria in the POME may possibly be linked with contaminations from poor sanitation in the mills (Okechalu et al. 2011), and intermittent disinfection of the environment. Besides, it may also be due to the handling process and the existing environmental conditions in the mills. The presence and growth of viable bacteria and fungi in POME may possibly be associated with the fact that POME is rich in carbohydrates, proteins, nitrogenous compounds, lipids, minerals, cellulose, hemicelluloses and lignin (Hii et al. 2012). The microbes isolated in the present study conceivably derive their nutrients from the aforementioned compounds in raw POME.

The microbial species found in POME has the prospective to degrade carbon source present in the POME. Bala et al. (2014b) and Bala (2016) has reported that *Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB and *Bacillus subtilis* 106PB showed high lipase activity on solid media indicating their ability for degrading lipid (oil) as carbon source and producing lipase enzyme. The types of organisms isolated in the present study were also identified as oil degrading microorganisms by Bharathi and Vasudevan (2001) and Rahman et al. (2002) because of their ability to hydrolyse lipid (oil). Biodegradation is connected with the capability of bacteria and fungi to grow on and degrade carbon sources in industrial wastewaters (Haimann 1995). The high organic matter in palm oil mill wastewater possibly will have played an essential role in the abundance of aerobic and facultative anaerobic microbial strains in the present study.

### **Genetic Identification of Bacteria and Fungi Isolates in POME Sample**

Tables 1 and 2 present the microorganisms isolated from POME based on 16S rRNA gene and 18S rRNA genes for bacteria and fungi respectively. Identification of isolates was performed by determining nucleotide sequences of 16S rRNA and 18S rRNA genes for bacteria and fungi in that order. The isolates were identified by sequences analysis of 16S rRNA and 18S rRNA genes. Sequencing of the 16S rRNA and 18S rRNA of the microbial strains suggest that they were known as *Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, *Providencia vermicola* 104PB, *Klebsiella pneumoniae* 105PB, *Bacillus subtilis* 106PB, *Aspergillus fumigatus* 107PF, *Aspergillus nomius* 108PF,

*Aspergillus niger* 109PF and *Meyerozyma guilliermondii* 110PF. Plates and Figures showing identified bacteria and fungi in POME sample is presented in Appendix A to H.

The results from the present study revealed that the microbes isolated are comparable to those found in areas polluted with wastewaters (Abass *et al.* 2012; Soleimaninanadegani & Manshad 2014; Bala *et al.* 2015a) and crude oil or petroleum hydrocarbons (Okereke *et al.* 2007). Bala *et al.* (2012) had also reported the isolation of *Bacillus subtilis* from industrial wastewater. Conversely, *Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, *Bacillus subtilis* 106PB, *Aspergillus fumigatus* 107PF and *Aspergillus niger* 109PF are lipase and cellulase producing organisms isolated from the present study.

The development of spores makes POME microorganisms to be quiescent and highly resistant to lethal consequence of boiling, dry heating and ultra violet radiation from the sunlight (Okechalu *et al.* 2011). Palm oil mill wastewater is a possible habitat for lipolytic and cellulolytic bacteria and fungi since it is rich in nutrients such as lipids (oil) and cellulosic materials (Ohimain *et al.* 2012a; 2012b; Bala 2016).

Ohimain *et al.* (2012a) isolated lipase and cellulase producing *Bacillus* sp from POME collected from palm oil processing environment. Asikong (1994) identified *Aspergillus* sp. as fungal species linked with lipase and cellulase production. *Aspergillus* sp. is particularly reported to be good producers of cellulase and lipase. These enzymes are responsible for the breakdown of cellulose and oil in POME (Wong *et al.* 2008). *Aspergillus niger* and *Aspergillus fumigatus* have been well-known for their capability to survive in oily wastewater such as Palm oil mill wastewater due to the presence of nutrients such as lipids (oil). Fungi are particularly aerobic and can also grow under environmental strained conditions such as low pH and poor nutrient status. Lipase facilitates the hydrolysis of lipid causing succeeding breakdown into fatty acid and alcohol (Guehi *et al.* 2007; Ghosh *et al.* 1996). Other researchers have also isolated comparable microbes aforementioned above at 28°C-37°C from POME sample (Bhumibhamon *et al.* 2002; Ohimain *et al.* 2012a; 2012b; Okwute 2013; Soleimaninanadegani & Manshad 2014; Bala 2016).

The prevalence of these microbes (bacteria and fungi) in palm oil mill wastewater may perhaps be due to their capability to make use of oil and cellulose as their sole carbon source which has been formerly reported by Ojumu *et al.* (2005), Bala *et al.* (2014b), Bala *et al.* (2015b), and Bala (2016). The use of POME as a carbon source by these microorganisms has been reported by Wu *et al.* (2007), Sira *et al.* (2010) and Bala (2016). The presence of *Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, *Bacillus subtilis* 106PB, *Aspergillus fumigatus* 107PF and *Aspergillus niger* 109PF isolated from POME sample in the current study revealed that these microorganisms are capable of biodegradation of oily wastewaters as reported by other researchers (Ohimain *et al.* 2012a; 2012b; 2012c; Nwuche & Ogbonna 2011).

Microorganisms present in POME have been used for the treatment of wastewaters such as palm oil mill wastewater and olive oil mill wastewater for the reduction of COD (Oswal *et al.* 2002; Ohimain *et al.* 2012a; Kamal *et al.* 2011; Neoh *et al.* 2013; Nawawi *et al.* 2010; Ahmad *et al.* 2011; Bala *et al.* 2014c; Bala *et al.* 2015a; Bala 2016). During degradation process, oil and cellulose in POME are broken down by effective microbes which make use of the organic waste present in palm oil mill wastewater and degrades these organic matters into water and carbondioxide (Singh *et al.* 2010; Jameel & Olanrewaju 2011). *Aspergillus fumigatus* 107PF, *Aspergillus niger* 109PF, *Micrococcus luteus* 101PB, *Stenotrophomonas maltophilia* 102PB, *Bacillus cereus* 103PB, and *Bacillus subtilis* 106PB have been isolated for POME with potential to degraded oil and cellulose (Bala *et al.* 2014b; Bala *et al.* 2015b; Bala 2016). The aforesaid microbes thus exhibited comparable biodegradation potential with published literatures. The oily habitat in palm oil mill wastewater possibly will make available a good environment for lipolytic microorganisms to grow due to the oil present in the wastewater which serves as carbon source. However, the present of these microbes in POME are useful in degrading contaminated pollutants in wastewaters such as crude oil (hydrocarbon) (Ohimain *et al.* 2012a; Soleimaninanadegani & Manshad 2014). Palm oil mill wastewater is inhabited by dissimilar types of microbes which plays a fundamental task in the biotreatment, bioremediation and biodegradation of oil-containing wastewaters (Hassen-Aboushiba *et al.* 2013; Tan *et al.* 2015).

**Table 5:** Cultural characteristics of bacteria isolated from POME.

Organisms	Characteristics	Gram's reaction
<i>Micrococcus luteus</i> 101PB	Circular, pinhead colonies which are convex with entire margins. Colonies produces a bright yellow, nondiffusable pigment	Positive cocci
<i>Stenotrophomonas maltophilia</i> 102PB	circular, smooth, convex, moist and pigmented colonies	Negative rod
<i>Bacillus cereus</i> 103PB	Large, irregular, opaque colonies. Smooth and moist colonies, whitish to cream	Positive rod
<i>Providencia vermicola</i> 104PB	Colonies are circular, shining, slimy, convex, and opaque with a brownish centre. Brown pigment is produced, colouring the medium around the colonies. Colonies are smooth with entire edges.	Negative rod
<i>Klebsiella pneumoniae</i> 105PB	Distinctive yeasty odor and bacterial colonies have a viscous/mucoid appearance	Negative rod
<i>Bacillus subtilis</i> 106PB	Dry, flat, and irregular, with lobate margins; colonies round or irregular; surface dull; become thick and opaque; whitish	Positive rod

Conversely, in view of the fact that most of the microbes domiciled in POME form spores, it facilitate their survival and continued existence in harsh or stressed normal conditions of palm oil mill wastewater such as absence of air or free oxygen (anaerobiosis), soaring concentration of oil and grease (Okechalu *et al.*

2011; Ugoji 1997), and acidity (Leslie-Grady *et al.* 1999; Breccari *et al.* 1996; Poh & Chong 2009; Ugoji 1997). This corroborates with the study of Bala *et al.* (2015a) who reported in their investigation a low pH of 4.74 from raw palm oil mill wastewater in Malaysia. Under anaerobic conditions, methane and carbon dioxide are produced (Ugoji 1997). The anaerobic microflora inhabitant of palm oil mill wastewater sludge may well be valuable for the manufacture of biohydrogen and biogas production by fermentation during treatment (Vijayaraghavan & Ahmad 2006; Atif *et al.* 2005; Ismail *et al.* 2010). Table 5 revealed cultural characteristics of bacteria isolated from palm oil mill wastewater while Table 6 revealed microscopic, macroscopic morphology and cultural characteristics of fungi isolated from palm oil mill wastewater.

**Table 6:** Microscopic, macroscopic morphology and cultural characteristics of fungi isolated from POME.

Organisms	Type of organisms	Microscopic morphology	Macroscopic morphology
<i>Aspergillus fumigatus</i> 107PF	Filamentous mold	Presence of rough conidiophore, with uni/biseriate phialides whose vesicle is round with radiate head. Brownish sclerotia were also observed	Presence of blue-green to yellow coloration from surface
<i>Aspergillus nomius</i> 108PF	Filamentous mold	Presence of septate hyphae and colourless and rough conidiophores with swollen vesicles	A brownish colour with a creamy edge that appears golden in the reverse of the septate
<i>Aspergillus niger</i> 109PF	Filamentous mold	Presence of septate hyphae, long and smooth conidiophores, and long unbranched sporangiospores with large, round head	Brownish-black mycelium with dark spores and often appears golden on the reverse side
<i>Meyerozyma guilliermondii</i> 110PF	Yeast	Clusters of small blastospores along the pseudohyphae and particularly at septal points. Pseudohyphae are short and few in number	Colonies are flat, moist, smooth, and cream to whitish in colour

## CONCLUSION

Results from the current study revealed the presence of diverse types of microorganisms domiciled in palm oil mill wastewater. This conclusion suggests that microorganisms thrive well in palm oil mill wastewater. The investigation provides insight on the exploitation of microbial strains in biotreatment of industrial agricultural based wastewaters such as palm oil mill wastewater. The diversity of microbial strains isolated from palm oil mill wastewater provides a basis to promote better understanding of the types and nature of microorganisms domicile in palm oil mill wastewater. This will provide evidence on the microbiota

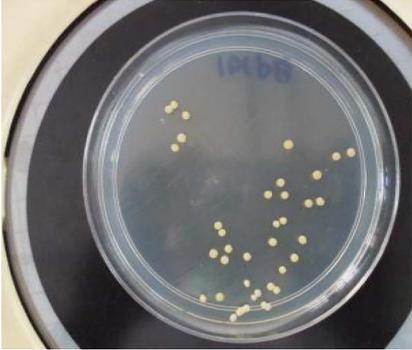
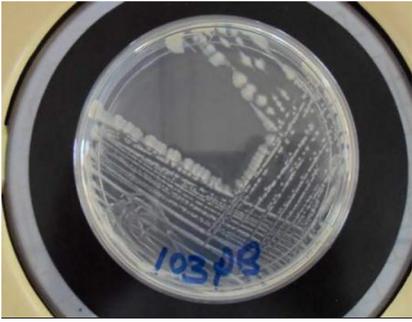
characteristics of palm oil mill wastewater. Conversely, this signifies the optimism for identification of native microbes from palm oil mill wastewater for biodegradation and bioremediation of industrial wastewaters. Study on metagenomic and transcriptomics characterisation is required for further identification of microbial strains diversity using Next-Generation Sequencing (NGS).

## **ACKNOWLEDGEMENTS**

The authors would like to thank Universiti Sains Malaysia for the financial support under Research University (RUI) Grant No. 1001/PTEKIND/814147.

**APPENDIX A**

Identified bacteria in POME sample.

Strains	Image	Gram's reaction
<p><i>Micrococcus luteus</i> 101PB (Pure culture)</p>		<p>Gram positive cocci</p>
<p><i>Stenotrophomonas maltophilia</i> 102PB (Pure culture)</p>		<p>Gram negative rod</p>
<p><i>Bacillus cereus</i> 103PB (Pure culture)</p>		<p>Gram positive rod</p>

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Strains	Image	Gram's reaction
<i>Providencia vermicola</i> 104PB (Pure culture)		Gram negative rod
<i>Klebsiella pneumoniae</i> 105PB (Pure culture)		Gram negative rod
<i>Bacillus subtilis</i> 106PB. (Pure culture)		Gram positive rod

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## APPENDIX B

### Identified fungi in POME sample

*Aspergillus fumigatus* 107PF  
(Microscopic staining)

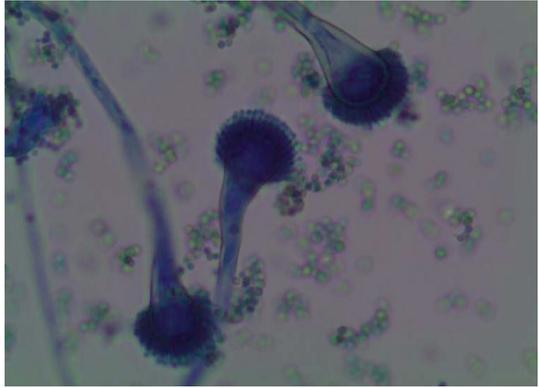


Plate 7

*Aspergillus nomius* 108PF  
(Microscopic staining)

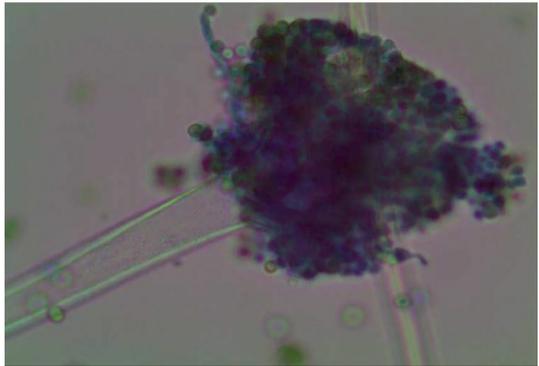


Plate 8

*Aspergillus niger* 109PF  
(Microscopic staining)

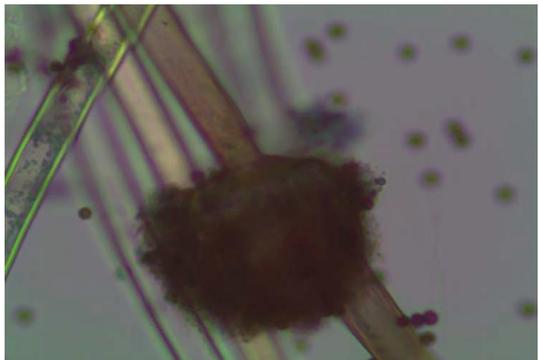


Plate 9

*Jeremiah David Bala et al.*

*Meyerozyma guilliermondii* 110PF  
(Microscopic staining)



Plate 10

*Aspergillus fumigatus* 107PF  
(Pure culture)

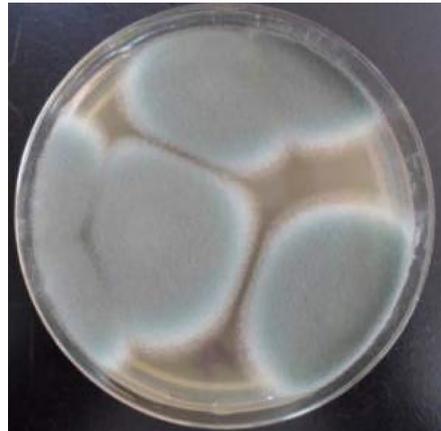


Plate 11

*Aspergillus nomius* 108PF  
(Pure culture)



Plate 12

*Aspergillus niger* 109PF  
(Pure culture)

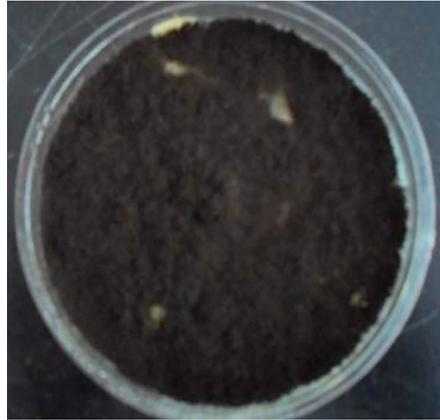


Plate 13

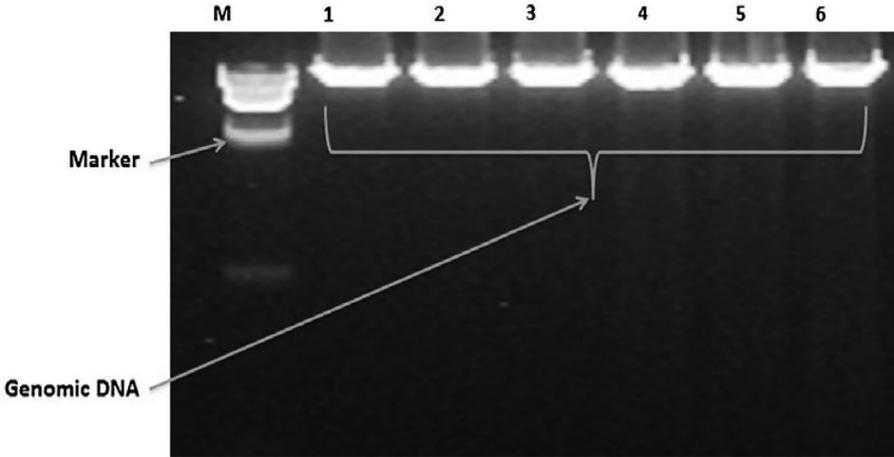
*Meyerozyma guilliermondii* 110PF  
(Pure culture)



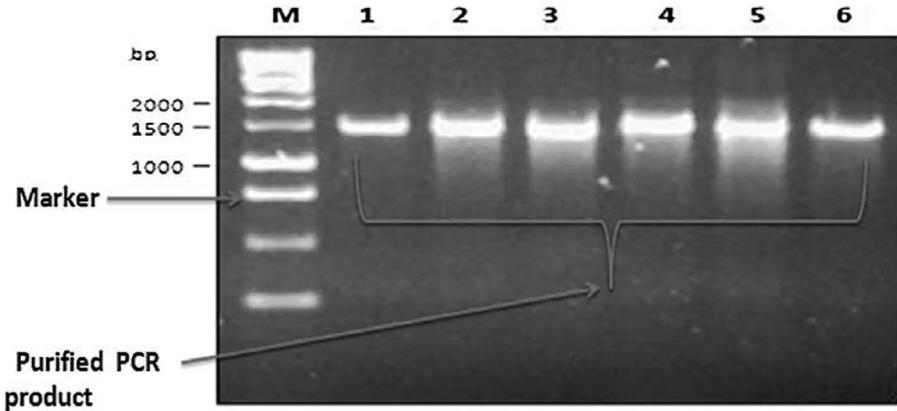
Plate 14

### APPENDIX C

Plates showing genomic DNA and purified PCR product of bacteria isolated from POME



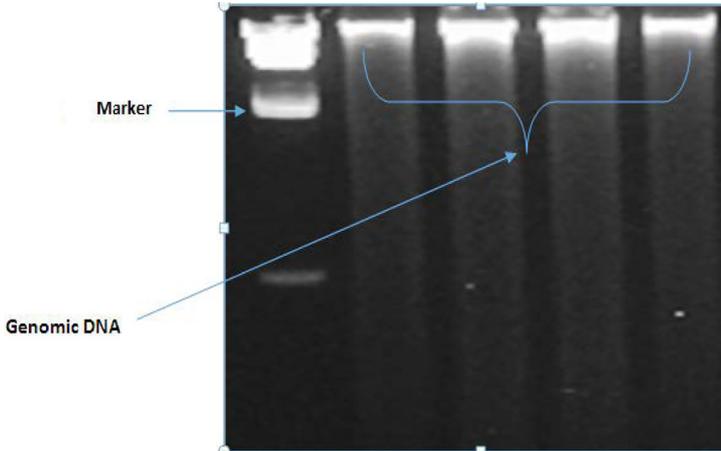
**Plate 15:** Gel picture of genomic DNA: **Lane 1:** 101PB; **2:** 102PB; **3:** 103PB; **4:** 104PB; **5:** 105PB; **6:** 106PB; **M:** Lambda/HindIII marker



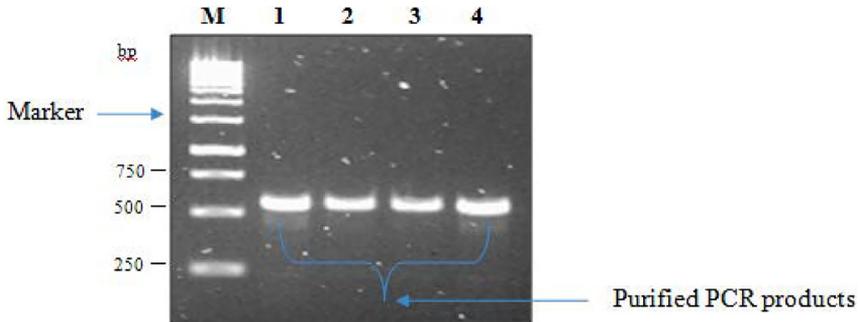
**Plate 16:** Gel picture of purified PCR product: **Lane 1:** 101PB; **2:** 102PB; **3:** 103PB; **4:** 104PB; **5:** 105PB; **6:** 106PB; **M:** 1 kb marker (Fermentas).

## APPENDIX D

Plates showing genomic DNA and purified PCR product of fungi isolated from POME



**Plate 17: Gel picture of genomic DNA: Lane 1: 107 PF; 2: 108 PF; 3: 109 PF; 4: 110 PF; M: Lambda/HindIII marker.**



**Plate 18: Gel picture of purified PCR product: Lane 1: 107 PF; 2: 108 PF; 3: 109 PF; 4: 110 PF; M: 1 kb marker (Fermentas).**

## APPENDIX E

### DNA sequence of bacterial strains isolated from POME

TCGAACGATGAAGCCCAGCTTGCTGGGTGGATTAGTGCGAACGGGTGAGTAACACGGAGTAA  
CCTGCCCTTAACTCTGGGATAAGCCTGGGAACTGGGTCTAATACCGGATAGGAGCGCCACCG  
CATGGTGGGTGTTGGAAAGATTATCGGTTTTGGATGGACTCGCGGCCTATCAGCTTGTGGTGA  
GGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGG  
ACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAA  
GCCTGATGCAGCGACGCCCGCTGAGGGATGACGGCCTCGGGTTGTAACCTCTTTCAGTAGG  
GAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCG  
GTAATACGTAGGGTGCAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGT  
GCGTCTGTCTGAAAAGTCCGGGGCTTAAACCCGGATCTGCGGTGGGTACGGGCAGACTAGAGT  
GCAGTAGGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCG  
ATGGCGAAGGCAGGCTCTGGGCTGTAACCTGACGCTGAGGAGCGAAAGCATGGGAGCGAACA  
GGATTAGATACCCTGGTAGTCCATGCCGTAACGTTGGGCACTAGGTGTGGGGACCATTCCACGG  
TTCCGCGCCGAGCTAACGCATTAAGTGCCCGCCTGGGAGTACGGCCGCAAGGCTAAACT  
CAAAGGAATTGACGGGGGCCCGACAAGCGGGGAGCATGCGGATTAATTGATGCAACGCGA  
AGAACCTTACCAAGGCTTGACATGTTCTCGATCGCCGTAGAGATACGGTTTTCCCTTTGGGGCGG  
GTTACAGGTGGTGCATGTTTGTCTGACGCTCGTGTCTGAGATGTTGGGTTAAGTCCCGCAAC  
GAGCGCAACCCTCGTTCATGTTGCCAGCAGCTCGTGGTGGGGACTCATGGGAGACTGCCGGG  
GTCAACTCGGAGGAAGGTGAGGACGACGTCAAATCATCATGCCCTTATGTCTTGGCTTCACG  
CATGCTACAATGGCCGGTACAATGGGTTGCGATACTGTGAGGTGGAGCTAATCCAAAAAGCCGG  
TCTCAGTTCGGATTGGGCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATC  
AGCAACGCTGCGGTGAATACGTTCCCGGGCCTGTACACACCGCCCGTCAAGTCACGAAAGTCG  
GTAACACCCGAAGCCGGTGGCCTAACCC

**Figure 1: *Micrococcus luteus* 101 PB (Accession NO. AB539843.1)**

GCTTGCTCTCTGGGTGGCGAGTGCGGACGGGTGAGGAATACATCGGAATCTACTCTGTCTGG  
GGGATAACGTAGGGAACTTACGCTAATACCGCATAACGACTACGGGTGAAAGCAGGGGACCTTC  
GGGCTTGCAGGATTGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCACCAA  
GGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAAGTGGAGACAGGTCCAGA  
CTCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCG  
CGTGGGTGAAGAAGGCCCTTCGGGTTGTAAGCCCTTTTTGTTGGGAAAGAAATCCAGCTGGCTAA  
TACCCGGTTGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGG  
TAATACGAAGGGTGAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCCTAGGTGGTCTTTA  
AGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGATACTGGGCGACTAGAATGT  
GGTAGAGGGTAGCGGAATTCCTGGTGTAGCAGTGAAATGCGTAGAGATCAGGAGGAACATCCAT  
GGCGAAGGCAGCTACCTGGACCAACATTGACACTGAGGCACGAAAGCGTGGGAGCAAAACAGG  
ATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCAG  
CAGTATCGAAGCTAACGCGTTAAGTTCCGCCGCTGGGAGTACGGTTCGCAAGACTGAAACTCAA  
AGGAATTGACGGGGGCCCGCACAAAGCGGTGGAGTATGTGGTTTAAATTCGATGCAACGCGAAGAA  
CCTTACCTGGCCTTGACATGTCGAGAACCTTCCAGAGATGGATTGGTGCCTTCGGGAACTCGAAC  
ACAGGCTGCTGATGGCTGCTGTCAGCTCGTGTCTGAGATGTTGGGTTAAGTCCCGCAACGAGC  
GCAACCCTTGTCTTAGTTGCCAGCAGCTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAA  
ACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACAGGTAC  
TACAATGGTAGGGACAGAGGGCTGCAAGCCGGCGACGGTAAGCCAATCCAGAAACCCTATCTC  
AGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCA  
TTGCTGCGGTGAATACGTTCCCGGGCCTGTACACACCGCCCGTACACCATGGGAGTTTGTG  
CACCAGAAGCAGGTAGCTTAACTTCGGGAGGGC

**Figure 2: *Stenotrophomonas maltophilia* 102PB (Accession No. JQ 619623.1)**

TGCAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACA  
CGTGGGTAACCTGCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATT  
TTGAAACCGCATGGTTCCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCGCGTCG  
CATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTG  
ATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTT  
CCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTGCTAA  
AACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCA  
GAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGTGGCAAGCGTTATCCGGA  
ATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCACGGCTCAACC  
GTGGAGGGTCATTGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGAATTCCATGTGTAG  
CGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGGCGACTTTCGGTCTGTAAGT  
ACACTGAGGCGCGAAAGCGTGGGGAGCAACAGGATTAGATACCCTGGTAGTCCACGCCGTAA  
CGATGAGTGCCTAAGTGTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTAAACCGCATTAAGCACTCC  
GCCTGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAGCGG  
TGGAGCATGTGGTTAATTGGAAGCAACGCGAAGAACCCTACCAGGTCTTGACATCCTCTGACAA  
CCCTAGAGATAGGGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTACAGCTC  
GTGTCGTGAGATGTTGGGTTAAGTCCCAGCAGGCGCAACCCTTGATCTTAGTTGCCATCATT  
AGTTGGGCACTTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGATGACGCTCAAATC  
ATCATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACC  
GCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACAT  
GAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTA  
CACACCGCCCGTACACCACGAGAGTTTGAACACCCGAAGTCCGGTGGGGTAACCTTTTGGAGC  
CAGCCGC

**Figure 3: *Bacillus cereus* 103PB (Accession No. JF 432000.1)**

GTCGAGCGGTAACAGGGGAAGCTTGCTTCCCGCTGACGAGCGGCGGACGGGTGAGTAATGTAT  
GGGGATCTGCCGATAGAGGGGGATAACTACTGGAAACGGTGGCTAATACCGCATAATCTCTTAG  
GAGCAAAGCAGGGGAACCTTCGGTCTTGCCTATCGGATGAACCCATATGGGATTAGCTAGTAGG  
TGGGGTAATGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGATCAGCCACACTGG  
GACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCA  
AGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCCTAGGGTTGTAAAGTACTTTTCAGTCGG  
GAGGAAGGCGTTGATGTTAATATCATCAACGATTGACGTTACCGACAGAAGAAGCACCAGGCTAAC  
TCCGTGCCAGCAGCCGCGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAG  
CGCACGCAGGCGGTTGATTAAGTTAGATGTGAAATCCCCGGGCTTAACCTGGGAATGGCATCTAA  
GACTGGTCAGCTAGAGTCTTGATAGAGGGGGGTAGAATCCATGTGTAGCGGTGAAATGCGTAGA  
GATGTGGAGGAATACCGGTGGCGAAGGCGGCCCTTGACAAAGACTGACGCTCAGGTGCGAA  
AGCGTGGGAGCAACAGGATTAGATACCCTGGTAGTCCACGCTGTAACAGATGTCGATTTGGAG  
GTTGTGCCCTTGGGCGTGGCTTCCGGAGCTAACGCGTTAATCGACCGCCTGGGGAGTACGG  
CCGCAAGGTTAAACTCAAATGAATTGACGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAA  
TTCGATGCAACGCGAAGAACCCTTACCTACTCTTGACATCCAGAGAATTTAGCAGAGATGCTTTAGT  
GCCTTCGGGAACCTGAGACAGGTGCTGCATGGCTGTCGTGACGCTCGTGTGTAATGTTGGG  
TTAAGTCCCAGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGATTCCGGTCCGGAACCTCAAAG  
GAGACTGCCGGTGATAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAG  
TAGGGCTACACACGTGCTACAATGGCGTATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGAA  
CTCATAAAGTACGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCCGGAATCGCTA  
GTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTGTACACACCGCCCGTACAC  
CATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGG

**Figure 4: *Providencia vermicola* 104PB (Accession No. KC775772.1)**

AGGTTAAGGCTACCTACTTCTTTTGAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGC  
CCGGGAACGTATTCACCGTAGCATTCTGATCTACGATTACTAGCGATTCCGACTTCATGGAGTCGA  
GTTGCGAGACTCCAATCCGGACTACGACATACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTT  
CTTTTGTATATGCCATTGTAGCACGTGTGTAGCCCTGTTGTCGTAAGGGCCATGATGACTTGACGTC  
ATCCCCACCTTCTCCAGTTTACTACTGGCAGTCTCCTTTGAGTTCGGGCTAACCGCTGGCAA

CAAAGGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATTTACACAACACGAGCTGACGAC  
AGCCATGCAGCACCTGTCTCACAGTTCGCCGAAGGCACCAATCCATCTCTGGAAAGTTCTGTGGAT  
GTCAAAGACCAGGTAAGGTTCTTCGCGTTGCATCGAATTAACCACATGCTCCACCGCTTGTGCGG  
GCCCCCGTCAATTCATTTGAGTTTTAACCTTTCGCGCCGTAATCCAGGCGGTGATTTAACGCG  
TTAGCTCCGGAAGCCACGCCTCAAGGGCACAACTCCAAATCGACATCGTTTACGGCGTGGACT  
AACCAGGGTATCTAATCCTGTTTGTCCCCACGCTTTCGACCTGAGCGTCAGTCTTTGTCCAG  
GGGGCCGCTTCGCCACCGGTATTCTCCAGATCTCTACGCATTTACCGCTACACCTGGAATT  
CTACCCCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAGTTCAGGTTGAGCCCGGG  
GATTTACATCCGACTTGACAGACCGCCTGCGTGCGCTTTACGCCAGTAATCCGATTAACGCT  
TGCACCCTCCGATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACG  
TCAATCGACAAGGTTATTAACCTTACCGCCTTCTCCCCGCTGAAAGTGCTTTACAACCCGAAGG  
CCTTCTTACACACGCGGCATGGCTGCATCAGGCTTTCGCGCCATTGTGCAATATTTCCCACTGCT  
GCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCACTCTCAGACCAGC  
TAGGGATCGTCGCTAGGTGAGCCGTTACCCACCTACTAGCTAATCCCATCTGGGCACATCTGA  
TGCCATGAGGCCGAAGGTCCCCCACTTTGGTCTTTCGACATTATGCGGTATTAGCTACCGTTTC  
CAGTAGTTATCCCCCTCCATCAGGCAGTTCCAGACATTACTACACCCGTCGCCCGCTCGTAC  
CCGAGAGCAAGCTCTCTGTGCTACCG

**Figure 5: *Klebsiella pneumoniae* 105PB (Accession No. GU128173.1)**

GCTCCTAAAAGGTTACCTACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGT  
GTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGCGATTCCAGCTTC  
ACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTGGGATTGGCTTAACCTCG  
CGGTTTCGCTGCCCTTTGTCTGCCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCGATGA  
TGATTTGACGTCATCCCACTTCTCCGTTTGTACCGGCAGTCACCTTAGAGTGCCCAACTG  
AATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACAC  
GAGCTGACGACAACCATGCACCCTGTCACTCTGCCCCGAAGGGGACGTCCTATCTCTAGGA  
TTGTACAGAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCAATTAACCACATGCTCCA  
CCGCTTGTGCGGGCCCCGTCATTCCTTTGAGTTTCAGTCTTTCGACCGTACTCCCAAGGCGG  
AGTGCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCTAACACTTAGCACTCATCGTT  
TACGGCGTGGACTACCAGGGTATCTAATCCTGTTTCGCTCCCAACGCTTTCGCTCCTCAGCGTCAG  
TTACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTACCGCTAC  
ACGTGGAATTCACCTCTCTTCTGCACTCAAGTTCCCAAGTTTCCAATGACCTCCCCGGTTG  
AGCCGGGGGCTTTACATCAGACTTAAGAAACCGCCTGCGAGCCCTTACGCCAATAATTCCG  
GACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTT  
AGGTACCCTCAAGGTGCCGCCCTATTTGAACGGCATTGTTCTTCCCTAACCAACAGAGCTTACG  
ATCCGAAAACCTTCACTACGCGCGGCTTGTCCGTCAGACTTTCGTCATTGCGGAAGATTC  
CCTACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAAGTGTGGCCGATCACCTCT  
CAGGTGCGCTACGCATCGTCGCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGG  
GTCCATCTGTAAGTGGTAGCCGAAGCCACCTTTTATGTCTGAACCATGCGGTTCAAACAACCATC  
CGGTATTAGCCCCGGTTTCCCGGAGTTATCCCAAGTCTTACAGGCAGGTTACCCACGTGTTACTCA  
CCCGTCCGCCGTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGAC

**Figure 6: *Bacillus subtilis* 106PB (Accession No. KF624694.1)**

## APPENDIX F

### DNA sequence of fungal strains isolated from POME

CCTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCTCCC  
ACCCGTGTCTATCGTACCTTGTGCTTCGGCGGGCCCGCCGTTTCGACGGCCCGGGGAGGC  
CTTGCGCCCCCGGGCCCGCCCGCCGAAGACCCCAACATGAACGCTGTTCTGAAAGTATGCA  
GTCTGAGTTGATTATCGTAATCAGTTAAAACCTTCAACAACGGATCTCTTGGTTCGGGCATCGATG  
AAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAA  
CGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTATTGCTGCCCTCAAG  
CACGGCTTGTGTGTTGGGCCCCCGTCCCCCTCTCCCGGGGACGGGCCCGAAAGGCAGCGGC  
GGCACC CGTCCGGTCTCGAGCGTATGGGGCTTTGTACCTGCTCTGTAGGCCCGGCCGGCG  
CCAGCCGACACCCAACTTTATTTTTCTAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAAC  
TTAAGCATATCAATAAGCGGAGGA

**Figure 7:** *Aspergillus fumigatus* 107PF (Accession No. EU664467.1)

TTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGTTCCTAGCGAGCCCAACCTCC  
CACCCGTGTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCGAAGGCCCGGGGGGCATC  
CGCCCCCGGGCCCGCCCGCCGGAGACACCACGAACCTCTGAACGATCTAGTGAAGTCTGAGT  
TGATTGTATCGCAATCAGTTAAAACCTTCAACAATGGATCTCTTGGTTCGGGCATCGATGAAGAAC  
GCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCGTGAATCATCGAGTCTTTGAACGCAC  
ATTGCGCCCCCTGGTATTCGGGGGGGCATGCCTGTCCGAGCGTCTATTGCTGCCATCAAGCACG  
GCTTGTGTGTTGGGTGCTGCCCCCCCTCCGGGGGGGGACGGGCCCTAAAGGCAGCGGGC  
GCACCGCTCCGATCCTCGAGCGTATGGGGCTTTGTACCCCGCTCTGTAGGCCCGGCCGGCG  
TTGCCGAACGCAAAACAACATTCTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAA  
CTTAAGCATATCAATAAGCGGAGGA

**Figure 8:** *Aspergillus nomius* 108PF (Accession No. DQ467991.1)

TTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGTCTTTGGGCCCAACCTCCCAT  
CCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCCGGCCCGGGGGGGGCGCC  
TCTGCCCCCCGGGCCCGTGCCTCGCCGGAGACCCCAACACGAACACTGTCTGAAAGCGTGCAGT  
CTGAGTTGATTGAATGCAATCAGTTAAAACCTTCAACAATGGATCTCTTGGTTCGGGCATCGATGA  
AGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAAC  
GCACATTGCGCCCCCTGGTATTCCGGGGGGGCATGCCTGTCCGAGCGTCTATTGCTGCCCTCAAGC  
CCGGCTTGTGTGTTGGGTGCGCCGTCCCCCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGGC  
GCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTACATGCTCTGTAGGATTGGCCGGCGCC  
TGCCGACGTTTTCCAACATTCTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACT  
TAAGCATATCAATAAGCGGAGG

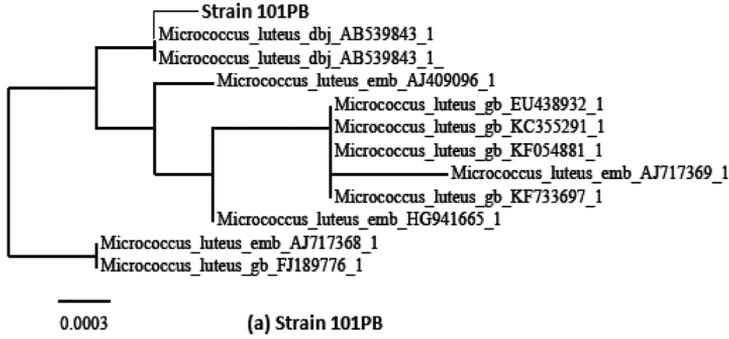
**Figure 9:** *Aspergillus niger* 109PF (Accession No. KC119204.1)

AACCTGCGGAAGGATCATTACAGTATTCTTTTCCAGCGCTTAACTGCGCGGGCAAAAACCTTAC  
ACACAGTGTCTTTTTGATACAGAACTTTGCTTTGGTTTGGCTTAGAGATAGTTGGCCAGAGG  
TTTAAACAAAACACAAATTTAATTATTTTTACAGTTAGTCAAATTTGAATTAATCTTCAAAACTTTCAAC  
AACGGATCTCTTGGTTCGTCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCA  
GATTTTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCAGAGGGCATGCCT  
GTTTGAGCGTCAATTTCTCTCAAAACCCCGGGTTTGGTATTGAGTGATACTTTAGTCGGACTAG  
GCGTTTGTCTTGAAGTATTGGCATGGGTAGTACTAGATAGTGTGTCGACCTCTCAATGTATTAG  
GTTTATCCAACCTCGTTGAATGGTGTGGCGGGATATTTCTGGTATTGTTGGCCCGGCTTACAACAA  
CAAACAAGTTTACCTCAAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAG  
GA

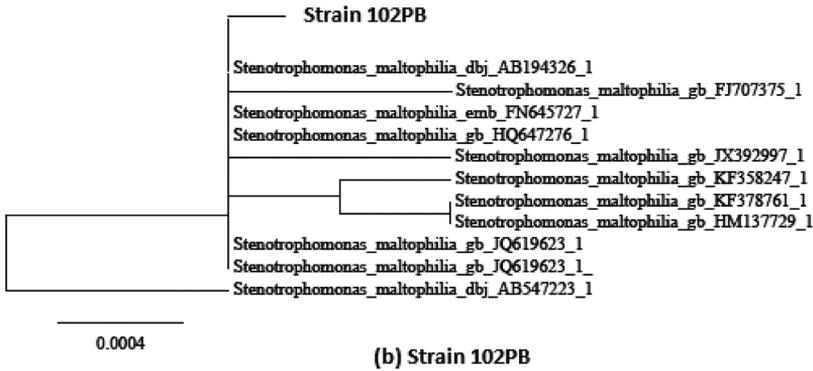
**Figure 10:** *Meyerozyma guilliermondii* 110PF (Accession No. JN183444.1).

## APPENDIX G

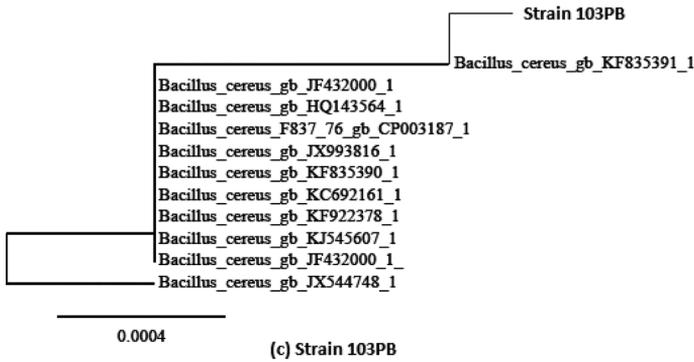
Phylogenetic trees of the identified bacterial isolates from POME



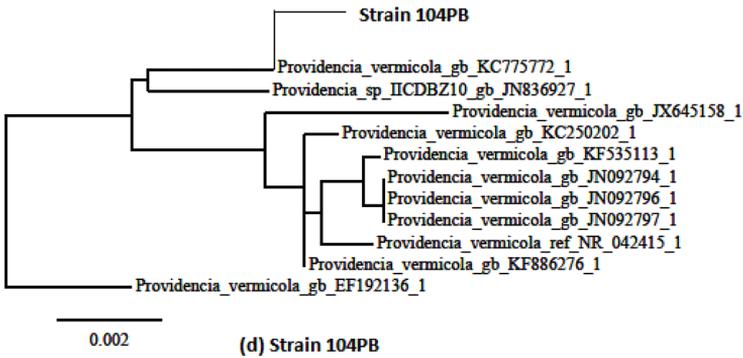
**Figure 11:** Phylogenetic tree of *Micrococcus luteus* 101PB based on 16S rRNA gene sequence comparisons



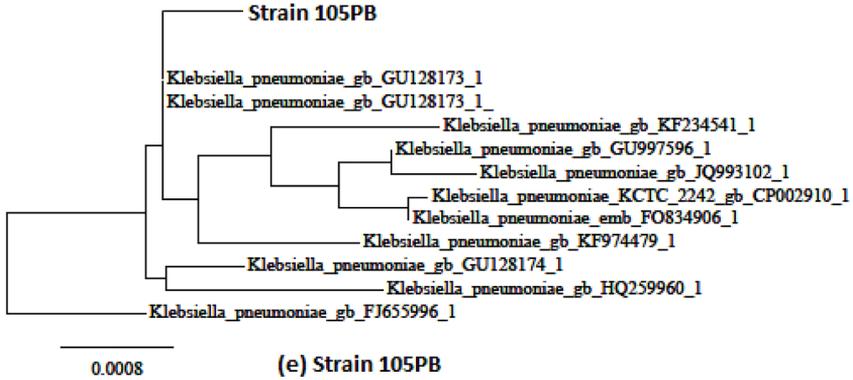
**Figure 12:** Phylogenetic tree of *Stenotrophomonas maltophilia* 102PB based on 16S rRNA gene sequence comparisons



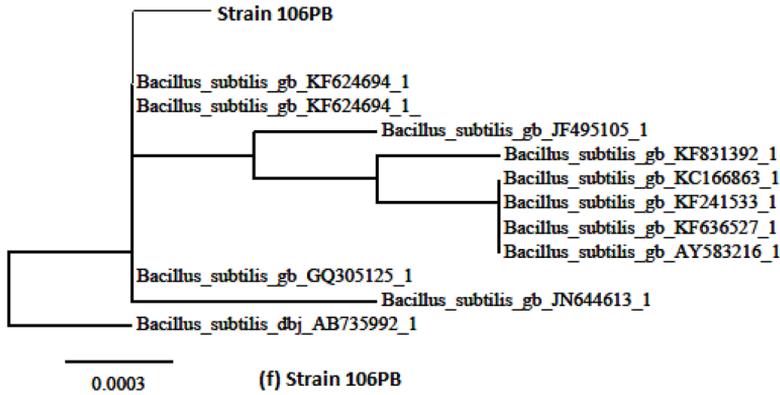
**Figure 13:** Phylogenetic tree of *Bacillus cereus* 103PB based on 16S rRNA gene sequence comparisons



**Figure 14:** Phylogenetic tree of *Providencia vermicola* 104PB based on 16S rRNA gene sequence comparisons



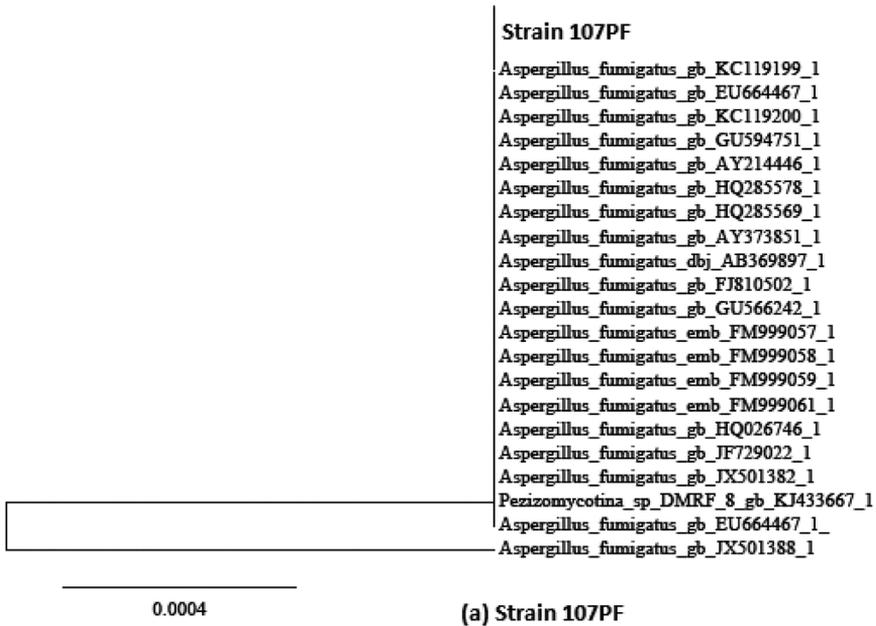
**Figure 15:** Phylogenetic tree of *Klebsiella pneumoniae* 105PB, based on 16S rRNA gene sequence comparisons



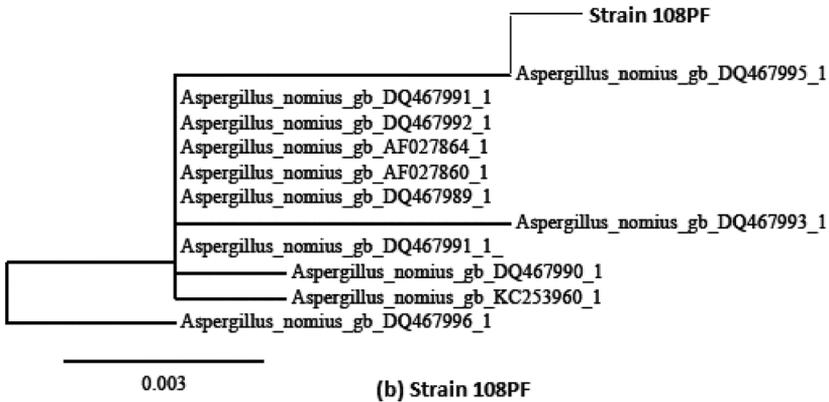
**Figure 16:** Phylogenetic tree of *Bacillus subtilis* 106PB based on 16S rRNA gene sequence comparisons

## APPENDIX H

Phylogenetic trees of the identified fungal isolates from POME



**Figure 17:** Phylogenetic tree of *Aspergillus fumigatus* 107PF based on 18S rRNA gene sequence comparisons



**Figure 18:** Phylogenetic tree of *Aspergillus nomius* 108PF based on 18S rRNA gene sequence comparisons

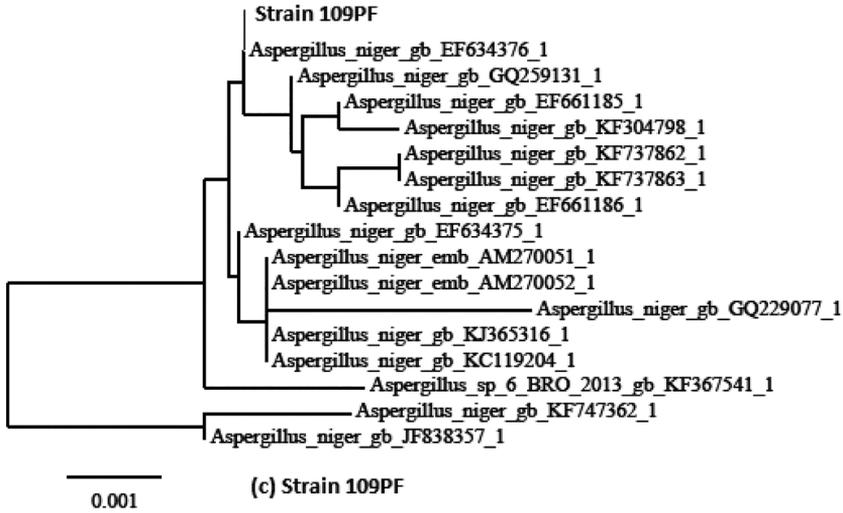


Figure 19: Phylogenetic tree of *Aspergillus niger* 109PF based on 18S rRNA gene sequence comparisons

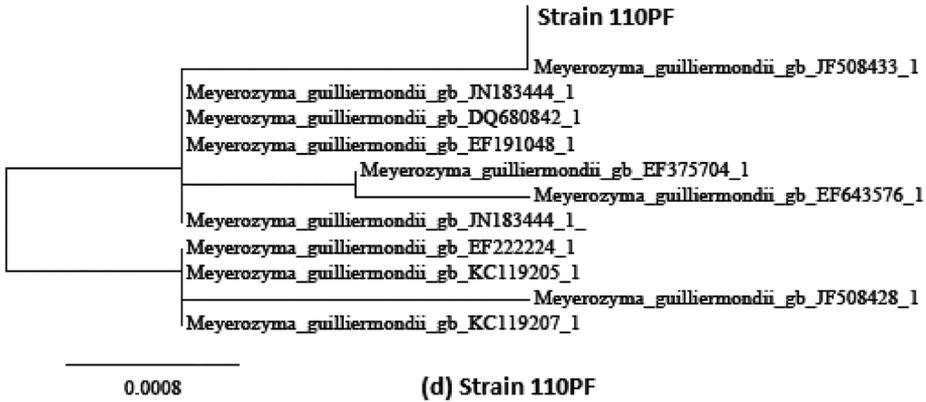


Figure 20: Phylogenetic tree of *Meyerozyma guilliermondii* 110PF based on 18S rRNA gene sequence comparisons

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