Microbiota of Palm Oil Mill Wastewater in Malaysia

Article *in* Tropical Life Sciences Research · January 2018

DOI: 10.21315/tlsr2018.29.2.10

CITATIONS
4

READS
1,150

4 authors, including:

Japareng Lalung
Universiti Sains Malaysia
47 PUBLICATIONS **417** CITATIONS
SEE PROFILE

Adel Al-Gheethi
Universiti Tun Hussein Onn Malaysia
265 PUBLICATIONS **1,466** CITATIONS
SEE PROFILE

Norli Ismail
Universiti Sains Malaysia
174 PUBLICATIONS **2,609** CITATIONS
SEE PROFILE

Accepted author version posted online: 30 January 2018

MICROBIOTA OF PALM OIL MILL WASTEWATER IN MALAYSIA

[1*] Bala, J.D. [1]Lalung, J., [2]Adel A.S. Al-Gheethi., [1]Kaizar H., and [1]Norli, I

[1]Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia (USM), 11800, Pulau, Penang, Malaysia.

[2]Micro-pollution Research Centre (MPRC), Department of Water and Environmental Engineering, Faculty of Civil & Environmental Engineering, Universiti Tun Hussein Onn Malaysia, Batu Pahat, Johor, Malaysia.

*Corrresponding author: jerrybrown316@yahoo.com

Abstract

This study was aimed at identifying indigenous microorganisms from palm oil mill effluent 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 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 8.2×10^5- 9.1×10^6 cfu/mL and 1.4×10^3- 3.4×10^3 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 palm oil mill effluent (POME). Therefore, this denotes that isolating native microorganisms from palm oil mill effluent (POME) is imperative for effectual bioremediation, biotreatment and biodegradation of industrial wastewaters.

Key words: Biodegradation; Industry; Malaysia; MALPOM; Microbiota; 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; Bala et al., 2014b; Bala et al., 2014c Bala et al., 2015a; Bala et al., 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, 2014). Palm oil mill wastewater is produced during oil extraction processes in palm oil mill industries. Palm oil mill effluent (POME) is an extremely polluting wastewater that contaminates the environment when released directly into rivers, streams or lakes devoid of treatment.

Palm oil mill effluent; 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 fiber 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-organized 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 industry in Pinang, Malaysia.

MATERIALS AND METHODS

Sample Collection and Preservation

Raw palm oil mill effluent (POME) was collected aseptically from MALPOM Sdn. Bhd. Pinang Malaysia palm oil mill industry in a sterile microbiological container (20 liters) 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 (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-Utilizing and Cellulose Utilizing 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 homogenized suspension and thereafter, ten-fold (10-fold) serial dilution was made by aseptically transferring one milliliter (1 mL) of the homogenized 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-sterilized 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 utilizing and cellulose utilizing 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 hours. 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 hours 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 Utilizing Bacteria

The mineral salt medium (MSM) (oil agar medium) for palm oil utilizing bacteria was prepared according to the mineral salts medium (MSM) composition of Zajic and Supplisson, (1972). The composition of the medium was NH_4Cl (4.0 g), K_2HPO_4 (1.8 g), KH_2PO_4 (1.2 g), $MgSO_4.7H_2O$ (0.2 g), NaCl (0.1 g), $FeSO_4$ (0.01 g), 15 g agar and distilled water, 1 litre). The medium was used for isolation, enumeration and identification of palm oil-utilizing 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 µm 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-Utilizing and Cellulose Utilizing Fungi from POME

The standard procedures for serial dilution aforementioned for bacterial isolation were followed for fungal isolation. Thereafter, using a sterile pipette, 0.1mL aliquots of the dilutions were aseptically removed with a sterile pipette and separately spread plated with flamed-sterilized glass spreader (bent glass rod) on well-dried Potato Dextrose Agar (PDA), oil agar (Palm Oil Agar (POA) Mineral Salts Medium (MSM) for fungi and Carboxymethyl cellulose (CMC) agar plates for fungi in triplicates for the enumeration of viable heterotrophic fungi, palm oil utilizing and cellulose utilizing 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 minutes in the laminar flow before been 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 Utilizing Fungi

The mineral salt medium (MSM) (oil agar medium) for palm oil utilizing fungi was prepared according to the mineral salts medium (MSM) composition of Mills *et al*. (1978) as modified by Okpokwasili and Okorie (1988).The composition of the medium was NaCl, 10.0g; $MgSO_4.7H_2O$, 0.42g; KCl, 0.29g; KH_2PO_4, 0.83g; Na_2HPO_4, 1.25g; $NaNO_3$, 0.42g; agar, 20g; distilled water, 1 litre and pH of 7.2.The medium was used for isolation, enumeration and identification of palm oil-utilizing

fungi (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 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 3) 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 hours (bacteria), 2 – 3 days (fungi) at 37°C and 28°C respectively.

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 4) for amplifying the DNA. Genetic identification of the pure cultures of fungal isolated from POME were sent to Centre for Chemical Biology (CCB), Universiti Sains Malaysia for sequencing of the 18S rRNA gene.

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 1 and oil degrading bacteria (ODB) and oil degrading fungi (ODF) are presented in Table 2.

The population of total heterotrophic bacteria (THB) ranged from 9.5×10^5 – 7.9×10^6 cfu/mL. The total heterotrophic fungi (THF) ranged from 2.1×10^4– 6.4×10^4 cfu/mL. Total viable heterotrophic indigenous (authochthonous) microbial population on CMC agar ranged from 8.2×10^5- 9.1×10^6

cfu/mL and 1.4 x 10^3- 3.4 x 10^3 cfu/mL for bacteria and fungi respectively. The microbial population of oil degrading bacteria (ODB) ranged from 6.4 x 10^5 – 4.8 x 10^6 cfu/mL and the oil degrading fungi (ODF) ranged from 2.8 x 10^3 – 4.7 x 10^4 cfu/mL (Tables 1&2). 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 x 10^6 cfu/g, 9.5 x 10^2 cfu/g, 1.2 x 10^2 cfu/g and 4.0 x 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 x 10^6cfu/mL and 1.0 x 10^3 cfu/mL correspondingly in POME.

In a related study, Okwute and Isu (2007a) and Okwute and Isu (2007b) have reported total aerobic bacterial populations of 9.6 x 10^8 cfu/mL, 1.64 x 10^9 cfu/mL and 1.07 x 10^9 cfu/mL in POME samples. In addition, Okwute (2013) have also confirmed the population of THB, THF and ODB as 4.0 x 10^9 cfu/mL, 2.6 x 10^3 cfu/mL and 2.6 x 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 x 10^8 cfu/mL as well as Wu et al. (2009) who revealed in their study the count of 6.65 x 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 x 10^5 – 2.0 x 10^6 cfu/mL and total heterotrophic fungi (THF) ranged from 3.1 – 5.7 x 10^4 cfu/mL while the oil degrading bacteria (ODB) ranged from 6.5 x 10^5 - 2.0 x 10^6 cfu/mL and the oil degrading fungi (ODF) ranged from 3.1 – 5.6 x 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 3 and 4 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 Table 5.

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 and 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; b; 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; b; Okwute, 2013; Soleimaninanadegani and 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), 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; b; c; Nwuche and 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 and Olanrewaju, 2011). *Aspergillus fumigatu*s 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 and 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).

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 and 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 and Ahmad, 2006; Atif et al., 2005; Ismail et al., 2010). Table 6 revealed cultural characteristics of bacteria isolated from palm oil mill wastewater while Table 7 revealed microscopic, macroscopic morphology and cultural characteristics of fungi isolated from palm oil mill wastewater.

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 characterization 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.

REFERENCES

Abass AO, Jameel TA, Muyibi AS, Abdul Karim IM, Alam Z (2012)Investigation of the viability of selected microorganisms on the biodegradation of palm oil mill effluents (POME). Int J Chem Environ Engineer 3(3): 182-186.

Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM (2012) Microalgae and wastewater treatment. Saudi J Biol Sci 19: 257–275.

Ahmad AL, Ismail S, Bhatia S. (2005) Optimization of coagulation- flocculation process for palm oil mill effluent using response surface methodology. Environ Sci Technol 39(8): 2828-2834.

Ahmad MN, Mokhtar MN, Baharuddin AS (2011) Changes in physicochemical and microbial community during co-composting of oil palm frond with palm oil mill effluent anaerobic sludge. Bio Res 6 (4): 4762–4780.

APHA (2005) Standard Methods for the Examination of Water and Wastewater, in, 21st edn, American Public Health Association (APHA), Washington, DC.

Awotoye OO, Dada AC, Arawomo GAO (2011) Impact of palm oil processing effluent discharge on the quality of receiving soil and river in South Western Nigeria. J Appl Sci Res 7(2): 111-118.

Asikong BE (1994) Studies on extracellular lipases of three fungi isolated from mouldy cocoa seed and palm fruit. M.Sc. Thesis. University of Calabar, Nigeria.

Atif AAY, Razi AF, Ngan MA, Morimoto M, Iyuke SE, Veziroglu NT (2005) Fed batch production of hydrogen from palm oil mill effluent using anaerobic microflora. Int J Hydrogen Energy30: 1393–1397.

Bergey DH, Holt JG, Krieg NR, Sneath PHA (1994) Bergey's Manual of Determinative Bacteriology (9th edn.) Lippincott Williams and Wilkins. ISBN 0-683-00603-7

Bala JD, Yusuf IZ, Tahir F (2012) Bacteriological assessment of pharmaceutical wastewater and its public health implications in Nigeria. IUP J Biotechnol 6(1): 34-49.

Bala, JD., Lalung, J and Ismail, N (2014a). Biodegradation of palm oil mill effluent (POME) by bacteria. *International Journal of Scientific and Research Publications* 4(3): 502-511

Bala, JD., Lalung, J and Ismail, N (2014b). Biodegradation potential and removal of oil and grease by bacteria isolated from Palm oil mill effluent (POME*). Proceedings of the International Conference on Beneficial Microbes ICOBM, 2014. Microbes for the benefits of Mankind.* May 27-29, 2014, PARKROYAL Penang Resort, Penang, Malaysia. School of Industrial Technology, Universiti Sains Malaysia, 2014. Pages 138-144 ISBN 978-967-394-186-5.

Bala, JD., Lalung, J and Ismail, N (2014c). Palm oil mill effluent (POME) treatment "Microbial communities in an anaerobic digester": A Review. *International Journal of Scientific and Research Publications,* 4(6): 2250-3153

Bala, JD., Lalung, J and Ismail, N (2015a). Studies on the reduction of organic load from palm oil mill effluent (POME) by bacterial strains. International Journal of Recycling of Organic Waste in Agriculture, 4(1): 1-10. DOI 10.1007/S40093-014-0079-6.

Bala, JD., Lalung, J., AL-Gheethi, AAS and Ismail, N (2015b). Reduction of oil and grease by fungi isolated from Palm oil mill effluent (POME*). Proceedings of the 4th ICERT 2015: International Conference on Environmental Research and Technology: Exploring the Frontiers in Environmental Science and Technology Research,* 27-29, May 2015, Parkroyal Hotel Penang, Malaysia. School of Industrial Technology, Universiti Sains Malaysia, 2015. Pages 79-91 ISBN 978-967-394-211-4.

Bala, JD (2016). Aerobic treatment and biodegradation of palm oil mill effluent by indigenous microorganisms. PhD Thesis. Environmental Technology Division, School of Industrial Technology, Universiti Sains Malaysia (USM), 11800 Pulau, Pinang, Malaysia.

Bharathi S, Vasudevan N (2001) Utilization of petroleum hydrocarbons by *Pseudomonas fluorescens* isolated from petroleum contaminated soil. Environ. Int. 26: 413–414.

Bhumibhamon O, Koprasertsak A, Funthong S (2002) Biotreatment of high fat and oil wastewater by lipase producing microorganisms. Kasetsart J 36: 261- 267.

Breccari M, Bonemazzi F, Majone M, Riccardi C (1996) Interaction between acidogenesis and methanogenesis in the anaerobic treatment of olive oil mill effluents. Water Res 30: 183–189.

Ghosh P, Saxena R, Gupta R, Yadav R, Davidson S (1996) Microbial lipases: production and applications. Sci Prog79:119-158.

Guehi TS, Dingkuhn M, Cros E, Fourny G, Ratomahenina R, Moulin G, Vidal AC (2007) Identification and lipase-producing abilities of moulds isolated from ivorian raw cocoa beans. Res J Agr Biol Sci3:838-843.

Haimann RA (1995) Fungal technologies for the treatment of hazardous waste. Environ Prog14 (3): 201-203.

Hassen-Aboushiba, A., Ramli, R., Sofian-Azirun, M. (2013) Ecological characteristics of POME ponds with reference to study some of their invertebrate species in Peninsular Malaysia. Journal of Animal and Plant Sciences 23 (5):1305-1315.

Hii KL, Yeap SP, Mashitah MD (2012) Cellulase production from palm oil mill effluent in Malaysia: Economical and technical perspectives. Eng Life Sci 12 (1): 7–28.

Ismail I, Hassan MA, Abdul Rahman NA, Soon CS (2010) Thermophilic biohydrogen production from palm oil mill effluent (POME) using suspended mixed culture, Biomass and Bioenergy, 34 (1): 42-47.

Jameel AT, Olanrewaju AA (2011) Aerobic biodegradation of oil and grease in palm oil mill effluent using consortium of microorganisms In: M.D.Z, Alam, A.T, Jameel and A, Amid, (eds). *Current research and development in biotechnology engineering at International Islamic University Malaysia* (IIUM) Vol. III. IIUM Press, Kuala Lumpur, pp. 43-51. ISBN 9789674181444.

Kamal SA, Jahim JM, Anuar N (2011) Pre-treatment effect of palm oil mill effluent (POME) during hydrogen production by a local isolate clostridium butyricum, Int J Adv Sci, Eng Infor Technol 2: 54–60.

Leslie-Grady CP, Daigger GT, Lim HC. (1999) Biological Wastewater Treatment, second ed. CRC Press. Revised & Expanded.

Ma AN (2000) Environmental Management for the Oil Palm Industry. Palm Oil Dev 30: 1-10.

Mills AL, Breuil C, Colwell RR (1978) Enumeration of petroleum-degrading marine and estuarine microorganisms by the most-probable number. Can J Microbiol 24: 552-557.

Mohammed RR, Ketabachi MR, McKay G (2014) Combined magnetic field and adsorption process for treatment of biologically treated palm oil mill effluent (POME). Chem Eng J 243: 31–42.

Nawawi WMFW, Jamal P, Alam MZ (2010) Utilization of sludge palm oil as a novel substrate for biosurfactant production. BioresTechnol 101(23): 9241– 9247.

Neoh CH, Yahya A, Adnan R, Majid ZA, Ibrahim Z (2013) Optimization of decolorization of palm oil mill effluent (POME) by growing cultures of *Aspergillus fumigatus* using response surface methodology. Environ Sci Poll Res 20:2912-2923.

Nwuche CO, Ogbonna JC (2011) Isolation of lipase producing fungi from palm oil mill effluent (POME) dump sites at Nsukka. Braz Arch Biol Technol 54:113-116.

Ohimain EI, Olukole CD, Izah SC, Eke RA, Okonkwo AC (2012a) Microbiology of Palm Oil Mill Effluents. J Microbiol Biotech Res 2 (6): 852-857.

Ohimain EI, Seiyaboh EI, Izah SC, Oghenegueke V, Perewarebo T (2012b) Some selected physico-chemical and heavy metal properties of palm oil mill effluents. Greener J Phys Sci 2: 131–137.

Ohimain EI, Daokoru-Olukole C, Izah SC, Alaka EE (2012c) Assessment of the quality of crude palm oil produced by smallholder processors in Rivers State, Nigeria. Nig J Agr Food Environ 8(2): 28 – 34.

Ojumu TV, Bello OO, Sonibare JA, Solomon BO (2005) Evaluation of microbial systems for bioremediation of petroleum refinery effluents in Nigeria. Afr J Biotechnol 4 (1): 31-35.

Okechalu JN, Dashen MM, Lar PM, Okechalu B, Gushop T (2011) Microbiological quality and chemical characteristics of palm oil sold within Jos Metropolis, Plateau State, Nigeria. J Microbiol Biotechnol Res 1(2): 107-112.

Okereke JN, Obiekezie SO, Obasi KO (2007) Microbial flora of oil-spilled sites in Egbema, Imo State, Nigeria. Afr J Biotechnol6(8): 991 - 993.

Okpokwasili GC, Okorie BB (1988) Biodeterioration potentials of microorganisms isolated from car engine lubricating oil. Tribol Int 21 (4): 215 - 220.

Okwute LO, Isu NR (2007a) The environmental impacts of palm oil mill effluent (POME) on some physicochemical parameters and total aerobic bioload of soil at a dump site in Anyigba, Kogi State, Nigeria. Afr J Agric Res 2(12): 656 – 662.

Okwute LO, Isu NR (2007b) Impact analysis of palm oil mill effluent on the aerobic bacterial density and ammonium oxidizers in a dumpsite in Anyigba, Kogi State.Afr J Biotechnol 6 (2): 116-119.

Okwute LO (2013) Bioremediation of soil polluted with palm oil mill effluent (POME) in Kogi State using microorganisms found in chicken dropping and cow dung. PhD Thesis. Federal University of Technology Minna, Nigeria. Department of Microbiology.

Oswal N, Sarma PM, Zinjarde SS, Pant A (2002) Palm oil mill effluent treatment by a tropical marine yeast.Biores Technol 85(1): 35-37.

Poh PE, Chong MF (2009) Development of anaerobic digestion methods for palm oil mill effluent (POME) treatment. Biores Technol 100:1-9.

Rahman KSM, Rahman JT, Lakshmanaperumalsamy P, Banat LM (2002) Towards Efficient Crude Oil Degradation by a Mixed Bacterial Consortium, Biores. Technol 85: 257 – 261.

Rupani PF, Singh RP, Ibrahim MH, Esa N (2010) Review of Current Palm Oil Mill Effluent (POME) Treatment Methods: Vermicomposting as a Sustainable Practice. World Appl Sci J 11 (1): 70-81.

Serikovna SZ, Serikovich KS, Sakenovna AS, Murzakhmetovich SS, Khamitovich AK (2013)Screening of lipid degrading microorganisms for wastewater treatment Malaysian J Microbiol 9(3): 219-226.

Shaaban AM, Haroun BM, Ibraheem IBM (2004) Assessment of impact of *Microcystis aeruginosa and Chlorella vulgaris* in the uptake of some heavy metals from culture media. In: Proc. 3rd Int. Conf. Biol. Sci. Fac. Sci., Tanta Univ., 3: 433– 450.

Singh R, Ibrahim MH, Esa N, Iliyana M (2010) Composting of waste from palm oil mill: a sustainable waste management practice. Rev Environ Sci Biotechnol 9 (4):331-344.

Sira P, Orathai P, Ratana R, Boonyarach K, Pastra S, Sumaeth C (2010) Biosurfactant production by *Pseudomonas aeruginosa* SP4 using sequencing batch reactor: Effect of oil-to-glucose ratio. Biochem Eng J 49: 185-191.

Soleimaninanadegani M, Manshad S (2014) Enhancement of biodegradation of palm oil mill effluents by local isolated microorganisms. Int Scholarly Res Notices 2014:1–8.

Tan, K.M., Liew, W.L., Muda, K., Kassim, M.A (2015). Microbiological Characteristics of Palm Oil Mill Effluent. Conference Paper # 855. *International Congress on Chemical, Biological and Environmental Sciences* (ICCBES). May 7-9, 2015, Kyoto Research Park, Kyoto, Japan. Conference Program page 93. ISBN 978-986-87417-8-2.

Ugoji EO (1997) Anaerobic digestion of palm oil mill effluent and its utilization as fertilizer for environmental protection. Renewable Energy 10: 291– 294.

Vijayaraghavan K, Ahmad D (2006) Biohydrogen generation from palm oil mill effluent using anaerobic contact filter. Int J Hydrogen Energy 31: 1284–1291. Wong KM, Nor AA, Suraini A, Vikineswary S, Hassan MA (2008) Enzymatic hydrolysis of palm oil mill effluent solid using mixed cellulases from locally isolated fungi. Res J Microbiol 3 (6): 474-481.

Wong KM, Nor AA, Suraini A, Vikineswary S, Hassan MA (2008) Enzymatic hydrolysis of palm oil mill effluent solid using mixed cellulases from locally isolated fungi. Res J Microbiol 3 (6): 474-481.

Wu TY, Mohammad AW, Jahim JM, Anuar N (2007) Palm oil mill effluent (POME) treatment and bioresources recovery using ultrafiltration membrane: effect of pressure on membrane fouling. Biochem Eng J 35: 309–317.

Wu TY, Mohammad AW, Jahim JM, Anuar N (2009) A holistic approach to managing palm oil mill effluent (POME): Biotechnological advances in the sustainable reuse of POME. Biotechnol Adv 27: 40–52.

Zajic JE, Supplisson B (1972) Emulsification and degradation of Bunker C fuel oil by microorganisms. Biotechnol Bioeng 14: 331-334.

Table 1: 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 2: 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	$2.8 \times 10^3 – 4.7 \times 10^4$

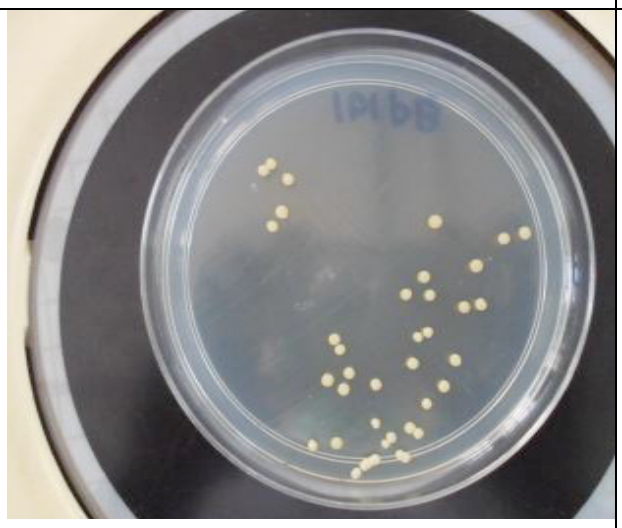
Table 3: Genetic Identification of bacterial isolates in POME

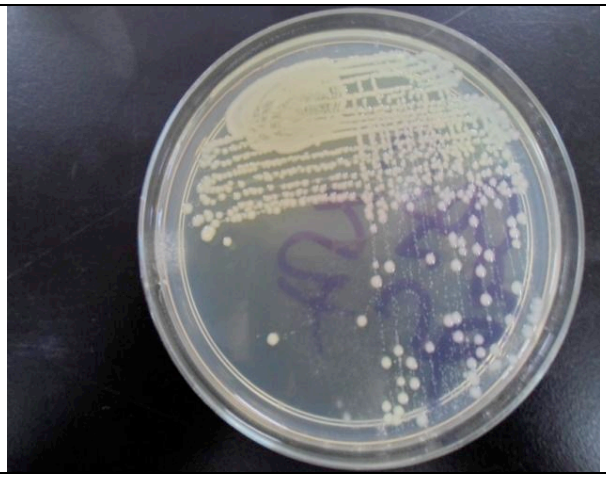
	Bacteria
Nucleotide Sequences	16S rRNA gene
Sequences of Primers	**27F: 5'- AGAGTTTGATCMTGGCTCAG-3'** **1492R: 5'-GGGTTACCTTGTTACGACTT-3'**
Strains	*Micrococcus luteus* 101PB **(Accession No. AB539843.1),** *Stenotrophomonas maltophilia* 102PB (**Accession No. JQ 619623.1),** *Bacillus cereus* 103PB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

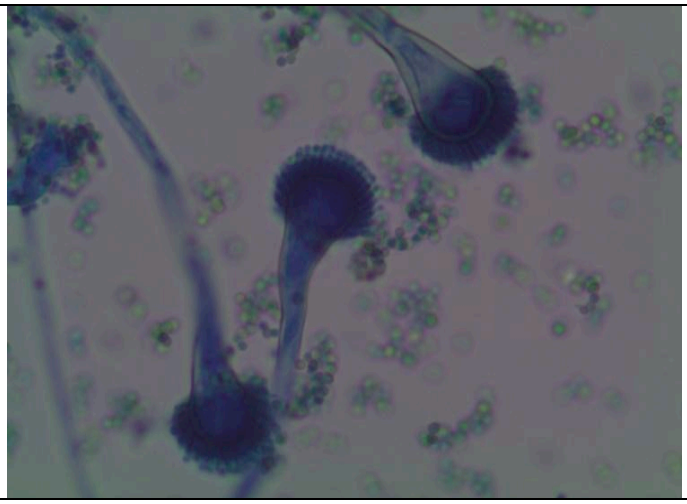
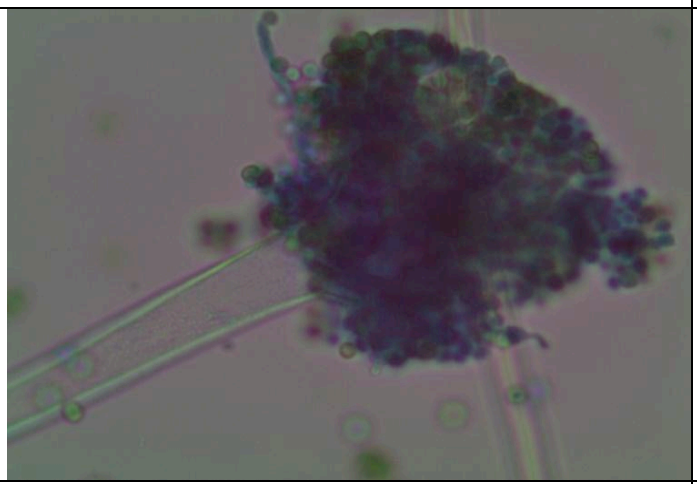
Table 4: Genetic Identification of fungal isolates in POME

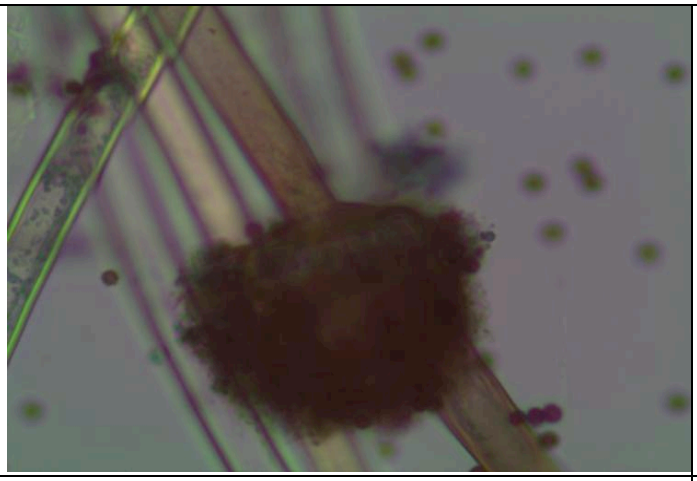
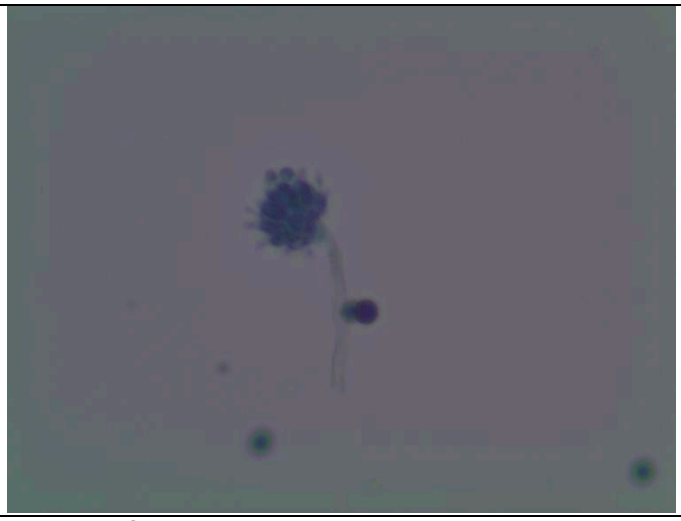
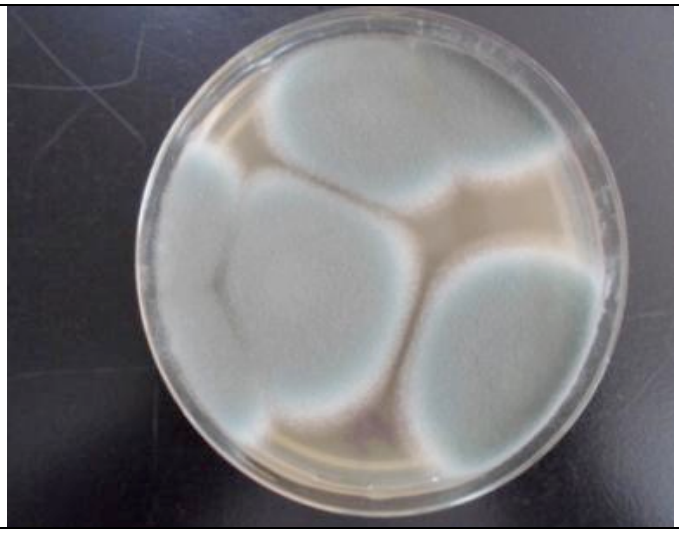
	Fungi
Nucleotide Sequences	18S rRNA genes
Sequences of Primers	**ITS1 F: 5'-TCCGTAGGTGAACCTGCGG -3'** **ITS4 R: 5'-TCCTCCGCTTATTGATATGC-3'.**
Strains	*Aspergillus fumigatus* 107PF **(Accession No. EU664467.1)**, *Aspergillus nomius* 108PF**(Accession No. DQ467991.1)**, *Aspergillus niger* 109PF**(Accession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

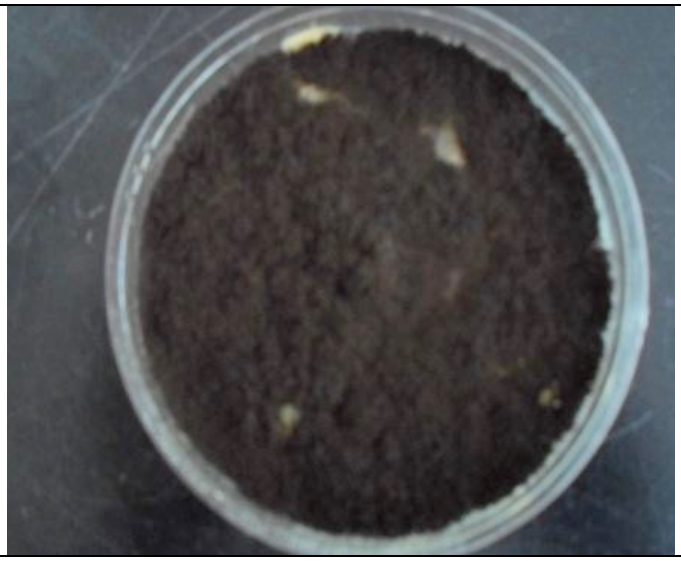
Table 5: Identified bacteria and fungi in POME sample

Strains	Image	Gram's reaction
Micrococcus luteus 101PB (Pure culture)		Gram positive cocci
	Plate 1 (Supplementary data).	
Stenotrophomonas maltophilia 102PB (Pure culture)		Gram negative rod
	Plate 2 (Supplementary data).	

Bacillus cereus 103PB (Pure culture)		Gram positive rod
	Plate 3 (Supplementary data).	
Providencia vermicola 104PB (Pure culture)		Gram negative rod
	Plate 4 (Supplementary data)	
Klebsiella pneumoniae 105PB (Pure culture)		Gram negative rod
	Plate 5 (Supplementary data).	

Bacillus subtilis 106PB. (Pure culture)		Gram positive rod
	Plate 6 (Supplementary data).	
Identified fungi in POME sample		
Aspergillus fumigatus 107PF (Microscopic staining)		
	Plate 7 (Supplementary data).	
Aspergillus nomius 108PF (Microscopic staining)		
	Plate 8 (Supplementary data).	

Aspergillus niger 109PF (Microscopic staining)		
	Plate 9 (Supplementary data).	
Meyerozyma guilliermondii 110PF (Microscopic staining)		
	Plate 10 (Supplementary data).	
Aspergillus fumigatus 107PF (Pure culture)		
	Plate 11 (Supplementary data).	

Aspergillus nomius 108PF (Pure culture)		
	Plate 12 (Supplementary data).	
Aspergillus niger 109PF (Pure culture)		
	Plate 13 (Supplementary data).	
Meyerozyma guilliermondii 110PF (Pure culture)		
	Plate 14 (Supplementary data).	
Plates showing genomic DNA and purified PCR product of bacteria isolated from POME		

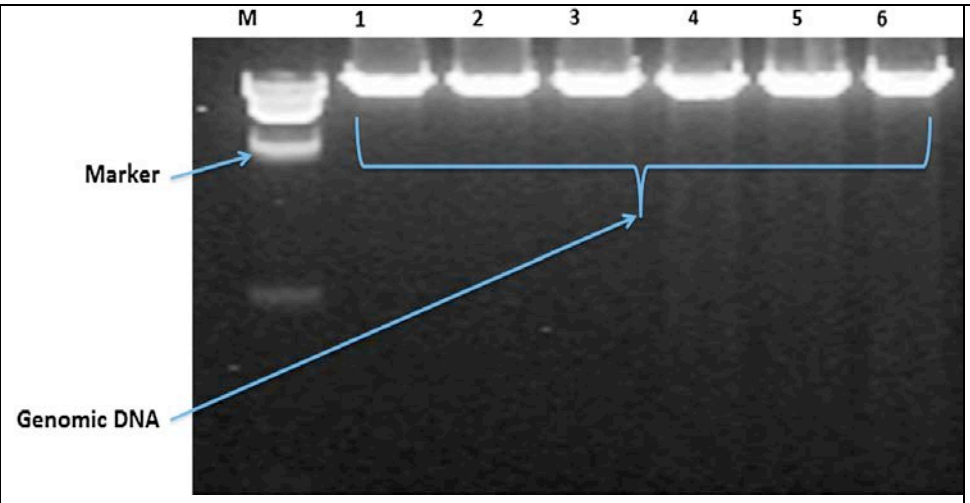


Plate 15: **Gel picture of genomic DNA: Lane 1**: 101PB; **2**: 102PB; **3**: 103PB; **4**: 104PB; **5**: 105PB; **6**: 106PB; M: Lambda/HindIII marker. **(**Supplementary data).

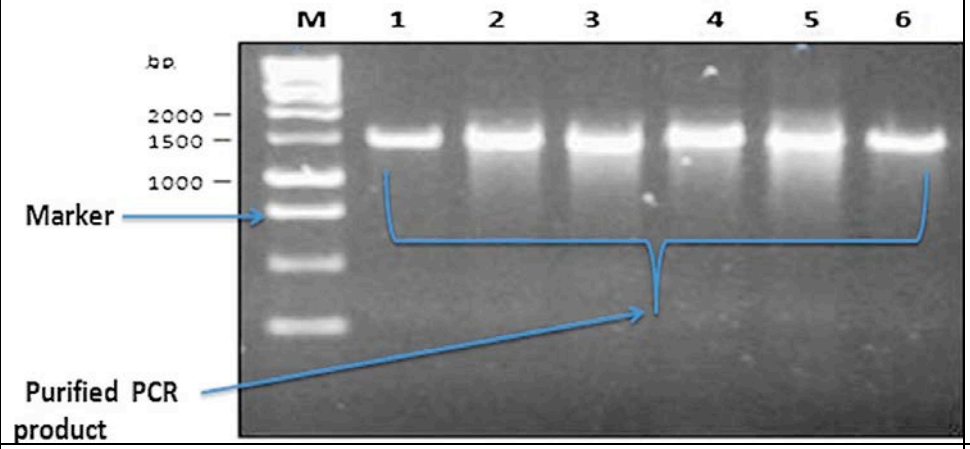


Plate 16:Gel picture of purified PCR product: Lane 1: 101PB; **2**: 102PB; **3**: 103PB; **4**: 104PB; **5**: 105PB; **6**: 106PB; M: 1 kb marker (Fermentas). **(**Supplementary data).

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

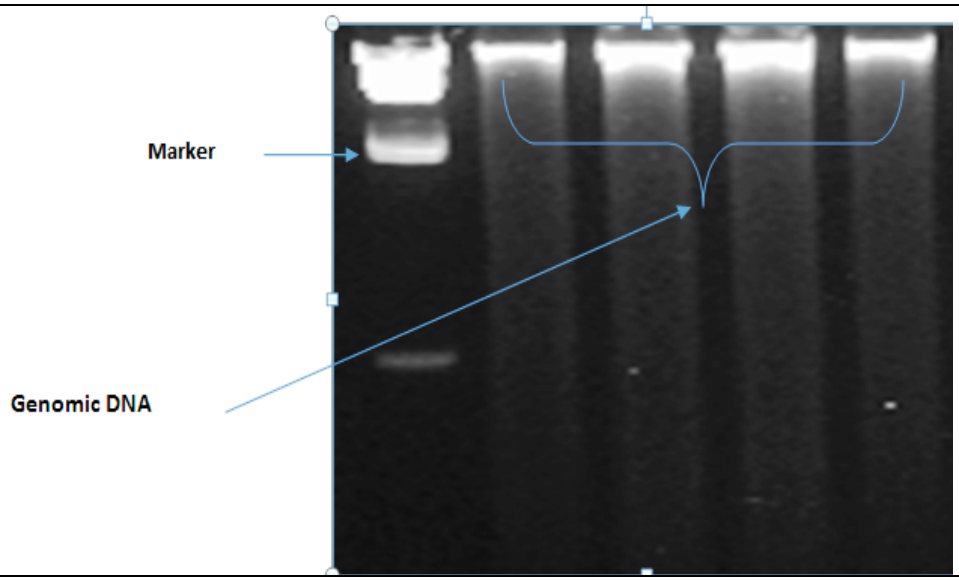


Plate 17: Gel picture of genomic DNA: Lane 1: 107 PF; **2:** 108 PF; **3:** 109 PF; **4:** 110 PF; **M:** Lambda/HindIII marker. **(**Supplementary data).

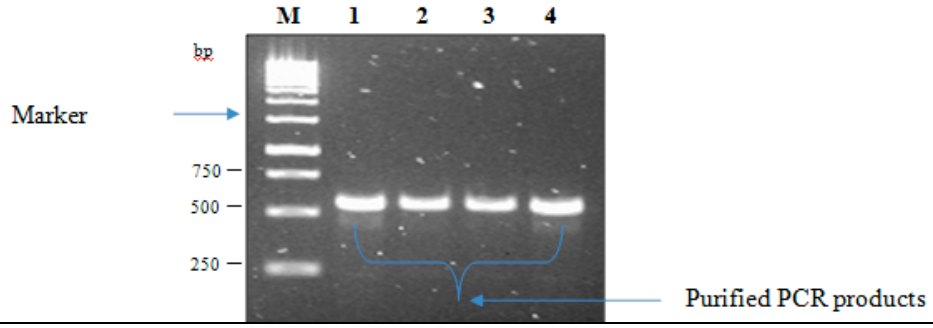


Plate 18: Gel picture of purified PCR product: Lane 1: 107 PF; **2:** 108 PF; **3:** 109 PF; **4:** 110 PF; **M:** 1 kb marker (Fermentas). **(**Supplementary data).

DNA sequence of bacterial strains isolated from POME (Supplementary data)

TCGAACGATGAAGCCCAGCTTGCTGGGTGGATTAGTGGCGAACGGGTGAGTAACACGT
GAGTAACCTGCCCTTAACTCTGGGATAAGCCTGGGAAACTGGGTCTAATACCGGATAGG
AGCGCCCACCGCATGGTGGGTGTTGGAAAGATTTATCGGTTTTGGATGGACTCGCGGC
CTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGA
GAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGC
AGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATG
ACGGCCTTCGGGTTGTAAACCTCTTTCAGTAGGGAAGAAGCGAAAGTGACGGTACCTGC
AGAAGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCG
TTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGTCGTGAAA
GTCCGGGGCTTAACCCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGCAGTAGGG
GAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATG
GCGAAGGCAGGTCTCTGGGCTGTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGA
ACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGTTGGGCACTAGGTGTGGGGAC
CATTCCACGGTTTCCGCGCCGCAGCTAACGCATTAAGTGCCCCGCCTGGGGAGTACGG
CCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGCGGAGCATGC
GGATTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATGTTCTCGATCGCCG
TAGAGATACGGTTTCCCCTTTGGGGCGGGTTCACAGGTGGTGCATGGTTGTCGTCAGCT
CGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTCCATGTTGCC
AGCACGTCGTGGTGGGGACTCATGGGAGACTGCCGGGGTCAACTCGGAGGAAGGTGA
GGACGACGTCAAATCATCATGCCCCTTATGTCTTGGGCTTCACGCATGCTACAATGGCC
GGTACAATGGGTTGCGATACTGTGAGGTGGAGCTAATCCCAAAAAGCCGGTCTCAGTTC
GGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCGCTAGTAATCGCAGATCAG
CAACGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCAAGTCACGAAA
GTCGGTAACACCCGAAGCCGGTGGCCTAACCC

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

GCTTGCTCTCTGGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACTCTG
TCGTGGGGGATAACGTAGGGAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAG
CAGGGGACCTTCGGGCCTTGCGCGATTGAATGAGCCGATGTCGGATTAGCTAGTTGGC
GGGGTAAAGGCCCACCAAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCAC
ACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGAC
AATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTA
AAGCCCTTTTGTTGGGAAAGAAATCCAGCTGGCTAATACCCGGTTGGGATGACGGTACC
CAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAA
GCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTCGTTTAAGTCCGTTGTG
AAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGATACTGGGCGACTAGAATGTGGTAG
AGGGTAGCGGAATTCCTGGTGTAGCAGTGAAATGCGTAGAGATCAGGAGGAACATCCAT
GGCGAAGGCAGCTACCTGGACCAACATTGACACTGAGGCACGAAAGCGTGGGGAGCAA
ACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGC
AATTTGGCACGCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTC
GCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGT
TTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGACATGTCGAGAACTTTCCAGA
GATGGATTGGTGCCTTCGGGAACTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCG
TGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAG

CACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGG
ATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTACTACAATGGTAGG
GACAGAGGGCTGCAAGCCGGCGACGGTAAGCCAATCCCAGAAACCCTATCTCAGTCCG
GATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAGATCAGCA
TTGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTT
TGTTGCACCAGAAGCAGGTAGCTTAACCTTCGGGAGGGC

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

TGCAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGA
GTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAAT
ACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTT
ATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACG
ATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGA
CTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCA
ACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAA
GTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTA
CGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGT
AAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGA
GGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAG
CGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTG
TAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGT
CCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGT
TAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATT
GACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAAC
CTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCTCCTTCGGGAGCA
GAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCC
CGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTTAGTTGGGCACTCTAAGGTGA
CTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATG
ACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGAGCTGCAAGACCGCGAGGTG
GAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAA
GCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTT
GTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGGTGGGGTAACC
TTTTGGAGCCAGCCGC

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

GTCGAGCGGTAACAGGGGAAGCTTGCTTCCCGCTGACGAGCGGCGGACGGGTGAGTA
ATGTATGGGGATCTGCCCGATAGAGGGGGATAACTACTGGAAACGGTGGCTAATACCG
CATAATCTCTTAGGAGCAAAGCAGGGGAACTTCGGTCCTTGCGCTATCGGATGAACCCA
TATGGGATTAGCTAGTAGGTGGGGTAATGGCTCACCTAGGCGACGATCCCTAGCTGGTC
TGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCA
GCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGA
AGAAGGCCCTAGGGTTGTAAAGTACTTTCAGTCGGGAGGAAGGCGTTGATGCTAATATC
ATCAACGATTGACGTTACCGACAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGC
GGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGC
GGTTGATTAAGTTAGATGTGAAATCCCCGGGCTTAACCTGGGAATGGCATCTAAGACTG
GTCAGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCATGTGTAGCGGTGAAATGCGTAGA
GATGTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGG
TGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGAT
GTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAATCGAC
CGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACA
AGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACA
TCCAGAGAATTTAGCAGAGATGCTTTAGTGCCTTCGGGAACTCTGAGACAGGTGCTGCA
TGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC
TTATCCTTTGTTGCCAGCGATTCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACC
GGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACG
TGCTACAATGGCGTATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGAACTCATAAAG
TACGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTA
ATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA
CACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGGGAGGG

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

AGGTTAAGGCTACCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTA
CAAGGCCCGGGAACGTATTCACCGTAGCATTCTGATCTACGATTACTAGCGATTCCGAC
TTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACATACTTTATGAGGTCCGCTT
GCTCTCGCGAGGTCGCTTCTCTTTGTATATGCCATTGTAGCACGTGTGTAGCCCTGGTC
GTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGTCT
CCTTTGAGTTCCCGGCCTAACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGTTGCGG
GACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCACA
GTTCCCGAAGGCACCAATCCATCTCTGGAAAGTTCTGTGGATGTCAAGACCAGGTAAGG
TTCTTCGCGTTGCATCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAA
TTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGATTTAACGCGTTAGCT
CCGGAAGCCACGCCTCAAGGGCACAACCTCCAAATCGACATCGTTTACGGCGTGGACT
AACCAGGGTATCTAATCCTGTTTGCTCCCCCACGCTTTCGCACCTGAGCGTCAGTCTTTG
TCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCCAGATCTCTACGCATTTCACCGCTA
CACCTGGAATTCTACCCCCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAGTTCC
CAGGTTGAGCCCGGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCGCTTTACG
CCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGT
TAGCCGGTGCTTCTTCTGCGGGTAACGTCAATCGACAAGGTTATTAACCTTACCGCCTTC
CTCCCCGCTGAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATGGCTGC
ATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGAC
CGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTA
GGTGAGCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCATGAGG
CCCGAAGGTCCCCCACTTTGGTCTTGCGACATTATGCGGTATTAGCTACCGTTTCCAGTA
GTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTACACCCGTCCGCCGCTCGTCA
CCCGAGAGCAAGCTCTCTGTGCTACCG

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

GCTCCTAAAAGGTTACCTCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGC
GGTGTGTACAAGGCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACTAGC
GATTCCAGCTTCACGCAGTCGAGTTGCAGACTGCGATCCGAACTGAGAACAGATTTGTG
GGATTGGCTTAACCTCGCGGTTTCGCTGCCCTTTGTTCTGCCCATTGTAGCACGTGTGT
AGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTC
ACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGC
TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACC
TGTCACTCTGCCCCCGAAGGGGACGTCCTATCTCTAGGATTGTCAGAGGATGTCAAGAC
CTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGC
CCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGGCGGAGTGCTTAA
TGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCTAACACTTAGCACTCATCGTTTAC
GGCGTGGACTACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCTCCTCAGCGT
CAGTTACAGACCAGAGAGTCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTT
CACCGCTACACGTGGAATTCCACTCTCCTCTTCTGCACTCAAGTTCCCCAGTTTCCAATG
ACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGCGAGCC
CTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGC
ACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCGCCCTATTTGAACGG
CACTTGTTCTTCCCTAACAACAGAGCTTTACGATCCGAAAACCTTCATCACTCACGCGGC
GTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGA
GTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCAT
CGTCGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGTCCATCTGTA
AGTGGTAGCCGAAGCCACCTTTTATGTCTGAACCATGCGGTTCAAACAACCATCCGGTA
TTAGCCCCGGTTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTC
ACCCGTCCGCCGCTAACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGAC

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

DNA sequence of fungal strains isolated from POME

CCTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAA
CCTCCCACCCGTGTCTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGTTTCGACGGCC
GCCGGGGAGGCCTTGCGCCCCCGGGCCCGCGCCCGCCGAAGACCCCAACATGAACGC
TGTTCTGAAAGTATGCAGTCTGAGTTGATTATCGTAATCAGTTAAAACTTTCAACAACGGA

TCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGC
AGAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGG
GCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCACGGCTTGTGTGTTGGGCCCCCG
TCCCCCTCTCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGGTCCTC
GAGCGTATGGGGCTTTGTCACCTGCTCTGTAGGCCCGGCCGGCGCCAGCCGACACCCA
ACTTTATTTTTCTAAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATA
TCAATAAGCGGAGGA

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

TTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGTAGGGTTCCTAGCGAGCCCAA
CCTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCGCAAGGCCGCC
GGGGGGCATCCGCCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGAACGAT
CTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGATCTCTTG
GTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTC
CGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCC
TGTCCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCCCCC
CTCCGGGGGGGGGACGGGCCCTAAAGGCAGCGGCGGCACCGCGTCCGATCCTCGAG
CGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCTTGCCGAACGCAAAAC
AACCATTCTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATAT
CAATAAGGCGGAGGAA

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

TTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGTCCTTTGGGCCCAACCT
CCCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCG
GGGGGGCGCCTCTGCCCCCCGGGCCCGTGCCCGCCGGAGACCCCAACACGAACACTG
TCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAATGGAT
CTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCA
GAATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGG
CATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGT
CCCCCTCTCCGGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCGCGTCCGATCCTCG
AGCGTATGGGGCTTTGTCACATGCTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCC
AACCATTCTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATAT
CAATAAGCCGGAGG

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

AACCTGCGGAAGGATCATTACAGTATTCTTTTGCCAGCGCTTAACTGCGCGGCGAAAAA
CCTTACACACAGTGTCTTTTTGATACAGAACTCTTGCTTTGGTTTGGCCTAGAGATAGGT
TGGGCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACAGTTAGTCAAATTTTGAATT
AATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGA
AATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATCTTTGAACGCACATT
GCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAACCCC
CGGGTTTGGTATTGAGTGATACTCTTAGTCGGACTAGGCGTTTGCTTGAAAAGTATTGGC
ATGGGTAGTACTAGATAGTGCTGTCGACCTCTCAATGTATTAGGTTTATCCAACTCGTTG
AATGGTGTGGCGGGATATTTCTGGTATTGTTGGCCCGGCCTTACAACAACCAAACAAGT
TTGACCTCAAATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA

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

Phylogenetic trees of the identified bacterial isolates from POME

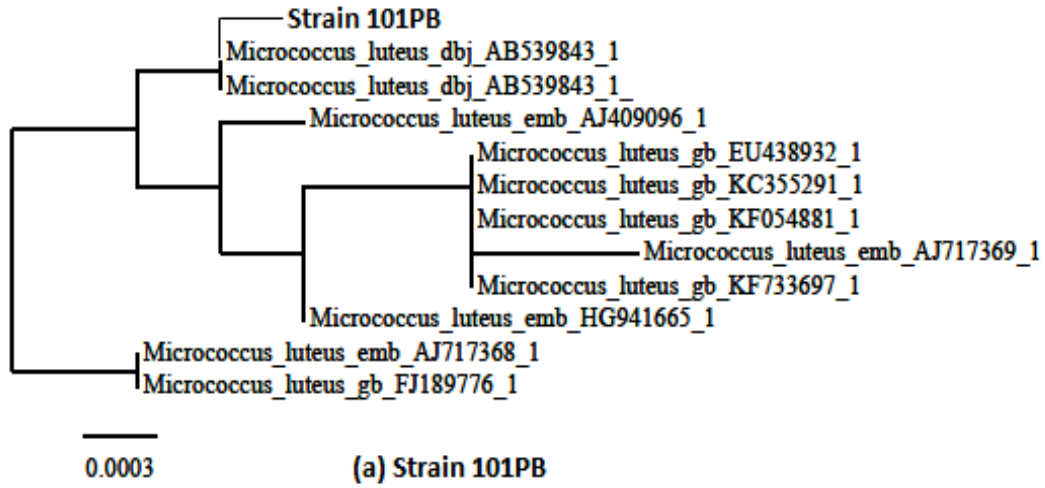


Figure 11: Phylogenetic tree of *Micrococcus luteus* 101PB based on 16S rRNA gene sequence comparisons

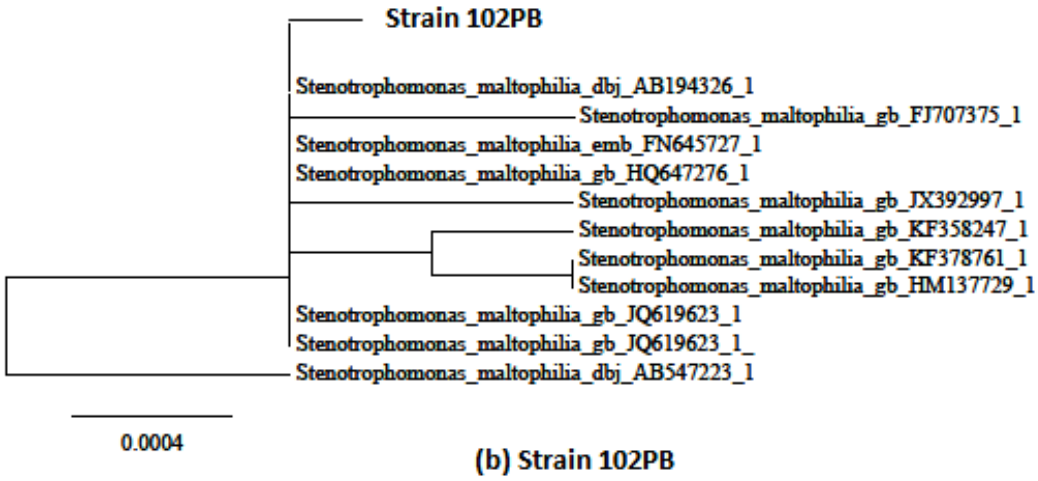


Figure 12: Phylogenetic tree of *Stenotrophomonas maltophilia* 102PB based on 16S rRNA gene sequence comparisons

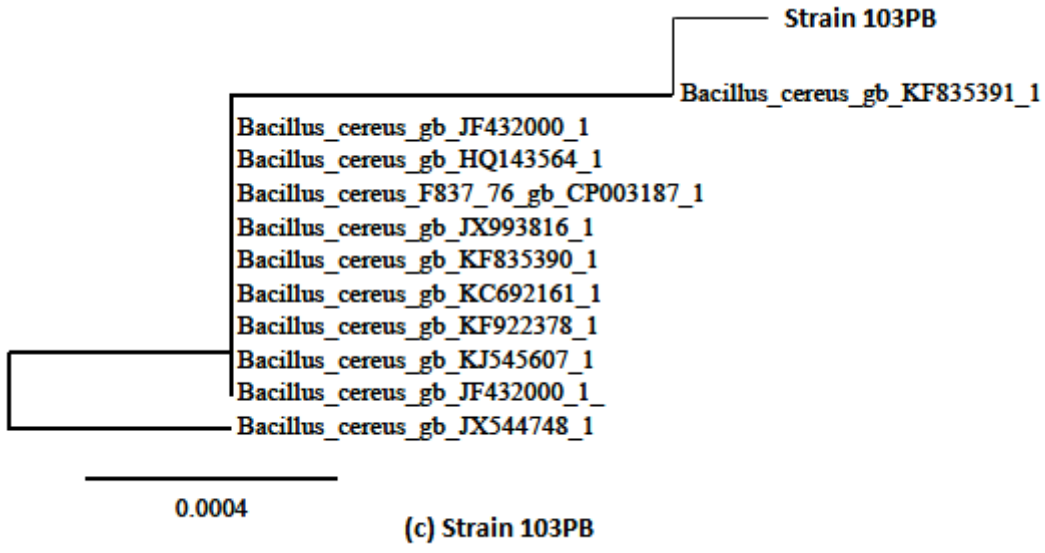


Figure 13: Phylogenetic tree of *Bacillus cereus* 103PB based on 16S rRNA gene sequence comparisons

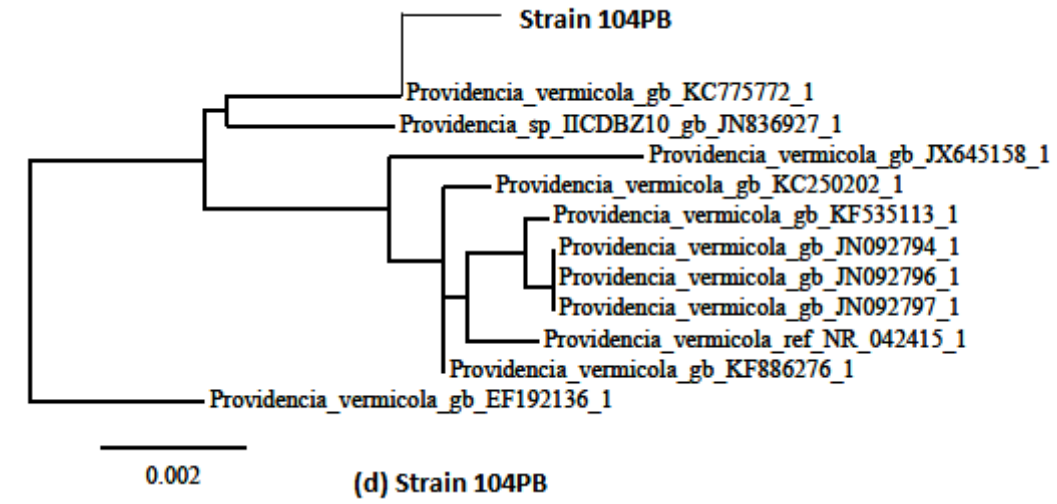


Figure 14: Phylogenetic tree of *Providencia vermicola* 104PB based on 16S rRNA gene sequence comparisons

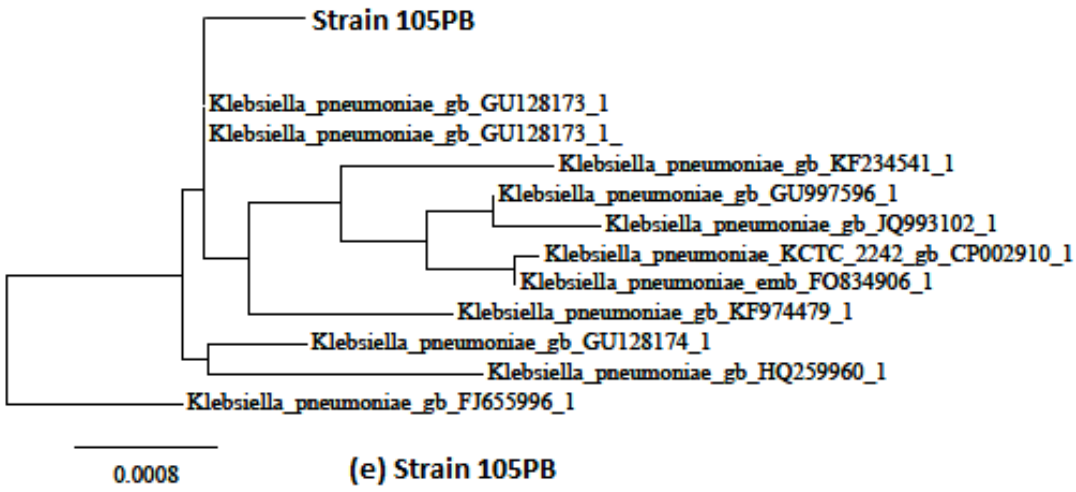


Figure 15: Phylogenetic tree of *Klebsiella pneumoniae* 105PB, based on 16S rRNA gene sequence comparisons

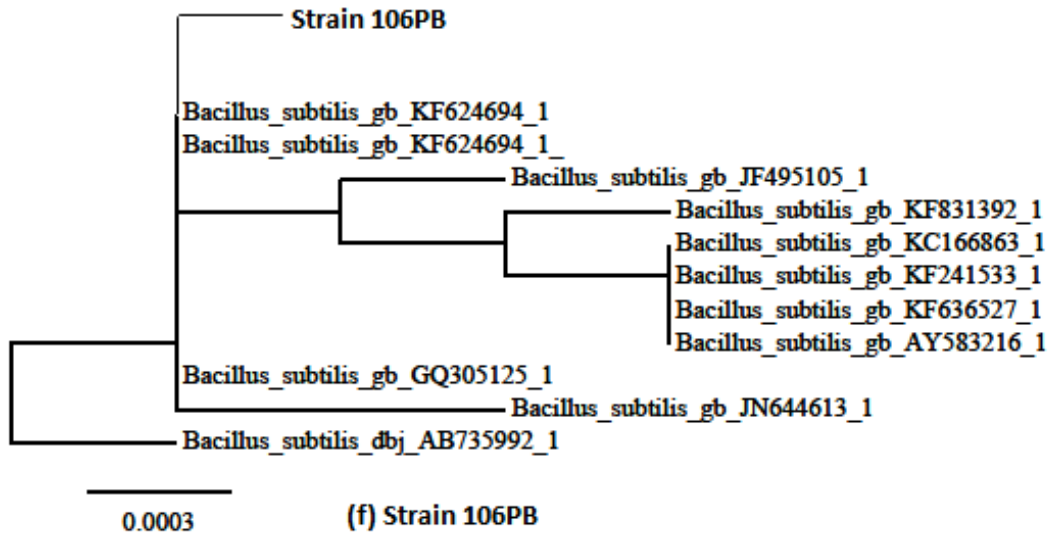


Figure 16: Phylogenetic tree of *Bacillus subtilis* 106PB based on 16S rRNA gene sequence comparisons

Phylogenetic trees of the identified fungal isolates from POME

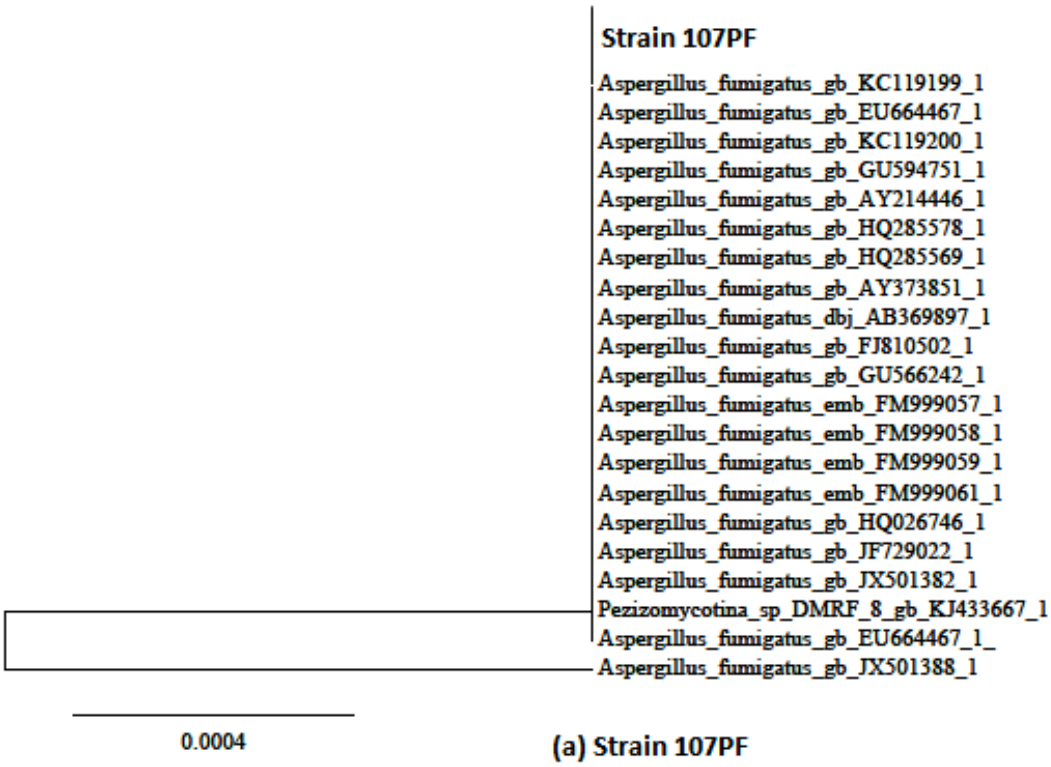


Figure 17: Phylogenetic tree of *Aspergillus fumigatus* 107PF based on 18S rRNA gene sequence comparisons

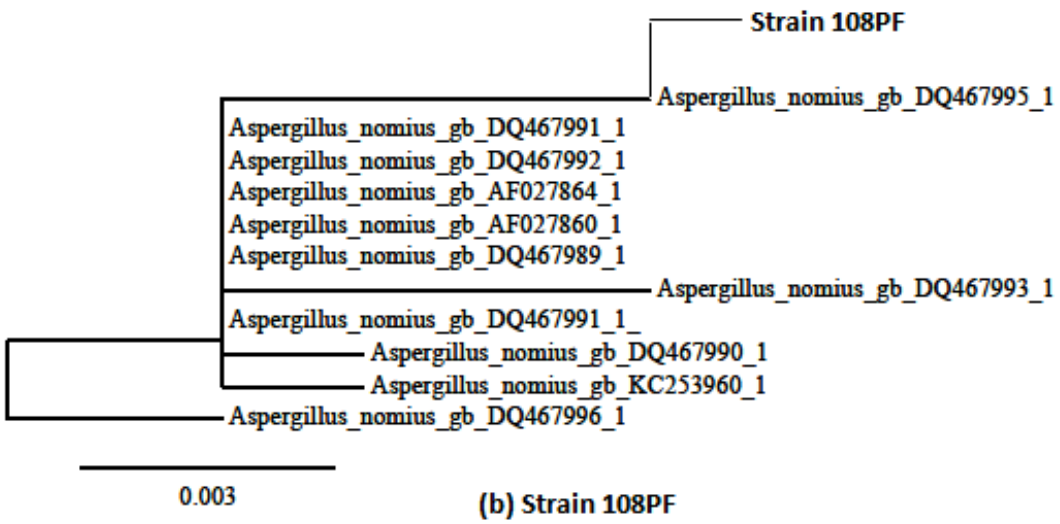


Figure 18: Phylogenetic tree of *Aspergillus nomius* 108PF based on 18S rRNA gene sequenc comparisons

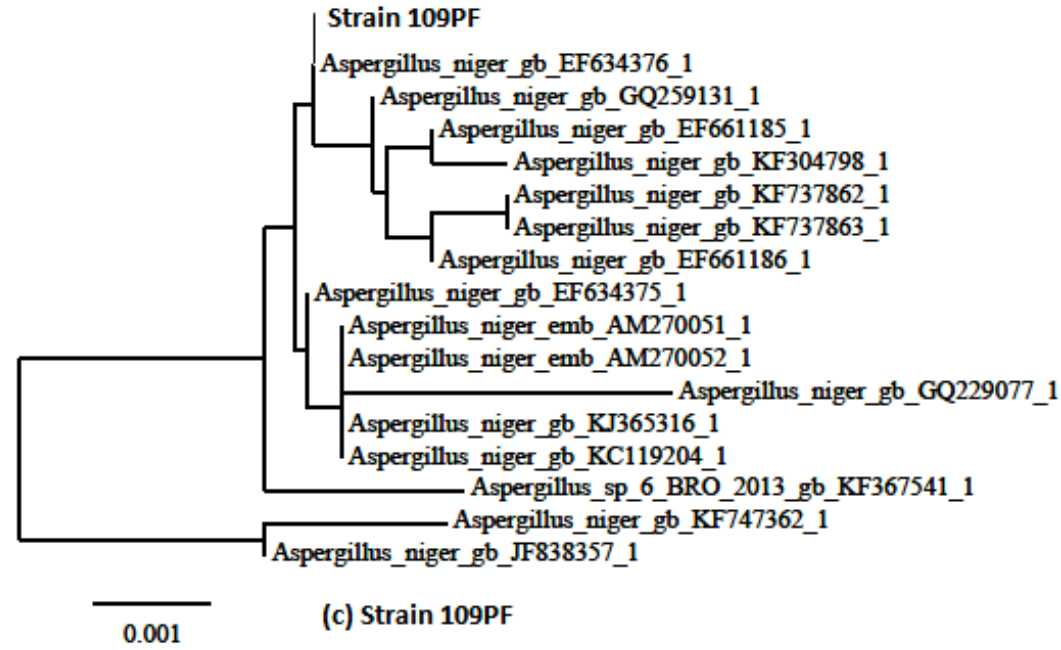


Figure 19: Phylogenetic tree of *Aspergillus niger* 109PF based on 18S rRNA gene sequence

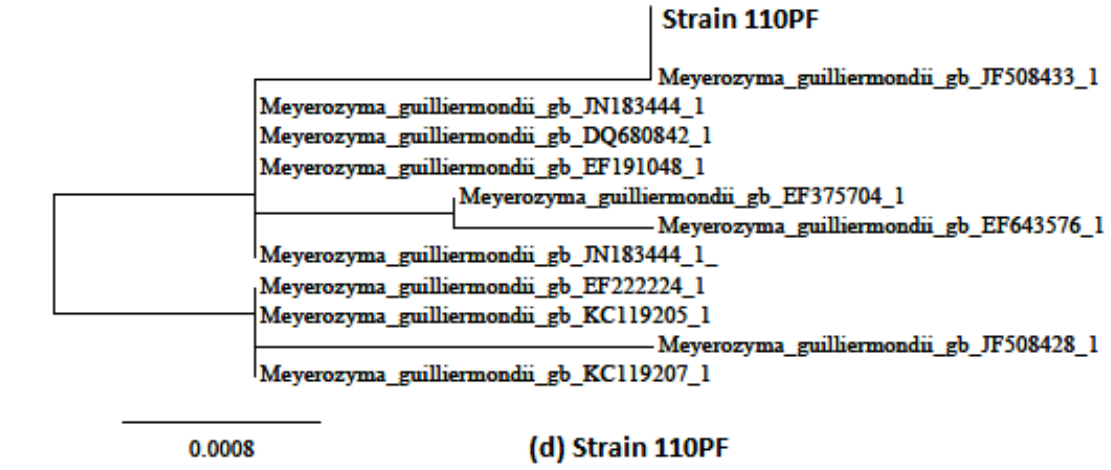


Figure 20: Phylogenetic tree of *Meyerozyma guilliermondii* 110PF based on 18S rRNA gene sequence comparisons

Table 6: Cultural characteristics of bacteria isolated from POME (Supplementary data)

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

Table 7: Microscopic, macroscopic morphology and cultural characteristics of fungi isolated from POME

Organisms	Type of organisms	Microscopic morphology	Macroscopic morphology
Aspergillus fumigatus 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
Aspergillus nomius 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
Aspergillus niger 109PF	Filamentous mold	Presence of septate hyphae, long and smooth conidiophores, and long unbranded sporangiospores with large, round head	Brownish-black mycelium with dark spores and often appears golden on the reverse side
Meyerozyma guilliermondii 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