Original Research

Volume 06, Issue 01, pp 41-53, March, 2024

ACHIEVERS JOURNAL OF SCIENTIFIC RESEARCH

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Biosurfactant Production by Some Microbes Isolated from Some Departmental General Offices at the University of Ilorin

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Submitted: January, 28, 2024, Revised: March 25, 2024, Accepted: March 29, 2024, Published: March 30, 2024

Abstract

Air contains large number of microorganisms including bacteria and fungi and their estimation is important as an index of cleanliness for any particular environment. Ability of a microorganism to produce biosurfactant is an indicator of its hydrocarbon degradation capability. The air microflora of five different offices at the faculty of Life Sciences in the University of Ilorin was examined by exposing plates on nutrient agar and potato dextrose agar at different locations in the offices. The bacterial load obtained ranged from 20,445 to 24,849 cfu/m^3 whiles the fungal load ranged from 5,269 to 10,144 cfu/m^3. A total of 6 bacterial and 10 fungal species were isolated at varying frequency of distribution. The bacteria isolated were *Pseudomonas aeruginosa, Micrococcus varians, Staphylococcus epidermidis, Bacillus circulans, Staphylococcus aureus* and *Bacillus subtilis.* The most predominant bacterial isolate was *Staphylococcus aureus* while the least was, *Bacillus circulans*. The fungi isolated were *Aspergillus niger, Penicillum chrysogenum, Cladosporium herbarum, Aspergillus nidulans, Mucor mucedo, Geotrichum candidum, Fusarium oxysporum* and *Rhizopus stolonifer*. The predominant fungal isolate was *Aspergillus niger* while *Fusarium oxysporum* was the least predominant. The highest emulsification index was observed in *Pseudomonas aureginosa* with an $E_{24\%}$ of 50% using crude oil as its carbon source. *Pseudomonas aeruginosa* and *Bacillus Bacillus subtilis* showed promise oof biosurfactant production and hence petroleum biodegradation. This will be explored further in our next study. Most of the microorganisms are opportunistic pathogens especially in immunocompromised individuals. Therefore, proper air sanitation is advised in offices to prevent infection.

Keywords: Air microflora, Bacteria, Biosurfactant, Fungi, Ilorin.

1.0 Introduction

The air in our atmosphere is made up of many gas molecules. The most common gases are nitrogen (78%), oxygen (about 21%), and argon(almost1%). Other molecules are present in the atmosphere as well, but in very small quantities (Ugboma *et al.*, 2021). The earth's atmosphere is full of microorganisms in the form of lichen, bacteria, fungi, and algae. The composition and concentration of these particles are generally related to man activities (Lacey and West, 2006).

Air quality of the environments is one of the main factors affecting health, wellbeing and productivity of people. Since most people spend 80% to 90% of their time inside (Spiru and Simona, 2017), one issue with indoor air quality is the presence of microorganisms including bacteria, mould, and viruses. Man inhales an average 14 m^3 of air per day. These make people highly exposed to indoor air environments.

Because of this, research on indoor microbes has gained popularity in recent years. The degree to which the air is clear, clean, and free of gaseous impurities like smoke, dust, and smog is referred to as good air quality. Poor air quality can affect or harm human health and the environment. These sources can seriously affect the overall air quality and can lead to severe health problems for humans (Wemedo *et al.*, 2012).

Air borne microorganisms are usually derived from various natural sources such as soil, animals and human activities which include sewage treatment, plants and animal rendering, fermentation process and agricultural activities all release microorganisms into air (Recer *et al.*, 2001). Several studies have identified human activities like talking sneezing and coughing as sources of microorganisms in air while other human activities such as vehicular transportation and human movements, washing in homes and business centers, flushing of toilets and sewages, sweeping of floors and roadsides can generate bio aerosols indirectly. Since microorganisms can lodge in or on dust particles, dust therefore is a potential source of bio aerosols (Chen and Hildermann, 2009).

Biosurfactants are biologically derived surface-active substances that are primarily produced as secondary metabolites by filamentous fungus, yeast, and bacteria. Due to their special amphiphilic composition, which combines hydrophobic and hydrophilic parts, which increases the bioavailability of water and lowers the surface tension. This provides emulsification activity (Nayarisseri *et al*., 2018; Meliani and Bensoltane, 2014). Biosurfactants have several advantages, including low toxicity, high biodegradability, low irritancy and compatibility with human skin (Karnwal *et al*., 2023). The ability of a microbe to produce biosurfactant is a pointer to its capability to degrade petroleum hydrocarbons.

The diverse structure of biosurfactants results from their different microbial origin, the substrate on which they are grown and cultivation conditions used (Santos *et al*., 2016). In recent years, there has been a substantial increase in the production of biosurfactants and their commercialization (Henkel and Hausmann, 2019).

There are basically five classes of biosurfactants: (i) lipopeptides, (ii) specific biosurfactants, (iii) glycolipids, and lipoproteins, (iv) polymeric surfactants and (v) phospholipids and fatty acids (Desai and Banat, 1997; Varjani and Upasani, 2017). The biosurfactant production is an important survival strategy by different microorganisms as it helps in uptake of hydrophobic substrates for surface associated modes of motility (Chrzanowski *et al*., 2012). Microbial surfactants are complex molecules, comprising a wide variety of chemical structures, such as glycolipids, lipopeptides, fatty acids, polysaccharides-protein complexes, peptides, phospholipids and neutral lipids (Banat *et al*., 2000). Numerous industrial sectors, including the oil, food, paper, pulp, pharmaceutical, and agricultural sectors, have found use for biosurfactants (Mukherjee *et al*., 2006). *Pseudomonas aeruginosa* is the preferred microorganism for the production of rhamnolipid type of biosurfactant utilizing glycerol, mannitol, fructose, glucose, and vegetable oils (Koch *et al*., 1991; Santos *et al*., 2002). Rhamnolipids are one of the most important glycolipid biosurfactants, which are produced by two bacterial species of *Pseudomonas aeruginosa* and *Burkholderia* (Fracchia *et al*., 2012).

Microorganisms especially bacteria represent an excellent source of biosurfactants, so that the isolation and characterization of the emulsifying capacity of biosurfactant molecules represents an important step for their future application in the areas of biotechnology (Singh *et al*., 2019; Volkering *et al*., 1997).
The ability of a microbe to produce biosurfactant is a pointer to its capability to degrade petroleum hydrocarbons. Hence, the present study focused on the biosurfactant production ability of microbes isolated from the air of various departmental offices at the University of Ilorin.

2.0 Materials and Methods

2.1 Air Sampling Procedure

The monitoring of air microflora was done by settling plate technique, on a daily basis. The exposure was carried out over the course of five days. On each occasion, duplicate plates were exposed. The exposure was done at different locations within the offices and placed on the sampling height 2m above the floor, which is approximately to be the human breathing zone. The nutrient agar plates were incubated at 37℃ for 24 hrs while the potato dextrose agar were incubated at room temperature (25℃) for 48-72 hrs. The numbers of colonies were counted and then characterized. Once colony forming units (CFU) were enumerated, CFU/m^3 were determined, taking into account the following equation described by Omeliansky (Borrego *et al.,* 2010; Gutarowska 2010).

$$N = 5a \times 10^4 (bt)^{-1} \quad \text{---------- Equation 1}$$

Where N=microbial CFU/m^3 of indoor air; a=number of colonies per Petri dish;
b=dish surface (cm^2); t=exposure time (min).

2.2 Characterization and identification of Bacteria Isolates

The characterization of each bacterial isolate was based on colonial morphology, cellular morphology and biochemical tests. Colonial morphology such as shape, colour, edge, elevation, surface texture and optical characteristics was observed macroscopically and recorded according to Fawole and Oso (2007). The cellular characteristics were determined by their reaction to Gram staining, endospores staining and motility test. In order to fully characterize and identify the isolates, the colonial and cellular morphologies were supplemented with routine biochemical tests such as coagulase test, catalase test, citrate utilization test, sugar fermentation test, oxygen relationship and Methyl red- Voges Proskaeur test. These results were compared with characteristics of organisms in Bergey's manual of determinative bacteriology (Holt *et al.,* 1984), to get the names of the bacterial isolates.

2.3 Characterisation and Identification of Fungal Isolates

The fungal isolate's morphological and microscopic characteristics were used for their characterisation (Onions *et al.,* 1981; Samson *et al.,* 2000; Fawole and Oso, 2007). Reference was made to Alexopoulos (1979) and Campbell and Stewart (1980) for identification.

2.4 Screening for biosurfactant producing bacteria

2.4.1 Haemolytic Assay

Each isolate was streaked on blood agar medium containing 5% v/v blood and incubated at 37 °C for 24–48 h. Haemolysis activity was indicated by the presence of clear zones around the colonies, indicating biosurfactant production (Samuel-Osamoka *et al*. 2018).

2.4.2 Drop Collapse Assay

Ten (10) µl of crude oil, engine oil and kerosene were used separately to thinly coat the wells of microtitre plates respectively. This was left undisturbed for 1 hour at 30 ℃ to form uniform thin coats in the well. The bacteria cultures were centrifuged at 3500 rpm for 10 minutes, then left to settle for the cell free supernatant to separate from the cell pellets. Then 10 µl of the centrifuged cell free supernatant of the cultures grown in nutrient broth (NB), were added separately to the center of the well. The presence of biosurfactant was detected from the drop collapsing from the oil coated well within 1-2 mins, while if the drop remains, it is negative (Dhiman and Meena 2016).

2.4.3 Oil displacement Assay

In oil displacement test, 40 µl of oil (crude oil, diesel or kerosine) was placed on the surface of 40 ml of distilled water in a Petri dish forming thin oil layer on it. After that, 10 µl of culture supernatant was gently placed on the centre of the oil layer. Clear zone formation by displacing oil indicates the presence of biosurfactant. The diameter of the clear zone on the oil surface was visualized under visible light and measured with a ruler after 30 seconds. This correlates to the surfactant activity and is also known as an oil displacement activity. The mathematical

representation of oil displacement test is calculated as; (mm) = diameter of the clear zone before oil (crude oil, diesel or kerosine) – diameter of the clear zone after addition of culture supernatant and oil (crude oil, diesel or kerosine) (Kurniati *et al.*, 2019; Safary *et al.*, 2010).

2.4.4 Emulsification Test

Emulsion activity of the culture supernatant was detected by addition of 0.5 ml hydrocarbons (engine oil, kerosene or crude oil) to an equal volume of cell free supernatant. This was mixed with a vortex for 2 minutes and allowed to stand for 24 hours at 35 ± 2 °C. The emulsification activity was observed after 24 h and it was calculated using the following formula:

$$E24\% = [Total\ height\ of\ the\ emulsifieid\ layer / Total\ height\ of\ the\ liquid\ layer \times 100] \quad \text{(Equation 2)}$$

This equation calculates the E24% value, as proposed by Khan *et al*. (2017). It represents the ratio of the total height of the emulsified layer to the total height of the liquid layer, expressed as a percentage.

3.0 RESULT AND DISCUSSION

3.1 Bacterial Isolates

Six bacterial isolates were obtained after a sampling period of five days. The bacterial isolates obtained from the sampling site were identified as *Pseudomonas aeruginosa, Micrococcus varians, Staphylococcus epidermidis, Bacillus circulans, Staphylococcus aureus* and *Bacillus subtilis.*

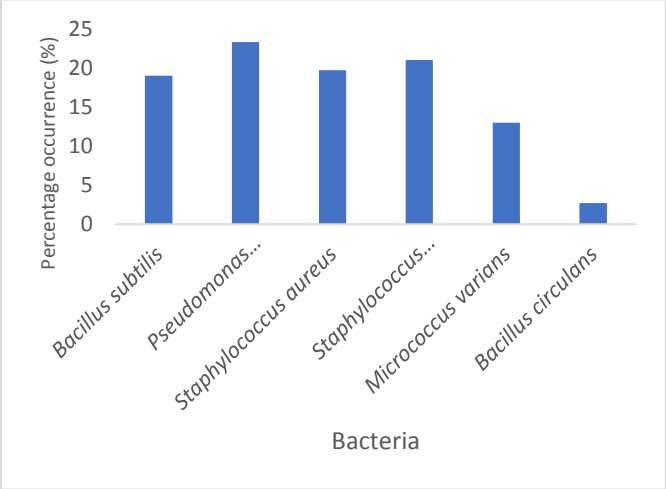

Figure 1: Percentage occurrence of bacterial isolates over the five days sampling period.

Table 1: Occurrence of Bacterial Isolates at the Sampling Site

S/N	Bacterial	Day 1	Day 2	Day 3	Day 4	Day 5
1	*Bacillus subtilis*	+	+	+	+	+
2	*Pseudomonas aeruginosa*	+	+	+	+	+
3	*Staphylococcus aureus*	+	+	+	+	+
4	*Staphylococcus epidermidis*	+	+	+	+	+
5	*Micrococcus varians*	+	+	+	+	+
6	*Bacillus circulans*	+	-	-	-	-

KEY: + = Present, - = Absent.

A total of five Gram positive and one Gram negative isolates were obtained. The Gram-positive organisms obtained include; *Micrococcus varians, Staphylococcus epidermidis, Bacillus circulans, Staphylococcus aureus* and *Bacillus subtilis* while *Pseudomonas aeruginosa* was Gram negative. The cellular arrangement of the isolates varied from singly to clusters and to chains.

The occurrences of the bacterial isolates are presented in Table 1. *Micrococcus varians, Staphylococcus epidermidis, Bacillus circulans, Staphylococcus aureus* and *Bacillus subtilis* were

isolated in the 1st, 2nd, 3rd, 4th, and 5th day while *Bacillus circulans* was isolated only on the first day. The overall frequency of occurrence in percentage of the isolated bacteria per week is shown in Figure 1.

Table 2 shows the number of bacterial isolates obtained at the sampling sites over the five days sampling period. Week one had the highest plate count while the lowest plate count was obtained in week two. From figure 1, it can be deduced that *Staphylococcus aureus* was the most predominant of all bacterial isolates with a percentage occurrence of 24% while *Bacillus circulans* which had a percentage occurrence of 3% was the least predominant.

Table 2: Number of bacteria in air at different sampling days

Bacterial Isolates	**Sampling Period**				
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Bacillus subtilis	5426 $cfum^{-3}$	3303 $cfum^{-3}$	3696 $cfum^{-3}$	4089 $cfum^{-3}$	4089 $cfum^{-3}$
Staphylococcus aureus	4639 $cfum^{-3}$	4482 $cfum^{-3}$	4953 $cfum^{-3}$	5111 $cfum^{-3}$	5504 $cfum^{-3}$
Pseudomonas aeruginosa	3774 $cfum^{-3}$	3932 $cfum^{-3}$	5347 $cfum^{-3}$	4010 $cfum^{-3}$	3774 $cfum^{-3}$
Staphylococcus epidermidis	5326 $cfum^{-3}$	3932 $cfum^{-3}$	4718 $cfum^{-3}$	4325 $cfum^{-3}$	3932 $cfum^{-3}$
Micrococcus varians	2752 $cfum^{-3}$	2595 $cfum^{-3}$	2831 $cfum^{-3}$	3460 $cfum^{-3}$	3145 $cfum^{-3}$
Bacillus circulans	2831 $cfum^{-3}$	0 $cfum^{-3}$	0 $cfum^{-3}$	0 $cfum^{-3}$	0 $cfum^{-3}$

3.2 Emulsification Index (E24) of Cell Free Supernatant of Test Organisms

Figure 2 shows the emulsification index (E24) of all 6 isolates grown in different hydrocarbon sources i.e., crude oil, kerosene and engine oil. The overall highest result was seen in *Pseudomonas aeruginosa* using crude oil as its carbon source and the lowest activity in *Micrococcus varians* using engine oil as the carbon source. The isolates showed the best growth in the presence of crude oil and poor growth in the presence of kerosene.

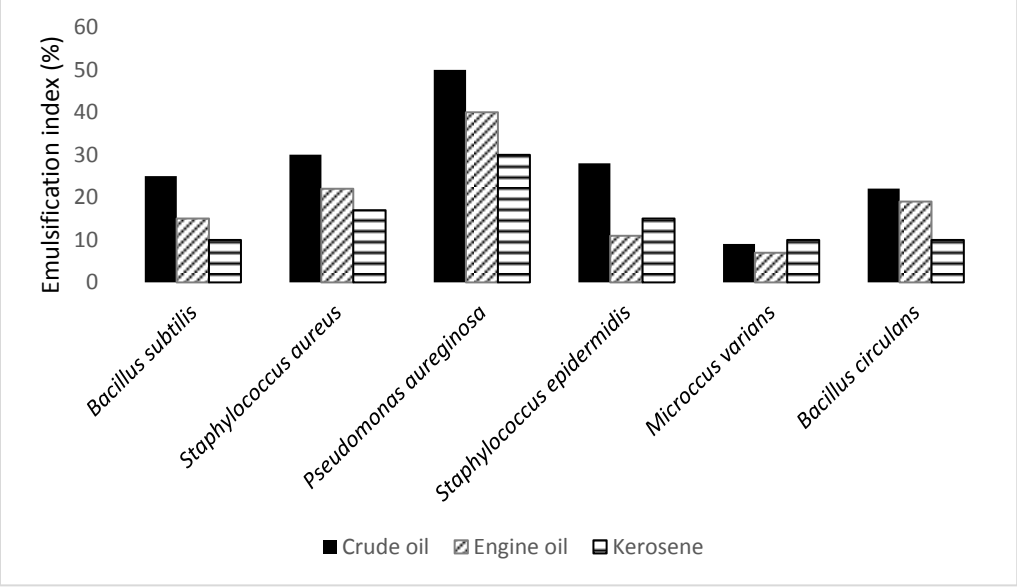

Figure 2: Emulsification index (E_{24}) of bacterial isolates in hydrocarbons

Table 3: Oil Spread Test, Drop Collapse Assay and Haemolytic Assay

Bacteria	Drop collapse			Oil spreading			Haemolysis
	C	E	K	C	E	K	
Bacillus subtilis	+++	++	-	++	-	+	β
Staphylococcus aureus	+	-	++	+	+	-	β
Pseudomonas aeruginosa	++	+	+	+++	+	++	β
Staphylococcus epidermidis	+	-	-	+	-	+	γ
Micrococcus varians	_	_	_	-	-	+	γ
Bacillus circulans	+	+	++	+	-	-	α

Key: C= Crude Oil, E= Engine Oil, K= Kerosene

Oil spreading assay: **'+'** - oil spreading with a clear zone of 0.5-1.5 cm, **'++'** - oil spreading with a clear zone of 1.6 to 2.5 cm, **'+++'** - oil spreading with a clear zone of 2.6 to 3.5 cm
Drop collapse assay: '+++'- drop collapse within 1 minute, **'++'**- drop collapse after 2 minute and '+' - drop collapse after 3 minutes of biosurfactant addition.
Haemolytic assay: 'A' - Alpha-haemolysis (α), **'β'**- Beta-haemolysis (β), **'γ'** gamma-haemolysis (γ).

3.3 Haemolytic Test

Among the six bacterial isolates, three were positive for biosurfactant production showing B-haemolysis, two partially haemolysed blood and one showed no haemolysis as shown in table 3. (Carrillo *et al.*,1996) discovered a correlation between haemolytic activity and the production of biosurfactants, and they suggested using blood agar lysis as the main technique for screening for the presence of biosurfactants. On the other hand, strains with positive haemolytic activity were found to be negative for biosurfactant production (Youssef *et al.*, 2004; Thavasi *et al.*, 2011). Furthermore, not all biosurfactants exhibit haemolytic activity, and haemolysis can also be caused by substances other than biosurfactants.

3.4 Drop Collapse Test

Drop collapse method is a sensitive and easy to perform method which requires small volume (~10µl) of culture broth or biosurfactant solution to test the surfactant property. Among the six (6) bacterial isolates screened, five (5) were positive for drop collapse activity (Table 3) namely *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis and Bacillus circulans. Micrococcus varians* was negative for drop collapse activity. Accuracy and reliability of results obtained in drop collapse assay in this study was similar to the results reported by Bodour and Miller-Maier (1998).

3.5 Oil Spread/Displacement Test

Oil spreading assay results were in corroboration with drop collapse assay results. These results confirmed the presence (for strains with positive results) and absence (for strains with negative results) of surface-active compound (biosurfactant) in the cell free culture broth. It was observed that six bacterial isolates were positive for the oil spreading assay, *Pseudomonas aeruginosa* showed highest oil spreading activity as showed in table 3. Similar results with drop collapse and oil spreading assay were reported by Youssef *et al.* (2004) while

screening bacteria for biosurfactant production and also recommended that both drop collapse and oil spreading assay methods as reliable techniques for testing biosurfactant production.

3.6 Fungal Isolates

A total number of ten different fungal isolates were obtained and identified (Table 4). These were *Aspergillus nidulans, Aspergillus niger, Mucor mucedo, Fusarium oxysporum, Rhizopus stolonifer, Geotrichum candidum, Penicillum chrysogenum, Cladosporium herbarum, Aspergillus flavus* and *Aspergillus versicolor.*

Table 4: occurrence of fungal Isolates at the sampling Sites

S/N	Fungal Isolates	Day 1	Day 2	Day 3	Day 4	Day 5
1	*Aspergillus nidulans*	+	-	+	+	+
2	*Aspergillus niger*	+	+	+	+	+
3	*Mucor mucedo*	+	+	+	-	+
4	*Fusarium oxysporum.*	+	+	+	+	-
5	*Rhizopus stolonifer*	+	+	+	-	-
6	*Geotrichum candidum.*	+	+	+	-	-
7	*Penicillum chrysogenum*	+	+	+	+	+
8	*Cladosporium herbarum*	+	+	+	+	-
9	*Aspergillus flavus*	+	+	+	-	-
10	*Aspergillus versicolor*	+	+	+	-	+

Key: + = Present, - = Absent

During the sampling period, the fungal isolates occurred at different frequencies over the period of isolation at the sampling site. Tables 4 and 5 show the frequency of occurrence and number of fungal isolates obtained while Figure 3 shows the percentage frequency of individual isolated over the five days sampling period. *Aspergillus niger* and *Penicillum chrysogenum* were both isolated in five of the five days of sampling. From Table 5 and Figure 3, it can be deduced that *Aspergillus niger* was the most predominant fungus as it had a percentage occurrence of 18%, and *Geotrichum candidum* was the least predominant with a percentage occurrence of 6%. Week three had the highest plate count with a total of 129 colonies while the lowest plate count was obtained in week five with 67 colonies.

The study showed that room ventilation plays a role in the distribution of microorganisms. The microbial isolates characterized and identified included six bacterial and ten fungal isolates, they include, *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus circulans, Micrococcus varians and Pseudomonas aeruginosa* for bacterial isolates, while the fungal isolates were *Aspergillus nidulans, Aspergillus niger, Penicillum chrysogenum, Cladosporium herbarum, Mucor mucedo, Geotrichum candidum, Fusarium oxysporum, Rhizopus stolonifer, Aspergillus flavus* and *Aspergillus versicolor.*

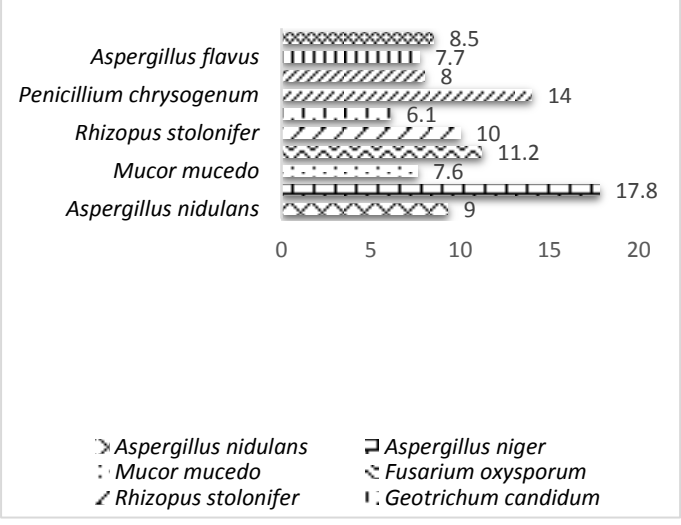

Figure 3: Overall percentage occurrence of isolated fungal isolates

Table 5: Number of fungi in air at different sampling days

S/N	Fungal Isolates	Sampling Period				
		Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
1	*Aspergillus nidulans*	629 cfum^{-3}	0 cfum^{-3}	944 cfum^{-3}	865 cfum^{-3}	1022 cfum^{-3}
2	*Aspergillus niger*	1808 cfum^{-3}	944 cfum^{-3}	1101 cfum^{-3}	1573 cfum^{-3}	1180 cfum^{-3}
3	*Mucor mucedo*	708 cfum^{-3}	472 cfum^{-3}	865 cfum^{-3}	0 cfum^{-3}	786 cfum^{-3}
4	*Fusarium oxysporum*	786 cfum^{-3}	944 cfum^{-3}	1022 cfum^{-3}	1415 cfum^{-3}	0 cfum^{-3}
5	*Rhizopus stolonifera*	944 cfum^{-3}	550 cfum^{-3}	944 cfum^{-3}	1258 cfum^{-3}	0 cfum^{-3}
6	*Geotrichum candidum*	629 cfum^{-3}	550 cfum^{-3}	1101 cfum^{-3}	0 cfum^{-3}	0 cfum^{-3}
7	*Penicillum chrysogenum*	865 cfum^{-3}	944 cfum^{-3}	1180 cfum^{-3}	1101 cfum^{-3}	1101 cfum^{-3}
8	*Cladosporium herbarum*	1022 cfum^{-3}	1101 cfum^{-3}	865 cfum^{-3}	0 cfum^{-3}	0 cfum^{-3}
9	*Aspergillus flavus*	865 cfum^{-3}	708 cfum^{-3}	1258 cfum^{-3}	0 cfum^{-3}	0 cfum^{-3}
10	*Aspergillus versicolor*	786 cfum^{-3}	315 cfum^{-3}	865 cfum^{-3}	0 cfum^{-3}	1180 cfum^{-3}

The most predominant fungal isolate was *Aspergillus niger* while *Fusarium oxysporum* were the least predominant. Presence of these organisms is affected by some factors which include level of activities in the sampling area or surrounding, therefore the member and type of organisms that fall on exposed plates depends on the time of exposure, the movement of individuals and the current content of the air passing at the time and method used in sampling (Sweeney and Dobson, 1998). The discharge of these infectious organisms into the environment are through sneezing, coughing, talking, contact with office materials and uncontrolled movement in and out of the office.

Staphylococcus aureus and *Bacillus subtilis*, were the most prevalent bacterial isolates. *Aspergillus niger* and *Penicillum chrysogenum* were the most prevalent fungal isolates. This finding agrees with Matković *et al.* (2007), who reported *Aspergillus spp.* and *Penicillium spp.* as the predominant genera of organisms isolated from the air. Ekhaise *et al.* (2008) reported *Aspergillus* species as the most common genus of fungi in the air environment. *Aspergillus* and other species of fungi have been implicated as pathogenic in causing several mycotic infections. The relatively high concentrations of fungi in the air environment of the sampled sites may pose only little health hazard to healthy individuals but would pose serious danger and special risk to immunosuppressed persons. Fungal spores from species of *Penicillium* have been implicated with allergies and elicit asthma in vulnerable individuals (Flannigan *et al.*, 1991).

Bacteria species isolated from the sampled air were identified as *Bacillus* sp, *Staphylococcus aureus*, *Pseudomonas* sp, and *Micrococcus* sp. Fungi species isolated from the sampled air were *Aspergillus* sp., *Mucor* sp., *Penicillium* sp., *and Fusarium* sp. This observation corroborates with that of Bowers *et al.* (2012) who reported having identified *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Enterococcus* in air samples of public buildings. A study by Genet *et al.* (2011) reported the presence of *Staphylococcus aureus*, *Enterobacter*, *Streptococcus pyogenes* and *Bacillus cereus* in air samples collected within operating rooms and surgical wards at Jimma University specialized hospital, Southwest Ethiopia.

People occupying or visiting enclosed spaces play an important role in the creation of indoor air micro-flora. Enclosed rooms and offices have the potential of placing human occupants at higher risk than the outdoors. This is because the rate of dilution is low. Enclosed spaces trap aerosols and allow them to buildup to potentially infectious levels. Most of the sampled offices have air conditioning systems, which does not

allow them to utilize the ultraviolet rays of sunlight. Since during the hot seasons the doors are always shut, the windows are closed and curtained, it becomes difficult for ultraviolet rays of sunlight to penetrate. Adetitun and Oladele, (2016) had reported that with the recent rise in community acquired nosocomial infections, more sanitaion is required in enclosed offices.

All of the six bacteria and ten fungi obtained from this research have been implicated as causal agents of food spoilage and diseases of animals and man. They are especially harmful as opportunistic pathogens in immune-compromised individuals, the aged and children. The pollution of the environment by pathogenic or opportunistic fungi and bacteria is an important factor affecting health. Diseases such as allergy, rhinitis, bronchial asthma, respiratory tract infection may develop in occupants or they may serve as latent hosts. The disease caused by these microorganisms is contacted through inhalation of their (Curtis *et al*., 2004).

Biosurfactant producing bacteria, such as *Pseudomonas aeruginosa* and *Bacillus subtilis* were potent producer which was confirmed by screening tests. *Pseudomonas aeruginosa* has been known to produce rhamnolipid type of biosurfactant having different biological properties (Lang and Wullbrandt, 1999). Surfactin, an important antimicrobial and antifungal agent is known to be produced from *Bacillus subtilis* (Kameda *et al*., 1974). Very recently, *Joanna et al*. (2018) reported a nonspecific synergistic antibacterial and anti-fungal effect of biogenic silver nanoparticles and biosurfactant produced by *Bacillus subtilis* towards environmental bacteria and fungi. These microorganisms have been widely recognized for their metabolic versatility and their ability to produce a variety of biosurfactants with diverse chemical structures.

Many of the biosurfactants known today have been studied to examine their possible technical applications (Nayak *et al*., 2009). Most of these applications involve their efficiency in bioremediation, dispersion of oil spills and enhanced oil recovery. Several species of *P. aeruginosa* and *B. Subtilis* produce rhamnolipid, a commonly isolated glycolipid biosurfactant and surfactin, a lipoprotein type biosurfactant, respectively; these two biosurfactants have been shown by Whang *et al*. (2008) to increase solubility and bioavailability of a petrochemical mixture and also stimulate indigenous microorganisms for enhanced biodegradation of diesel contaminated soil.

This work points to the interconnected nature of influencers of indoor air micro flora: human occupancy, activity and ventilation. Hospodsky *et al*. (2012) worked on the effect of human occupancy and microbial load and diversity. He and his co-workers realised that the classrooms had more microbes when occupied than when unoccupied. Kembel *et al*. (2012) researched on the influence of ventilation strategy on microbial load and diversity. In their study, they quantified relationships between building attributes and airborne bacterial communities at a health-care facility. They quantified airborne bacterial community structure and environmental conditions in patient rooms exposed to mechanical or window ventilation and in outdoor air. These workers discovered that the phylogenetic diversity of airborne bacterial communities was lower indoors than outdoors, and mechanically ventilated rooms contained less diverse microbial communities than did window-ventilated rooms. Bacterial communities in indoor environments contained many taxa that are absent or rare outdoors, including taxa closely related to potential human pathogens. Meadow *et al*. (2014) reported the effect of ventilation and human activity on indoor micro-flora. This study showed that anthropogenic activities.

Among the six bacterial isolates obtained in this work, the cultures of *Bacillus subtilis* and *Pseudomonas aeruginosa* were found to produce more biosurfactant than others. (Table 3). The results are agreeable with the work of Anandaraj and Thivakaran (2010); Priya and Usharani (2009); Jaysree *et al*. (2011); Tabatabaee *et al*. (2005).

4.0 CONCLUSION

To harness the potential of bacteria to produce biosurfactant, our future research will focus on optimizing biosurfactant production by these bacterial strains. Metabolic engineering approaches may be employed to enhance biosurfactant yields and focus their properties for specific applications. On the other hand, special attention should be given to the elimination and reduction of pathogenic microbes from indoor air via intermittent sanitation practices. The use of high efficient particulate air filters should be further emphasized indoors in addition to dilution through proper natural ventilation.

Acknowledgement
We thank the Heads of the Departments where we got our samples from for cooperating with us.

Conflict of interest
There is no conflict of interest assicated with this work.

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