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SCREENING AND GROWTH OPTIMIZATION OF BIOSURFACTANT-PRODUCING FUNGI IN AGRO-WASTE (SORGHUM BRAN) MEDIA FOR BIOREMEDIATION STRATEGIES

***Tomilayo Ruth Bukola, Omojasola Patricia Folakemi, Frank Olakunle Otuyelu, Adetitun David Olugbenga and Ridwan Tope Azeez**

Department of Microbiology, Faculty of Life Sciences, University of Ilorin, P.M.B 1515, Ilorin, Nigeria.

*Corresponding authors' email: tomilayo.rb@unilorin.edu.ng Phone: +2348035025834

ABSTRACT

Biosurfactants are valuable microbial amphiphilic molecules, possessing various structures and applications. Optimization of biosufactant production for bioremediation strategies can be enhanced using enriched growth medium for the potent microbes. This study aimed to investigate the biostimulation potential of Agro-waste-enriched media on domestic liquid waste polluted soil using fungal isolates with greater capability for biosurfactant production. Domestic liquid polluted soil samples taken from three different restaurants washing sites at university of Ilorin were analyzed using standard microbiological techniques. Fungal Isolates were morphologically and microscopically identified, screening assays (Haemolytic test, oil spread test, lipase activity and Emulsification Index) were conducted to investigate biosurfactant production potential; isolates with the highest biosurfactant potential were further examined for their growth on agro-enriched media: sorghum bran (PFSBM) using potato broth as control. The results revealed six fungi isolates and yeast (*Aspergillus niger*, *Aspergillus flavus* and *Aspergillus terreus*, *Penicillium chrysogenum*, *Penicillium notatum*, *Clasporium cladosporoides* and *Candida albicans*). *A. niger*, exhibited β-Haemolysis, 62.3% (E_{24}%); 1.1cm (oil spread) and 1.2cm (lipase activity) with the highest biomass growth on PFSBM and PSBM media from (0.885 - 5.206) x 10^{-2} and (0.931 – 5.526) x 10^{-2} respectively from day 0 to day 12 with a slight decreased on day 15; followed by *P. chrysogenum* and *A. flavus* in a decreasing order respectively. This study highlighted the possibility of using indigenous biosurfactant – producing fungi biostimulated with sorghum bran for bioremediation strategy of the domestic liquid polluted sites; offering a sustainable and ecofriendly alternative to conventional chemical surfactants.

Keywords: Biostimulation, Biosurfactants, Emulsification Index, Hydrophobic, Moulds

INTRODUCTION

Soil pollution by domestic liquid waste is a significant environmental issue that affects soil quality and ecosystem health. Domestic liquid waste, also known as domestic wastewater, includes sewage and wastewater from households (toilets, baths, showers, kitchens, and laundries), which typically contain organic matter, pathogens (Bacteria, viruses, and protozoa) that pose health risks to humans and animals when in contact with contaminated soil or water (WHO, 2013 and Shan *et al*., 2023), nutrients (such as nitrogen and phosphorus), detergents, and various household chemicals.

The infiltration of domestic liquid waste into soil can lead to several adverse environmental effects on soil structure and fertility, microbial communities, groundwater Contamination and health risks (Tamas *et al*., 2022).

The presence of domestic liquid waste in soil creates a unique environment where microorganisms, including fungi, must adapt to survive and thrive. These adaptations can lead to the production of biosurfactants—amphiphilic compounds produced by microorganisms that reduce surface tension and can emulsify hydrophobic pollutants (Sharma *et al*., 2022). Studying soils polluted by domestic liquid waste provides an opportunity to explore the potential of fungi to produce biosurfactants, which can be harnessed for environmental cleanup and various industrial applications (Raji and Bello, 2024).

Studies have shown that *Aspergillus terreus* was found to produce a biosurfactant with excellent emulsifying activity (Khan *et al*., 2023). Similarly, *Fusarium oxysporum* was reported to produce a biosurfactant with high surface activity and stability (Krishnaswamy, 2020).

The potential of moulds as biosurfactant producers lies in their high production yield, diverse metabolic capabilities, adaptability to various substrates, and environmental resilience. Moulds, such as species from the genera *Aspergillus* and *Penicillium*, are prolific producers of biosurfactants, often achieving higher yields compared to bacteria and yeast. This high yield can be attributed to their robust metabolic pathways and ability to utilize a wide range of substrates, including inexpensive and readily available waste materials. For instance, *Aspergillus niger* has been shown to produce significant quantities of biosurfactants when grown on agro-industrial wastes, which not only reduces production costs but also enhances sustainability (Liliya *et al*., 2024). Similarly, *Penicillium chrysogenum* can efficiently produce biosurfactants using substrates like molasses, leading to high yield and making the process economically viable (Marcelino *et al*.,2020).

The utilization of alternative and sustainable growth media is crucial for optimizing fungal cultivation processes. Sorghum bran, a byproduct of sorghum grain processing, has the potential to serve as an economical and environmentally friendly substrate for fungal growth. Thus, its effectiveness as a growth medium for specific fungi species needs to be largely explored.

MATERIALS AND METHODS

Site Description

The study sites were within the University of Ilorin, polluted highly with domestic liquid waste; ASUU eatery located besides the ASUU building, the school's general park and another eatery located along motion ground.

Sample Collection
Composite Samples were collected randomly in a sterile polythene bag at different points from each of the sites using a sterile hand trowel, each labeled A.S, M.G and P.E based on the initials of their respective site of collection. They were then immediately transported to the laboratory.

Isolation and Identification of fungi isolates
Serial dilutions of the collected soil samples were carried out, pure cultures of the fungal isolates were obtained (Fawole and Oso, 2007). Macroscopic and microscopic characterization (hyphae, conidiophore and spores features) was made, while identification was done by comparing the result with literatures (Indunil *et al*., 2020; Boddireddy and Singara, 2011).

Screening of fungal isolates for Biosurfactant Production
The biosurfactant producing ability of the prevalent isolates was investigated using some standard methods; hemolytic test, oil displacement test, Lipolytic test and emulsification index (Yehia *et al*., 2024).
Hemolytic Test: Fresh young culture colony of 3-day old fungal isolates was picked and placed on blood agar (37g/L Potato Dextrose Agar, 5% v/v cow blood) then incubated at room temp 28°C for 5-7days. A clear zone around the colony indicates biosurfactant production; (the presence of biosurfactant that completely lyse the red blood cells resulting to colonies surrounded by clear zone). However, complete hemolysis shown by the colonies on blood agar denote β-haemolysis; partial haemolysis (greenish zone) signify α-hemolysis; however, non-clear zone surrounding the colony in the medium signified Y- hemolysis, that is, absence of biosurfactant (inability of the concerned colony to produce biosurfactant (Liliya *et al*. 2024).
Oil Spread Test: About 40ml of distilled water was put into petri dish with a diameter of 10cm, thereafter 1ml of crude oil was dropped to form a thin oil layer on the surface of the water; afterwards, 50µl of cell free broth of the test organisms was dropped on to the surface center of the oil layer. Observed clear zone signified biosurfactant activity. The diameter of the cleared zone was measured, colonies with higher diameter sizes was recorded to have potential for biosurfactant production; showing higher surface activity (Liliya *et al*. 2024 and Yehia *et al*., 2024).

Lipolytic test
This test was carried out by preparing PDA according to manufacturer's instructions and supplementing it with 1% (V/V) paraffin oil. The pH of the medium was stabilized at 7.5 using 0.1M NaoH solution. The fungal isolates were then aseptically picked and dropped unto sterile distilled water in a test tube, shook and about 0.2ml was spread unto the surface of the medium; then, incubated at 28°c for 3 days. Positive result (ability to produce surfactant) showed the presence of clear zones (lipase production) around the colonies; the diameter of which was measured (Liepius *et al*., 2021 and Yayuk *et al*., 2019).

Emulsification Index test
The emulsification index of the culture samples were determined by adding 2ml of hydrocarbon (kerosene) to the same amount of culture supernatant, vortexed for 2 minutes to mix up, and left to stand for 24 hours at room temperature. Height of the emulsion layer formed was measured. The emulsification index E_{24} (%) is given as the ratio of the height of the emulsification layer to the liquid column multiplied by 100. Emulsification Index = (Length of emulsification layer/ length of liquid column) or (Emulsion height / Total height) × 100. Higher E_{24} value shows better emulsifying activity, reflecting effective biosurfactant production (Yehia *et al*., 2024 and Mansour *et al*., 2023).

Preparation of Composed Growth Medium for the Prevalent Fungi Isolates with Biosurfactant Producing Capability
Potato Broth
200g of peeled and sliced potatoes was boiled till softened in 1L of distilled water; strained through white cloth to extract the broth and distilled water was added up to make a litre. The following growth medium used was composed singly by adding specified grams of fructose, sorghum bran, and Dextrose to the prepared potato broth.
Growth medium 1 (PDSBM): 40ml of potato broth + 4g of sorghum bran + 4g of dextrose + 150ml of distilled water;
Growth medium 2 (PFSBM): 40ml of potato broth + 4g of fructose + 4g of sorghum bran + 200ml of distilled water;
Growth medium 3 (PDM): 40ml of potato broth + 4g of dextrose + 200ml of distilled water
Control Growth medium 4 (CPM): 40ml of potato broth + 200ml of distilled water
Each of the broths were divided into three, placed in a conical flask and sterilized in an autoclave at 121°C for 15 minutes. Each of the sterilized three portions is inoculated with different organism of the three different isolates. However, the control medium (CPM) is not inoculated; each medium was properly labeled based on their inoculum content.

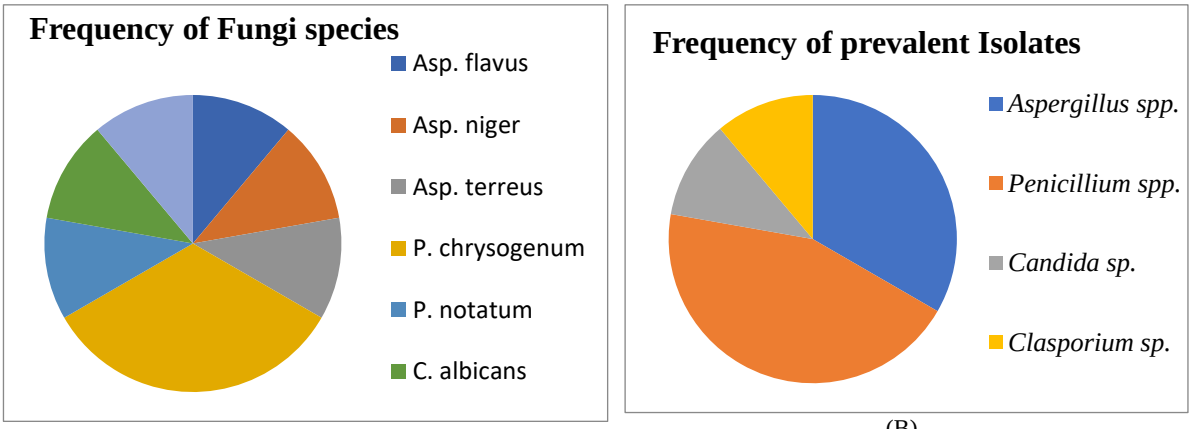
Standardization of the Inocula
The broth culture of the fungi isolates was standardized using MacFarland standard. Small inoculum was picked from a 5-7 days old culture into sterile distilled water in a test tube; the resulting microbial suspension was then put in a colorimeter; adjustment was made until the reading on the colorimeter was between 0.8 - 1.

Growth Measurement
Dry weight measurement of fungal mycelium in each of the medium was determined at every 3days interval for 15days using weight difference method. The mixture was thoroughly shaken while 20mls of each broth was taken and filtered through a pre-weighed filter and air dried at 80^0C for 1 hour. The difference in weight represents the weight of the mycelium (Sebiotimo *et al*., 2010 and Tomilayo *et al*., 2024).

RESULTS AND DISCUSSION
In this study, the morphological and microscopic features (Table 1) showed the seven identified prevalent fungal species isolates obtained: *Aspergillus flavus* (1PR), *Aspergillus terreus* (5PR) *Penicillium chrysogenum* (1ASR), *Penicillium notatum* (2MGR) and *Aspergillus niger* (2PR) *Clasporium cladosporoides* (4PR) *Candida albicans* (2ASR).

Table 1: Microscopic identification and Morphological characteristics

Isolates	Hyphae/mycelium	Conidiophore	Spores	Phialides	Probable Isolates
1PR	Septate hyphae	Erect, yellowish green and unbranched	Black Spherical spores with roughened surface	Flask shaped	*Aspergillus flavus*
2PR	Septate hyphae	Erect, black and unbranched	Yellowish green spherical spores	Flask shaped	*Aspergillus niger*
4PR	Septate hyphae	Erect, septate and branched	Bluish green spherical spores with smooth surface	Flask shaped and borne in clusters	*Clasporium cladosporoides*
5PR	Septate hyphae	Erect and branched	Brown oval shaped spores with slightly roughened surface	Flask shaped	*Aspergillus terreus*
2MGR	Septate hyphae	Erect, septate and branched	Bluish green ellipsoidal, spores with smooth surface	Flask shaped	*Penicillium notatum*
1ASR	Short chains of yeast cells, spherical in shape and forming buds	Not present	Not observed	Not present	*Penicillium chrysogenum*
2ASR	Oval yeast cells forming buds and appearing in clusters	Not present	Not observed	Not present	*Candida albicans*



(A) (B)

Figure 1: Chart A and B showing the frequency of the probable prevalent Fungi species in the sampled soil

Figure 1 however, showed that *Penicillium* spp. (*P. chrysogenum* and *P. notatum*) had the highest frequency followed by *Aspergillus* spp. (*A. niger*, *A. flavus* and *A. terreus*). Though, the result was in contrary to the research work of Tomilayo *et al*. (2024); whereby, *A. niger*, *A. flavus*, *P. chrysogenum* and *R. stolonifer* were obtained from crude oil polluted soil; Mohammed *et al*. (2023) from crude oil polluted soil, also obtained *Aspergillus* spp. as the most common strain among the 10 isolates recorded in his work; similarly, *A. niger* was found to be the most dominant fungal isolate in the analyzed ten soil samples by Mansour *et al*. (2023), followed by *A. terreus* and *A. egypticus*.

Table 2: Screening Test

Isolates	Haemolytic test	Oil displacement test	Lipase activity	% Emulsification Index (E_{24})
1PR	β haemolysis	0.6	1.4	56.1
2PR	β haemolysis	1.1	1.2	62.3
3PR	ϒ haemolysis	-	-	47.5
4PR	ϒ haemolysis	0.1	0.3	31.3
5PR	ϒ haemolysis	0.1	-	25.5
1MGR	ϒ haemolysis	-	0.2	32.8
2MGR	β haemolysis	0.4	-	32.5
1ASR	β haemolysis	1.3	0.8	58.5
2AS R	α haemolysis	0.5	1.8	53.8

The screening results (Table 2) showed that *A. niger* had the highest biosurfactant production potential; having β haemolysis, Emulsification index (E 24) % value of 62.3, 1.1 cm (oil displacement) and 1.2 cm (lipase activity); followed by *P. chrysogenum* and *A. flavus*; having β haemolysis; 1.3 cm, 0.8 cm, 58.5%, and 0.6 cm, 1.4 cm, 56.1% for oil displacement, lipase activity and Emulsification index (E_{24}) respectively. This result is in support of Mansour *et al.* (2023), whereby highest zone diameter was obtained with *A. niger*, although, followed by *A. flavus* and *P. chrysogenum* in a decreasing order. The screening result obtained in this study on *A. niger* might be due to the fact that the isolate could adapt better in a versatile environment and probably has the ability to adapt to more harsh conditions from where it is cultivated. However, the proliferation status of the isolate might have also been contributed by the genetic makeup acquisition from the environment as well as structural adaptive features such as hyphae and thallus that enhances its enablement. However, the result was as well in agreement with many researchers whereby the isolated *A. niger* but, from crude oil polluted site, had the best degradation potential (Tomilayo *et al*., 2024). Conversely, other prevalent fungi isolates do not showed total positive reaction all through the four screening test except yeast *Candida albicans* that showed α haemolysis; 0.5cm (oil displacement), 1.8cm (lipase activity) and 53.8 (E_{24}) %.

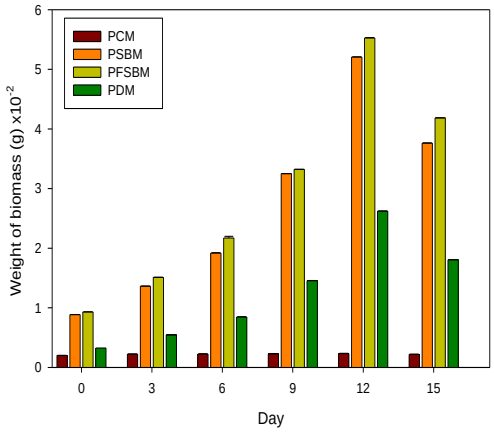


Figure 2: Growth rate *A. niger* (2PR) in three different agro-based enriched medium

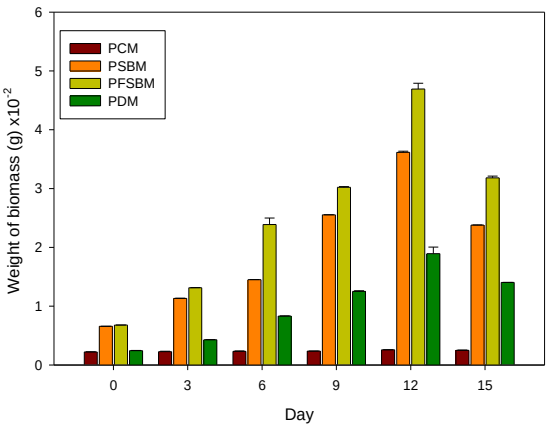


Figure 3: Growth rate of *A. flavus* (1PR) in three different agro based enriched media

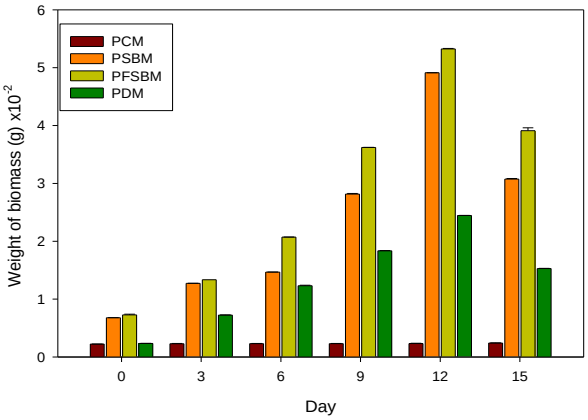


Figure 4: Growth rate of *P. chrysogenum* (1ASR) in three different agro based enriched growth media

However, the aim of this study in exploring the effectiveness of sorghum bran as sole carbon source for biostimulation strategy to remediate the domestic polluted sites was observed in the growth pattern of the three indigenous prevalent fungi isolates (*A. niger, A. flavus,* and *P. chrysogenum*) having the highest biosurfactant production potential. In this study, *A. niger*; also showed the highest biomass growth rate (g/l) in every of the composed enriched media; PSBM, PFSBM and PDM (Figure 2) ranging between (0.885 - 5.206) x 10^{-2}, (0.931 – 5.526) x 10^{-2} and (0.325 - 2.622) x 10^{-2}) respectively from day 0 to day 12 with a slight decreased on day 15. The declining might be as a result of essential nutrient depletion, possible accumulation of toxic metabolites; resulting in the dead cells getting decayed and the interwoven hyphae getting dissolved; thus became filterable through the filter pores (Tomilayo *et al*., 2024). However, the other two most prevalent isolates from the domestic liquid polluted soils, as well had the following growth rate range values in PSBM, PFSBM and PDM; (0.678 - 4.912) x 10^{-2}, (0.728 – 5.323) x 10^{-2} and (0.234 – 2.445) x 10^{-2} then; (0.658 – 3.615) x 10^{-2}, (0.678 – 4.691) x 10^{-2} and (0.245 – 1.894) x 10^{-2} as shown by *P. chrysogenum* (figure 4) and *A. flavus* (figure 3) respectively. But there was no significant increase in the values of the control medium from day 0 till day 15 (0.2 – 0.258) x 10^{-2}; this is because control medium had no inoculum, so, no growth is expected.

Nevertheless, the three fungal isolates (*A. niger, P. chrysogenum* and *A. flavus,*) having shown remarkable growth rate in the enriched growth medium proved that the biostimulation of the domestic liquid polluted soil using sorghum bran will be a sort of friendly clean up strategy of the studied environment; thus, boosting waste management.

Using sorghum bran enriched media to enhance the growth of *A. niger, P. chrysogenum,* and *A. flavus* agreed with work of Izebe *et al*., 2020 and Reddy *et al*., 2010; however, Izebe *et al*., researched on *Staph. aureus* and *Bacillus subtilis*.

The need for preparations of enriched culture media from local resources has led researchers into the use of readily available materials. This agreed to the research study by Jhanani and Krishnakumar (2025); who reported formulations from fruit peel extracts to grow *E. coli, Serratia* sp., *and P. aeruginosa*. Similarly, Sorghum bran been rich in essential nutrients (carbohydrates, proteins, and minerals) is crucial for fungal growth. The bran's high fiber content provides a suitable structure for mycelial development thus, facilitates its rapid expansion (Widowati and Luna 2022). Studies have also shown that sorghum bran can effectively support the growth of various microorganisms, making it a viable and cost friendly alternative to more expensive, commercially available media (Izebe *et al.,* 2020).

From this finding, it was observed that *A. niger* exhibited a robust growth rate on sorghum bran, comparable to that of traditional media (Potato dextrose broth). According to Khoddami *et al.,* (2023), the production of enzymes such as amylase and cellulase was significantly enhanced, indicating that sorghum bran not only supports growth but also promotes metabolic activity.

P. chrysogenum, known for its antibiotic production, also showed promising results when cultivated on sorghum bran. The nutrient-rich medium might as well support the synthesis of penicillin, with yields comparable to those obtained using standard media. The mycelial density was observed to be higher on sorghum bran, suggesting that the medium provides an optimal environment for fungal proliferation (Liliya *et al.,* 2024 and Thanigaivel *et al*., 2024),

The use of sorghum bran as a growth medium is cost-effective, reducing the reliance on expensive imported media. This is particularly beneficial for research and industrial applications in developing countries. Additionally, utilizing agricultural by-products like sorghum bran contributes to waste reduction and promotes ecofriendly sustainable approach in enhancing smooth cleanup of the polluted sites, having the indigenous moulds of high biosurfactant production potential.

CONCLUSION

Sorghum bran is a viable and sustainable alternative for fungal cultivation, offering significant benefits in terms of cost, environmental impact, and growth performance. However, further studies on its optimization are needed to fully harness its potential in various biotechnological applications and perpetual clean up strategy.

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