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Profiling Culturable Filamentous Fungi Isolated from *Cyperus polystachyos* (Many-Spiked Flat sedge) Rhizosphere Soil for Biosurfactant Production

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Abstract

The research examined culturable filamentous fungi from *Cyperus polystachyos* (many spiked flat sedge) plant rhizosphere soil for their potential as biosurfactant. Soil samples were collected from a crude oil contaminated site. Parameters investigated include: pH, electrical conductivity, nutrients and heavy metals concentrations, using standard soil analysis methods. Microorganisms were cultured on Potato Dextrose Agar using standard microbiology plate culture while the emulsification potential of the fungi was carried out for (28) days using the emulsification index E_{24}. The genera identified are: *Aspergillus sp*., *Cladosporium sp*., *Penicillium sp*., and *Fusarium sp*. The soil tested was slightly acidic (5.65). The fungi recorded various mycelium growth diameters with *Aspergillus sp*. recording the highest mycelia 47 mm. The emulsification activity showed *Penicillium sp*. with an E_{24} index of 50% > *Aspergillus sp*. E_{24} index of 10% > *Fusarium sp*. 3.45 % while *Cladosporium sp*. demonstrated no emulsification activity. These findings revealed the potential of *Penicillium* sp. for biosurfactant production. It is recommended that the culture conditions of the identified microorganisms be optimized, also molecularly characterized biosurfactants producers, and scale up production to enhance their commercial feasibility.

Keywords: *Aspergillus sp., Biosurfactant, Emulsification, Many-Spiked Flat Sedge, Rhizosphere,*

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I. INTRODUCTION

The desire for sustainable and eco-friendly substitutions in industrial processes has led to the exploration of biotechnological solutions derived from natural sources. Surfactants formed by tiny organisms have really caught global attention because they can carry out lots of functions such as breaking down oils, mixing up liquids, and keeping immiscible liquids stable; which is very important in many industrial procedures. Emulsifiers are like little helpers that keeps oil and water from separating by making the surface tension less and forming stable mixtures (Kawabata *et al*., 2021). Synthetic emulsifiers have been used in food processing, cosmetics, pharmaceuticals, and cleaning products; nevertheless, due to the negative impact on the environment the need for increasing concerns about their environmental impact and toxicity have prompted interest in finding natural, biodegradable alternatives (Chaturvedi *et al*., 2023). Among the microorganisms capable of producing such emulsifiers, filamentous fungi have emerged as a promising source, particularly due to their ability to synthesize a variety of extracellular biosurfactants with excellent emulsifying properties. Filamentous fungi, such as *Aspergillus*, *Penicillium*, *Trichoderma*, and *Fusarium*, have been documented to produce biosurfactants including glycolipids, phospholipids, lipopeptides, and glycoproteins that possess both hydrophilic and hydrophobic regions, which enable them to stabilize emulsions and interact with both aqueous and oily phases (Kawabata *et al*., 2021; Chaturvedi *et al*., 2023). Current research points out that fungal biosurfactants have lots of bioactivities such as fighting germs, cancer, and free radicals, making them very useful for all sorts of (Hasan *et al*., 2022). While studies on fungal emulsifiers utilizes well known fungal species, Further investigation points to the untapped sources such as the rhizosphere.

The rhizosphere, a narrow region of soil or substrate is a home to diverse microorganism. It is an important zone for plant and microbial interaction. Exudates from p serve as buffer for microbes in the rhizosphere prompting the release of secondary metabolites in the form of biosurfactants including biosurfactants. The synergic between plant roots and microorganisms is super important for natural chemical reactions taking place around the roots, and it’s a pretty untapped source for finding new emulsifiers and other life-

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changing substances (Jiang *et al*., 2023; Trabelsi *et al*., 2022). Fungi around the rhizosphere play a hung role in the breakdown of complex organic matter, recycling nutrients, and making the soil better for plants. Current research point to the fact that fungi in the rhizosphere produce secondary metabolites, including enzymes such as cellulases, lipases, and esterases, which play a vital role in the breakdown of complex organic matter, including oils and fats, into simpler, bioavailable forms (Santi *et al.,* 2022). Rhizosphere fungii have be found to be able to facilitates the bioavailability of hydrophobic compounds in the soil, enhancing the overall health of the ecosystem and promoting plant growth. Making them useful resources to both industries and biotechnological processes specially those focused on green environmental solutions.

Cyperus polystachyos, (Many-Spiked Flatsedge) a grasslike plant, is commonly found in tropical and subtropical wetlands. It is able to survive in nutrient lacking soil. This plant has a solid root network, which serve as habitat to lots of microorganisms. Fungal species inhabiting the rhizosphere of *Cyperus polystachyos* utilizs organic compounds present for growth and biosurfactant production. Enough research has been carried out on microbes around the roots of plants found in wetlands especially the filamentous fungi that are able to emulsify. Given that the rhizosphere plays a pivotal role in supporting microbial life and facilitating plant-microbe interactions, it is reasonable to assume that fungi inhabiting the rhizosphere of C. *polystachyos* may exhibit unique properties that are closely tied to their ecological function in nutrient cycling and organic matter degradation (Cai *et al.,* 2023; Zhao *et al.*, 2023). The desire for more fungi specie with the ability to produce biosurfactant in this environment can led to discovery of new emulsifier which have significant industrial and environmental applications, in bioremediation, agriculture, and waste management.

There are some environmental factors that affects the ability of fungi to produce a biodegradable molecule. They include nutrient availability, pH, temperature and presence of some specific mediums. The presence of hydrophobic substrates, such as oils or hydrocarbons, which act as a carbon source for fungi triggers the production of some emulsifiers. Some microbes such as *Trichoderma harzianum* has been shown to produce high levels of emulsifiers when grown on waste cooking oil, while *Aspergillus niger* and *Penicillium chrysogenum* have demonstrated emulsification activities when cultured on various lipid-rich media (Patel *et al.,* 2023; Aderemi *et al*., 2023). These fungi isolated from the rhizosphere would respond to the presence of carbon source that could trigger the production of emulsifiers and other bioactive metabolites. Research have also shown that change in growth environment can speed up the production and effectiveness of biogradable molecules (biosurfactant). (Kumar *et al.,* 2023). Thus, understanding the optimal growth conditions for fungal isolates from *Cyperus polystachyos* and their ability to produce biosurfactants will be crucial in harnessing their full potential for industrial applications.

The promise of fungal biosurfactants in industry is substantial, with uses in various areas. In the food business, they help make things like mayonnaise, salad dressing, and margarine, creamier, more stable, and last longer on the shelf. In fashion and pharmaceuticals, they are important for mixing up creams, lotions, and ointments. They are a safer and more eco-friendly option compared to usual chemical emulsifiers (Kumar *et al.*, 2023). Fungal emulsifier have been employed in bioremediation breaking down hydrophobic pollutants like petroleum hydrocarbons in the soil and water. The use of fungal biosurfactants in these applications offers several advantages over traditional chemical surfactants, including their biodegradability, low toxicity, and low environmental impact (Chaturvedi *et al.,* 2023; Rinaudo *et al*., 2024). In particular, biosurfactants produced by fungi isolated from plant rhizospheres, such as *Cyperus polystachyos*, are more suitable for bioremediation, where they could emulsify and degrade toxic organic pollutants in the environment, thus maintaining. (Zhao *et al*., 2023; Gao *et al*., 2024). They also aid agricultural practices by enhancing soil microbial health, promoting nutrient cycling, and control of soil borne pathogens. a stable environment (Santi *et al*., 2022).

However, with all the focus on green and earth-friendly technologies, finding new bioemulsifiers to replace synthetic ones is very important. The ability to discover important biosurfactants from the rhizosphere of *Cyperus polystachyos* could serve as an alternative. Whereas considerable progress has been made in understanding fungal biosurfactants, yet more fungal species needs to be explored to find or produce novel, functional emulsifiers under varying environmental conditions. The unique microbial community in the rhizosphere of *C. polystachyos* offers a promising and largely untapped resource for discovering new fungal strains with enhanced emulsifying properties (Tian *et al*., 2023; Patel *et al.,* 2023). This study was aimed at investigating the emulsification potential of filamentous fungi isolated from *Cyperus polystachyos* rhizosphere soil. This paper specifies objectives: (i) To isolate and identify filamentous fungi from *Cyperus polystachyos* rhizosphere soil (ii) To evaluate the emulsification potential of isolated fungi using hydrocarbon. (iii) To optimize emulsification conditions for selected fungal isolates.

II. Materials And Methods

A. Sample collection

Cyperus polystachyos plant with the rhizosphere soil zone was collected from Rumukparali waterside, Obio Akpor, Rivers state, Nigeria. The sample was collected with the knife and put into a sterile sample bag. It was then transferred to the laboratory for immediate investigation.

B. Physiochemical tests

The method used by Xiaojun *et al*. (2020) was adopted carrying out the physiochemical analysis. Electrical Conductivity (E C) Test, pH Measurement, Nitrate Test, Phosphate Test, Zinc Test, Iron Test, Chromium Test, Nickel Test, Aluminum Test.

C. Isolation of total heterotrophic fungi (THF)

This was carried out according to the method used by Singh (2023). Exactly 1g of the soil sample was suspended in 9ml of normal saline aseptically and transferred to another test tube with the use of a sterile syringe in a quantity of 1ml to form serial dilutions arriving at two dilutions (10^{-2} and 10^{-3}) for the purpose of this study. 0.1ml of each diluent was aseptically transferred to the potato dextrose agar which was prepared based on manufacturer's instructions. The inoculums were spread in the plate using a hockey stick already dipped in ethanol, flamed and cooled intermittently. Each dilution was spread plated in duplicates and incubated at 28±2˚C for 5 days. Incubated plates with fungal colonies count (CFU/g) ranging from 30-300 colonies were enumerated.

D. Microscopy of fungal characteristics using lactophenol blue

Performed as described by Elgarhi *et al*. (2020). A pure culture from the isolates is used in this experiment. The inoculating loop is aseptically used to pick up some part of the pure fungi strain which is placed on a slide that has been prepared with drops of lactophenol blue and then examined under a microscope to be able to see the microscopic characteristics of the organism.

E. Emulsification index E24; biosurfactant screening

The method described by Ali (2021) was adopted. The biosurfactant production assessment was carried out beginning from the preparation of the mineral salt agar, which is a 50ml of culture media composed of $MgSO_4 \cdot 7H_2O$ (0.5g/L), KH_2PO_4 (1g/L), $NaNO_3$(3g/L) and peptone (0.3g/L). Crude oil was mixed with 10ml of the mineral salt agar and stirred, one test tube for each isolate. The crude oil serves as the carbon source for the organism. Aseptically, the inoculating loop was used to pick a fragment of the respective isolates and put into the mixture already prepared (mineral salt agar and crude oil). This setup was allowed to stand for 14 and 28days, and the E_{24} was measured respectively. On the 14th and 28th day respectively, 4ml of the mixture was extracted with a sterile syringe and poured into a sterile test tube for the respective isolates. The test tubes were centrifuged for 15 minutes. On completion, 2ml of the cell-free supernatant was extracted and 1 ml of kerosene was added in a test tube. The test tubes containing the mixture were vortex mixed for 2 minutes and kept for 24 hours to observe emulsification activity. The emulsion activity was calculated using the formula below:

$$E_{24}=\frac{He}{Ht} \text{ X } 100$$

Where; He= height of the emulsion, Ht= total height of the mixture, and E_{24}= emulsification index after 24 hours (%).

III. RESULTS

A. Physiochemical analysis on the sample

The physicochemical characteristics of the rhizosphere soil of Cyperus polystachyos. The parameters measured include pH, electrical conductivity (EC in µS/cm), and concentrations of nitrate, phosphate, iron, zinc, chromium, aluminum, and nickel (all in mg/kg). The results are summarized in Table 1.

Table 1. Physiochemical Analysis on the Soil Sample

S/N	Parameters	Outcome
1	pH	5.76
2	Electrical conductivity (µS/cm)	318
3	Nitrate (mg/kg)	7.129
4	Phosphate (mg/kg)	1.312
5	Iron (mg/kg)	115.162
6	Zinc (mg/kg)	12.513
7	Chromium (mg/kg)	5.311
8	Aluminum (mg/kg)	2.478
9	Nickel (mg/kg)	17.620

B. Morphological characteristics of fungal isolates

Table 2 and Figure 1 show the morphological characteristics of the isolates and the tentative genera based on microscopic and macroscopic examination of the features. *Cladosporium* sp., *Penicillium* sp., *Fusarium* sp., and three distinct *Aspergillus* sp., were identified among the isolates.

C. Fungal Growth Measurement

Fungal growth measurement was carried out on the isolates. The growth of the fungal isolates was observed and the growth rate for day 2, 4, 6, 8 and 10 respectively from the 6mm cork borer were recorded. The isolate labelled OJF2 had no growth after being subcultured. The measurements in millimeters (mm) is shown in the Table 3.

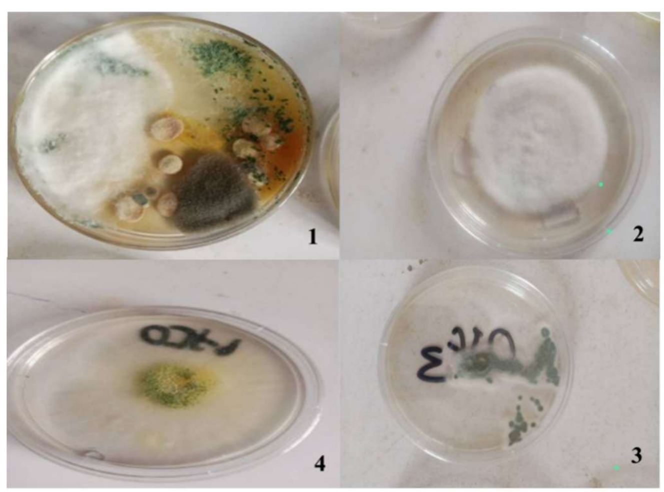

Figure 1. Isolated filamentous fungi on potato dextrose agar (PDA): 1, total fungi. 2, *Fusarium sp.* 3, *Penicillium sp*. 4, *Aspergillus sp*.

Table 2. Morphological characteristics of fungal isolates

Sample Code	Macroscopy	Microscopy	Tentative Isolate
OJF1	White-yellow aerial mycelium with dark spores, circular white margin with deep yellow cracked reverse.	Smooth colored conidia, globose shape, brown dark spores, bulbous vesicle, flask-shaped phialides	*Aspergillus sp.*
OJF2	Dark green, round, slow growing, dome shaped dry mycelium with deep yellow cracked reverse.	Single conidia, branched hyphae with spore	*Cladosporium sp.*
OJF3	Green dry mycelia rough surface, white margin, circular and dome shaped.	Flask-shaped brush-like conidiophore	*Penicillium sp.*
OJF4	Yellow reverse with white margin, yellow dark-green segmented dry mycelium	Dark spore, oval shaped thick hyphae	*Aspergillus sp.*
OJF5	White, thick fluffy aerial mycelia smooth and circular	Macroconidia presence of thick hyphae, slender, flask-shaped conidiophore	*Fusarium sp.*
OJF6	Green-white mycelia, circular downy colony.	Green globose spore in cluster, born on thick-walled long hyphae.	*Aspergillus sp.*

Table 3: Fungal growth measurement (mm).

	DAYS					
CODE	0	2	4	6	8	10
OJF1	6	17	21	28	37	42
OJF2	6	-	-	-	-	-
OJF3	6	10	15	20	26	34
OJF4	6	9	13	21	27	35
OJF5	6	17	23	28	32	38
OJF6	6	15	21	38	44	47

D. Emulsification potential

Figure 2 shows the respective emulsification potential of the isolated fungal species including; *Cladosporium, Penicillium, Fusarium,* and the three *Aspergillus* species after 24 hours on the 14^{th} and 28^{th} day respectively.

IV. DISCUSSION

The identified diversity of filamentous fungi emphasizes the abundant microbial life within the rhizosphere, with each fungal species displaying distinct emulsification properties. These fungi play important roles in clean-up of environmental pollutants. They are also producers of valuable bioproducts as observed in recent studies on their biotechnological potential (Piegza *et al*., 2021). The rhizosphere's nutrient-rich environment has long been identified as a hotspot for microbial interactions, the fungi genera *Aspergillus*, *Penicillium*, *Cladosporium*, and *Fusarium* were identified from the sample (table 2 and figure 1) some of which have been recognized for their capacity to produce biosurfactants (Desai and Banat, 2020).

The rhizosphere soil of *Cyperus polystachyos recorded a slightly acidic pH, with moderate electrical conductivity. There is the presence of growth promoting nutrient; nitrate and phosphate. While the phosphate present is sufficient, nitrate on the other hand is not; applying the C: N :P of 100 :10 :1 (*Zaman *et al*., 2025). The soil recorded traces of heavy metals with iron having the highest concentration of the heavy metals analyzed, then Nickel > Zinc > Chromium > Aluminum as can be seen in Table 1. The high concentration of heavy metals especially iron could be due to exposure to crude oil contamination.

The emulsification potential of the fungal isolates had *Penicillium* sp. exhibited the highest emulsification index E_{24} at 50% (figure 2), which is considerably higher than the 35% which was reported by Kiran *et al.* (2021) in marine-derived *Penicillium* isolates under comparable conditions. This could be attributed to differences in soil composition or fungal strain. In contrast, *Fusarium* sp. demonstrated 6.8% E_{24}, this is similar to the results of Santos *et al*. (2021), who reported low biosurfactant yields for *Fusarium* in similarly challenging environments slightly acidic pH. Meanwhile, *Aspergillus* sp., having an E_{24} of 10%, showed moderate potential when compared to *Penicillium*. This result is in agreement with the work of Desai and Banat (2020), where *Aspergillus* strains produced modest levels of biosurfactants in similar ecosystem.

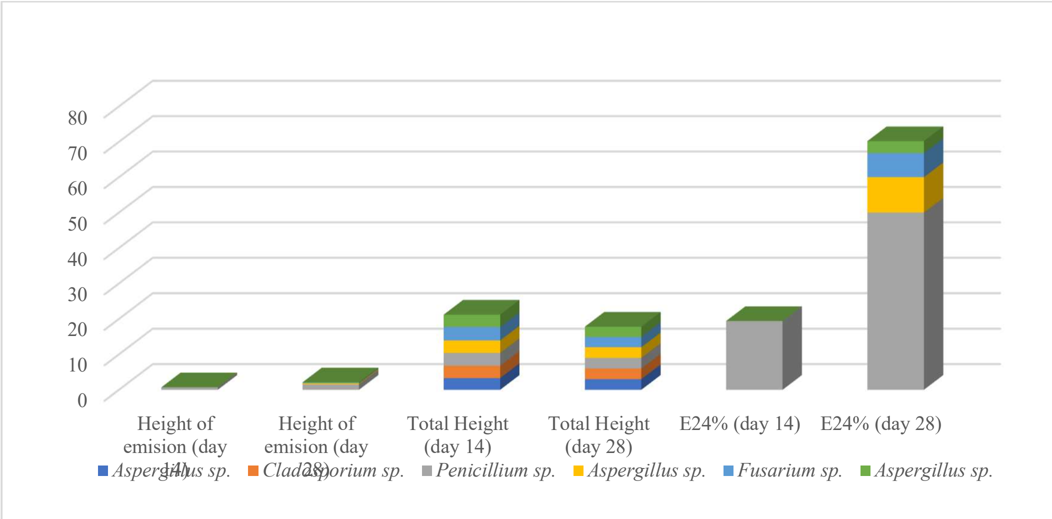

Figure 2 Emulsification Potential of Various Fungal Species

When comparing the mycelium growth of the various fungi isolates *Aspergillus sp.* > *Fusarium* sp. > *Penicillium sp. while Cladosporium sp. did not grow (Table 3). This is in agreement with other researchers who state that different environmental conditions affect the growth of the organisms (*Kiran *et al.,* 2021., Santos *et al*., 2021; Desai and Banat 2020).

Filamentous fungal biosurfactants producers and their environment show both ecological and industrial significance. For example, Ali *et al.* (2023) highlighted the role of biosurfactants in pollutant degradation, noting that these molecules enhance the bioavailability of hydrophobic contaminants. This is in agreement with our findings, where biosurfactant-producing fungi were isolated from a rhizosphere likely exposed to anthropogenic pollutants, given the presence of heavy metals. Kumar and colleagues in (2021) highlighted how fungal biosurfactants can boost farming by helping plants grow better and absorb nutrients, which could be useful for the root zone of *Cyperus polystachyos* rhizosphere. The outstanding performance of *Penicillium* sp. in this research, backed up its role in cleaning up the environment and boosting farming.

But there are some differences between our results and the findings of other researchers, which points to the fact that there are still some gaps in our knowledge. For example, in this study *Fusarium* sp. showed low emulsification potential but Santos *et al.* (2021) reported moderate biosurfactant production in strains of Fusarium under high pH condition. Also, moderate production of biosurfactant by *Aspergillus* sp. was reported by Desai and Banat, 2020 indicating strain-specific differences under

high-nutrient conditions. These differences highlight the importance of close examination of the genes and proteins of various filamentous fungi in the rhizosphere of the *Cyperus polystachyos* plant.

Hence, the comparative analysis of filamentous fungi from the *Cyperus polystachyos* rhizosphere reveals a complex interplay between soil conditions, fungal physiology, and metabolic output. While *Penicillium* sp. emerges as the most efficient biosurfactant producer in this study, *Aspergillus* and *Fusarium* demonstrate potential that could be harnessed through environmental optimization or genetic engineering. These findings both corroborate and expand upon existing literature, providing a nuanced understanding of how rhizosphere fungi can be leveraged for biotechnological and ecological applications. Further research is warranted to explore strain-specific traits, soil-microbe interactions, and scalable production techniques to fully realize the potential of these fungi.

V. Conclusion

Penicillium sp. is the most efficient biosurfactant producer in this research, but *Aspergillus* sp. and *Fusarium* sp. also show promise as biosurfactant producers but in less proportion. They can be applied to crude oil contaminated ecosystem to restore such site to a clean, health state.

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