Biosurfactant producing *Aspergillus, Penicillium,* and *Candida* performed higher biodegradation of diesel oil than a non-producing fungal strain

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ABSTRACT

The biosurfactant production can enhance the hydrocarbon biodegradation, as the hydrophobicity of these compounds reduces the degradation rates. Much of the attention was given to microbial hydrocarbon biodegradation, while limited work is present regarding the capacity of fungal biosurfactants for enhancing the remediation process. This research work identified the potential of biosurfactant production and hydrocarbon degradation of selected fungal strains belonging to *Aspergillus*, *Penicillium,* and *Candida* genera in contrast to a hydrocarbon-degrading and biosurfactant non-producing fungal strain. The highest biodegradation was noted for *Aspergillus niger* FA5 (90.70%), followed by *Penicillium chrysogenum* FP4 and *Aspergillus terreus* FP6 (87.40 and 85.03%, respectively), and lastly, *Candida* sp. FG2 (80.10%). Biosurfactant-producing hydrocarbon degrading fungal strains *A. niger* FA5, *P. chrysogenum* FP4, *A. terreus* FP6, and *Candida* sp. FG2 degraded hydrocarbons 1.32, 1.27, 1.24, and 1.18 times higher than non-producing *A. flavus* FP10 (68.60%). When the data were analyzed for correlation, hydrocarbon degradation was found negatively corelated to surface tension ($r = -0.747$, $p = 0.005$), while positively correlated with emulsification index ($r = 0.964$, $p < 0.001$), and cell hydrophobicity ($r = 0.835$, $p < 0.001$). The results indicate that fungi capable of attaching higher levels of hydrocarbons to the cell surface and effectively reducing surface tension were able to exhibit significant improvements in the rate of hydrocarbon degradation. Hence, it is concluded that if a fungus can produce biosurfactant that can improve hydrocarbon emulsification and reduce surface tension the hydrocarbon breakdown can be accelerated by up to 12 to 22%, compared to non-producers.

Keywords: Biosurfactant, fungi, *Aspergillus, Penicillium, Candida*, diesel oil, biodegradation, hydrophobicity.

INTRODUCTION

Pollution of petroleum hydrocarbons, having hydrophobic properties, induces deleterious impacts on territorial and aquatic systems [1]. If they remain in the environmental matric, these notorious contaminants are ingested or inhaled, leading to rapid absorption due to their physicochemical properties [2, 3]. Soil contamination with hydrocarbons goes hand in hand with industrialization. Hydrocarbons are recalcitrant, and due to their hydrophobicity, their natural degradation process is reduced. Thus, they persist in the environment. Biological methods used to treat petroleum hydrocarbons, bioremediation, and phytoremediation are gaining interest over time [4, 5]. The selected advantages are low finance, energy, labor, chemical recurring need to carry out the operation, and a natural and ecofriendly approach [6, 7]. Among many methods used in the bioremediation of contaminates, one of the adopted approaches is biosurfactant-producing microorganisms [8]. Biosurfactants are biological molecules that enhance the solubilization and degradation of hydrocarbon by reducing hydrocarbon surface tension and increasing emulsification [7, 8].

Biosurfactants present added advantages and benefits compared to artificial counterparts. These included renewability and production ease, reduced toxicity and environmental persistence, and resistance to extreme conditions like high pH and elevated temperature [9]. Despite having these advantages, their role in a commercial application is very scarce and insignificant. Though attention was given to bacterial biosurfactant production and their use in the biodegradation of hydrocarbon and other organic contaminants, the focus on fungal biosurfactants and their effectiveness have not been given due attention [10].

Vast potential and effectiveness against organic contamination of fungi were well portrayed by [7]. Fungi are known to produce enzymes specific and non-specific that perform chemical modification contaminants and improve their bioavailability [6]. It can be inferred that fungal strains capable of producing biosurfactants can also exacerbate the hydrocarbon remediation rate, as similar findings were noted in bacterial biosurfactant producers [11, 12]. Very few studies are available focusing on biosurfactant production by fungi. *Aspergillus*, *Candida*, *Torulopsis*, *Trichosporon*, and *Ustilago* are notable fungal genera from which some members are known for the production of sophorolipid glycolipid, glycolipoprotein lipoprotein, and protein-lipid-carbohydrate complex [12]. In a study conducted by Al-Otib [13], *Candida* strains isolated from

oil-reservoir soils were used for crude oil degradation. According to them, biosurfactant production is related to the type of hydrocarbon degradation. Similarly, *Aspergillus* sp. capable of producing extracellular enzymes capable of adsorbing, absorbing, and degrading hydrocarbons, notably crude oil and polycyclic aromatic hydrocarbon [14]. EL-Hanafy et al. [15] reported that isolates belonging to isolates of *Aspergillu*s and *Penicillium* were capable of performing hydrocarbon degradation. Ishaq et al. [16] proposed that biosurfactants produced by *Aspergillus flavus* can be utilized for bioremediation applications. Based on these findings, it would be highly interesting to assess the biosurfactant production and hydrocarbon degradation potential of selected fungal strains belonging to *Aspergillus*, *Penicillium,* and *Candida* genera, compared to a hydrocarbon-degrading biosurfactant non-producing (BNP) fungus.

MATERIALS AND METHODS

Preliminary substrate utilization test of selected fungal strains. Previously isolated 23 fungal strains that effectively utilized diesel oil previously [7] were subjected to preliminary substrate utilization (PSU) test against crude and spent engine oil, as a carbon source, in hydrocarbon-adoptive fungi (HAF) broth containing (g L^{-1}) KCl, 0.25; NaH_2PO_4, 0.1; NH_4NO_3, 1; with final pH 7. For this purpose, pure fungal cultures were prepared on potato dextrose agar (PDA) containing (g L^{-1}) D(+)-glucose, 20; potato infusion, 4; and technical agar, 15. After 7 days of incubation on PDA at 30 ºC, fungal cultures were used to prepare 2 mm agar plugs, which were used as inoculum for HAF broth in the PSU test. Fungal growth of each strain on both substrates was noted (3 for maximum, while 0 for no growth) after 13 days of incubation at 30 ºC. Selected strains were identified previously [7].

Drop collapse test. Fungal strains were selected based on the PSU test and were subjected to the screening of biosurfactant production with the help of the drop collapse method. For this purpose, the way proposed by Shatila et al. [17] was adopted. Briefly, 96 well microtiter plates, each well containing 7 µl of diesel oil, were equilibrated for 60 minutes at 30 ºC. A 20 µl of cell-free supernatant (CFS) of each fungal culture was added to the oil surface. The fungal CFSs were prepared by centrifugation, at 6000 g for 15 min, of 5 days old cultures, cultivated at 30 ºC in potato dextrose broth (PDB) containing (g/L) D(+)-glucose, 20; and potato infusion, 4; at final pH of 7. A negative control having only PDB was also placed for quality assurance. The drop collapse noted

the presence of extracellular biosurfactant collapsed, while no collapse considered the absence of such moieties.

Biosurfactant quantification and qualitative characterization. Each selected biosurfactant producing fungal strain was inoculated in 50 ml of PDB and incubated in a static condition at 30 ºC for 7 days. Each fungal culture, after the incubation, was washed thrice with centrifugation at 3500 rpm for 15 min. To facilitate washing, 2-3 drops of tween 20 were added into the fungal cultures before the centrifugation. Cells were retained and washed in 0.9% saline. Cell suspensions of 10^6 cells ml^{-1} concentrations were prepared for each selected strain [7]. These suspensions were used for biosurfactant production and biodegradation experiment. For biosurfactant production and quantification, 1 ml of each cell suspension with the known fungal cell concentration was inoculated in 50 ml of PBD containing diesel (2%) and was incubated in a static condition at 30 ºC for 7 days. After incubation, biosurfactant production by each of the fungal strains was harvested. CFSs were prepared by filtration of cultured PDB with Whatman filter paper no. 1, followed by centrifugation at 7000 g for 20 mins at 4 ºC. Acidification using 6 M HCl to pH 2 for each CFS was done and kept overnight for the precipitation of biosurfactant. The final extraction of the biosurfactant was performed using the acidified CFS and methanol (at a 1:1 ratio, v/v), using a separatory funnel [18]. Extracted biosurfactant yield is expressed in milligram per 50 milliliters (mg ml_{50}^{-1}) of PDB medium after solvent removal by evaporation at room temperature in a fume hood. The thin-layer chromatography (TLC) used the silica gel plates to characterize the biosurfactant [18].

Emulsification index (E_{24}). Fungal cultures were cultivated in PDB for 7 days at 30 ºC. After cultivation, the CFS of each strain was used for emulsification index (E_{24}) estimation. These CFSs were prepared by centrifugation of cultivated cultures at 7000 g for 20 min, and resultant CFSs were used. Briefly, an equal quantity of diesel was mixed with CFS at a 1:1 ratio. The mixture was left at room temperature for 24 hours after homogenization on the vortex at high speed for 2 min [19]. After 24 hours, the emulsified layer height (in mm) was noted and divided by the total height of the liquid column (in mm) and was used to express E_{24} in percentage.

Surface tension measurement. Fungal enrichment in HAF broth containing 1% diesel (w/v) for 15 days at 30 ºC was done, and cultivated HAF broths were used to prepare CFSs to reduce surface tension using the du Noüy ring tensiometer [20]. Briefly, surface tension initially

and after incubation, at room temperature (30 ± 2 ºC), of each fungal culture was noted by CFSs. The CFSs for each culture were prepared by centrifugation at 5000 g for 20 mins. Biosurfactant non-producer hydrocarbon-degrading strain was considered as the control for the surface tension reduction test.

Microbial Adhesion to Hydrocarbons. Cell hydrophobicity was noted using the microbial adhesion to hydrocarbons method described by Kuyukina at al. [21], and Rubtsova et al. [22]. Fungal cells were washed twice after incubation of HAF broth containing 1 % diesel (w/v) for 15 days at 30 ºC. Washing and resuspending were carried with fresh, sterilized HAF broth. The cell suspension was prepared with an optical density at 600 nm wavelength (OD_{600}) of 0.5. After standardization, 1 ml of washed cell suspensions were added with 50 ml of diesel oil and mixed on a vortexed at high speed for 3 min. The cellular hydrophobicity was expressed in the percentage of adherence to diesel and quantified by the following equation:

$$Cell\ hydrophobicity\ (\%) = 100\ \times \left(1 - \frac{aqueous\ phase\ OD}{initial\ cell\ suspension\ OD}\right)$$

The OD was taken at 600 nm at room temperature after the aqueous phase and diesel separation.

Biodegradation experiment. For the hydrocarbon biodegradation experiment, 1 ml of washed inoculum (as prepared for biosurfactant production) of each strain (biosurfactant producer and non-producer) was mixed with 50 ml PDB containing 2% diesel at pH 7 was maintained. Negative control of un-inoculated media was prepared. The experiment was conducted in static conditions for 30 days at 30 ºC. Using the dichloromethane (DCM) extraction method, a gravimetric analysis of the residual hydrocarbons was performed to quantify the hydrocarbon degradation [6,7]. After incubation, the residual hydrocarbons were briefly extracted from the media in DCM by preparing a mixture using a 1:1 ratio (media: DCM). The remaining total petroleum hydrocarbons (TPHs) were quantified gravimetrically by weighing the leftover diesel after biodegradation in pre-weighed screw vials. The extracts were placed on a hotplate at 80 ºC in a fume hood and kept undisturbed till constant weight was attained.

Statistical Analysis. All experiments were conducted in triplicate, and values are presented in mean with standard deviation. Statistical analysis (Normality test, One-way ANOVA, and Tukey

MR post hoc test) was conducted using Excel stat and SPSS 20. Biosurfactant yields were also compared between different fungal stains using one-way ANOVA followed by a post hoc Tukey MR test. After extraction, biosurfactant samples (0.1 g for each case) were dissolved in methanol. In order to determine whether there was a correlation between the studied variables, Pearson's correlation method was applied to the data after checking the distribution of the data. Pearson's coefficient of correlation (R) values were deemed significant if the p-value with significance was less than 0.05. Pearson's correlation plots (PCP) were used as a method of presenting the values to avoid a bloated data presentation.

RESULTS

Fungal strain selected after utilization of variant hydrocarbon sources. Among the per-isolated 23 fungal strains, six strains (*Aspergillus niger* FA5, *Aspergillus terreus* FP6, *Aspergillus flavus* FP10, *Candida* sp. FG1 and FG2, and *Penicillium chrysogenum* FP4) were shortlisted for further experimentation, due to their growth on HAF broth containing crude oil and spent engine oil (Table 1).

Screening, quantification, and characterization of biosurfactants production. There were five fungal stains among six selected strains, *A. niger* FA5, *A. terreus* FP6, and *Candida* sp. FG1 and FG2, and *P. chrysogenum* FP4 were favorable for biosurfactant production, as their CFSs collapsed in equilibrated microwell plates (Table 2). All biosurfactant-producing strains were subjected to quantification and characterization analysis. The amount of biosurfactant produced by selected fungal strains is presented in Table 2. The significantly highest biosurfactant producing fungal strain among the selected strains was *P. chrysogenum* FP4. The strain FP4 produced 232.98 mg ml_{50}^{-1} of biosurfactant, 153.4% higher than the *A. niger* FA5 strain. There was no statistically significant difference between *Candida* sp. stains FG1, and FG2 was observed, and they produced (mg ml_{50}^{-1}) 185.11 ±12.95 and 168.28 ±11.77, respectively, was 101.3 and 83 % higher than strain FA5, while *A. terreus* FP6 produced 131.35 ±7.41 mg ml_{50}^{-1} biosurfactant, 42.8 % higher than FA5. Qualitative characterization of biosurfactants produced by fungal strains with TLC revealed that biosurfactants produced were lipoprotein in nature, as the chromatogram mainly consisted of protein and lipid spots when stained with respective reagents.

Surface tension, emulsification index, and cell hydrophobicity measurements. Results of the surface tension test are presented in Table 2. Each sample's initial surface tension (ST) was 70.27 ± 1.39 mN m^{-1}. *A. niger* FA5 showed a highest reduction in surface tension (23.73 ±1.43 mN m^{-1}, which is 2.81 times lower than the biosurfactant non-producing *A. flavus* FP10. *P. chrysogenum* FP4 reduced the surface tension to 33.90 ± 2.04 mN m^{-1}, which is 1.97 times lower than the BNP FP10 strain. No significant difference in surface tension between *A. terreus* FP6 and *Candida* sp. FG2 was noted, with an ST of 36.23 ± 0.89 and 37.81 ±1.24 mN m^{-1}, respectively; ~1.8 times higher for both cases compared to the BNP FP10 strain. A minor ST reduction was noted for *Candida* sp. FG1, which was 41.59 ±1.37 mN m^{-1}, 1.6 times higher than the NPF strain.

Emulsification activity by *A. niger* FA5 (52.1% emulsion in PDB) was significantly higher than other strains, which was 1.79 times higher than the *Candida* sp. FG2 (Figure 1.a). While for other strains, emulsification indexes were; *P. chrysogenum* FP4; 45.73%, *A. terreus* FP6; 41.70%, and *Candida* sp. FG2; 30.10%, respectively. The emulsion formed due to biosurfactant in the CFS was stable, without any changes, at room temperature for more than one month. Similarly, for the assessment of cellular hydrophobicity, the same trend was noted, where statistically highest cell hydrophobicity was noted for *A. niger* FA5 and *P. chrysogenum* FP4; 85.50% and 85.07%, respectively (Figure 1. b), followed by *A. terreus* FP6; 81.70%, and least was noted for *Candida* sp. FG2, which was 66.77%.

Biodegradation experiment. The result of hydrocarbon degradation by fungi is presented in Figure 2. It depicted that strains capable of producing biosurfactants performed significantly higher biodegradation than non-producing strains (*A. flavus* FP10). A statistically significant difference between the hydrocarbon degradation percentages was noted among biosurfactant producing, non-producing, and negative control. Among biosurfactant producing strains, the highest biodegradation was noted for *A. niger* FA5 (90.70%), followed by *P. chrysogenum* FP4 and *A. terreus* FP6 (87.40 and 85.03%, respectively), and lastly *Candida* sp. FG2 (80.10%). The degradations by *A. niger* FA5, *P. chrysogenum* FP4, *A. terreus* FP6, and *Candida* sp. FG2 were 1.32, 1.27, 1.24, and 1.18 times higher than the degradation by BNP *A. flavus* FP10 (68.60%). In the negative control, ~1% of hydrocarbon reduction was noted.

Correlation analysis of the studied parameters. Pearson's correlation plots are presented in Figure 3. Analysis revealed that the emulsification index was significantly correlated with cell

hydrophobicity (strong positive correlation, $r = 0.893$, $p < 0.001$), hydrocarbon biodegradation (strong positive correlation, $r = 0.964$, $p < 0.001$), and surface tension (strong negative correlation, $r = -0.814$, $p = 0.001$). It suggests that fungi cause higher emulsification of hydrocarbons, thus increasing their biodegradation. Cell hydrophobicity also showed positive correlations with hydrocarbon biodegradation ($r = 0.835$, p 0.001). These results indicate that fungi capable of attaching more hydrocarbons to their cell surfaces have a significant impact on hydrocarbon degradation rates. Furthermore, the hydrocarbon biodegradation showed a significant correlation with surface tension (strong negative correlation, $r = -0.747$, $p = 0.005$), indicating that a reduction of surface tension would increase the bioavailability of hydrocarbons. All other correlations between the parameters studied were insignificant.

DISCUSSION

In this study, 23 fungal strains were tested for their growth on crude oil and spent engine oil. Six strains showed positive results. It was identified that isolated strains of *A. niger* FA5, *P. chrysogenum* FP4, *A. terreus* FP6, and *Candida* sp. FG1 and FG2 were capable of synthesizing biosurfactants. Similarly, *A. flavus* was identified as a non-biosurfactant-producing hydrocarbon-utilizing fungal strain. One of the most commonly reported fungi for the production of biosurfactants is *Candida* [23]. Other explored fungal genera for the production of biosurfactants include *Aspergillus, Ustilago,* and *Trichosporon* [12]. The amount of biosurfactant produced by microbes depends on the type and source of the microorganism [7, 23], physiochemical factors like the content of carbon, nitrogen, trace elements, temperature, and aeration [6, 24]. The highest amount of biosurfactant was produced by *Penicillium chrysogenum* FP4, followed by FG1>FG2>FP6>FA5. Due to rigid cell walls, fungi produce a greater amount of biosurfactant than bacteria [12]. The higher production of biosurfactants by fungi makes them favorable to produce biosurfactants at a commercial scale. Microbial biosurfactants can be categorized into five main types based on chemical composition: glycolipids, phospholipids, lipopeptides, lipoproteins, and polymeric biosurfactants [25]. In this study, all the identified fungal strains produced lipoprotein biosurfactants. Most of the species of *Candida* were reported to synthesize a sophorolipid

biosurfactant, which is a type of glycolipid [11, 12]. *Aspergillus* species was reported to produce glycolipid biosurfactants [12].

The hydrocarbon remediation potential of a biosurfactant's activity is highlighted by the fluctuations in the surface and interfacial tension of hydrocarbon mixtures. A promising microorganism for biosurfactant production should be capable of reducing the surface tension < 40 mN m^{-1} [25, 26]. Reduction in surface tension enhances the availability of hydrophobic molecules to microbes, thus resulting in an increased degradation rate. Further, a highly effective biosurfactant can lower the water surface tension from 72 to 27 mN m^{-1} [27]. Similar results were found that all selected fungal strains reduced surface tension efficiently. *A. niger* FA5 showed the highest surface tension reduction, followed by *P. chrysogenum* FP4. No significant difference between *A. terreus* FP6 and *Candida* sp. FG2 was noted. The most negligible surface tension reduction was noted for *Candida* sp. FG1.

Estimating the emulsification index is another notable approach that can be used to screen potential biosurfactant-producing microorganisms. Emulsification index is known to be associated with the production of biosurfactants and the degradation of hydrocarbons. Biosurfactants can enhance bioremediation through solubilization, mobilization, or emulsification [7]. Biosurfactants can facilitate the bioavailability of hydrocarbons through the emulsification of non-aqueous phase liquids. The higher the emulsification, the more the hydrocarbon availability for degradation. Emulsification activity by *A. niger* FA5 was significantly higher, followed by *P. chrysogenum* FP4; *A. terreus* FP6; and *Candida* sp. FG2.

The use of biosurfactants can enhance hydrocarbon degradation. It is achieved by enhancing microbe substrate accessibility and easing the interaction between the hydrophobic substrate and microbial cell surface. Due to improvements in the hydrophobicity of their surfaces, microbial cells associate easily with hydrophobic contaminants like hydrocarbons [23]. Hence, it can be inferred that hydrophobicity plays a significant role in microbe attachment to hydrophobic surfaces. This association is necessary as the microbial cell's hydrophobic nature is a degradation and growth rate-limiting factor in the environment containing hydrophobic contaminants [28]. The more hydrophobic a cell is, the more hydrocarbons will adhere to its surface. In this study, the

cellular hydrophobicity of *A. niger* FA5 and *P. chrysogenum* FP4 was ~85%, followed by *A. terreus* FP6; ~81%. The least was recorded for *Candida* sp. FG2, which was 66.77%.

The hydrocarbon degradation potential of fungal strain-producing biosurfactants was compared with non-biosurfactant producer fungal strains. It was observed that fungal strains producing biosurfactants have higher biodegradation rates than non-producing strains (*A. flavus* FP10). It was also observed that strains having the potential for higher surface tension reduction have higher biodegradation rates. The effectiveness of a surfactant is determined by its ability to reduce surface tension. Surface tension reduction enhances the bioavailability of hydrophobic compounds to microbes [30]. It was identified that *A. niger* FA5 has the highest biodegradation rate; 90.7%, with the highest reduction in surface tension; 23.73 mN m^{-1}. *A. flavus* FP10, a non-biosurfactant producer, has the lowest biodegradation than biosurfactant producing fungi, with a little surface tension reduction; 66.76 mN m^{-1}. Similarly, EL-Hanafy et al. [15] reported that fungal strain *A. oryzae* Y2 performed significantly better emulsification activity, microbial adhesion to hydrocarbons, and surface tension reduction, which led to the highest hydrocarbon degradation (99%), that 51% higher than the biosurfactant non-producing fungal strain. Correlation analysis indicates that only higher biosurfactant production does not guarantee hydrocarbon degradation, so specific degradation requires relevant types of biosurfactants. Al-Otib [13] also proposed that used oil showed the highest amount of biosurfactants produced by *Candida* (*C. parapsilosis, C. famata*) and *Rhodotorula* spp., while crude oil showed the highest production with *Candida krusei*. Hence, it can be inferred biosurfactant production and efficacy are dependent on the type of hydrocarbons present for degradation.

The ecological acceptability and environmental friendliness of biosurfactants are one of the most prominent advantages over synthetic surfactants [31]. Further, the biodegradation of hydrocarbon can be enhanced when a fungal strain produces biosurfactants [32]. Biosurfactant-producing and hydrocarbon-degrading fungal strains can increase hydrocarbon degradation by 12 to 22%, compared to fungi that cannot produce biosurfactants. Based on the results of this study, it is likely that the findings of Maamar et al. [33] agreed with the results of this study. The four fungal isolates (*Aspergillus terreus, Trichoderma harzianum, Penicillium citreonigrum,* and *Lulworthiales* sp.) that produced a high amount of biosurfactants also were those that were capable of efficiently degrading crude oil, indicating that biosurfactant production was well correlated with crude oil-

degradation ability. This experiment is a limited study as it was carried out in the lab. In field experiments, by observing the effects on soil fungi and degradation in natural conditions, the reduction in surface tension and impact on the overall microbial population can be observed. The application of readily degradable co-substrate and nutrients along with hydrocarbon will also be interesting to investigate. Ottman et al [34] reported that *Aspergillus fumigatus* capable of producing biosurfactant and hydrocarbon degradation performed up to 57% TPHs degradation. This was increased by 10.52% and reached 63% by the addition of nutrients in the form of the Bushnell Haas mineral. The effect of nutrient availability on biosurfactant production by fungi and its effect on bioremediation can also be studied. Synthetic surfactants can cause damage to the environment, are resistant to degradation, and accumulates in the natural ecosystem. There is a need to study the biochemistry, physiology, and genetics of strains capable of producing biosurfactants involved in hydrocarbon bioremediation. It can effectively enhance biosurfactants' field application.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that manuscript is original, and it is not published in any previous publications. The authors declare they have no conflicts of interest. This work does not contain a description of any studies using humans and animals as subjects.

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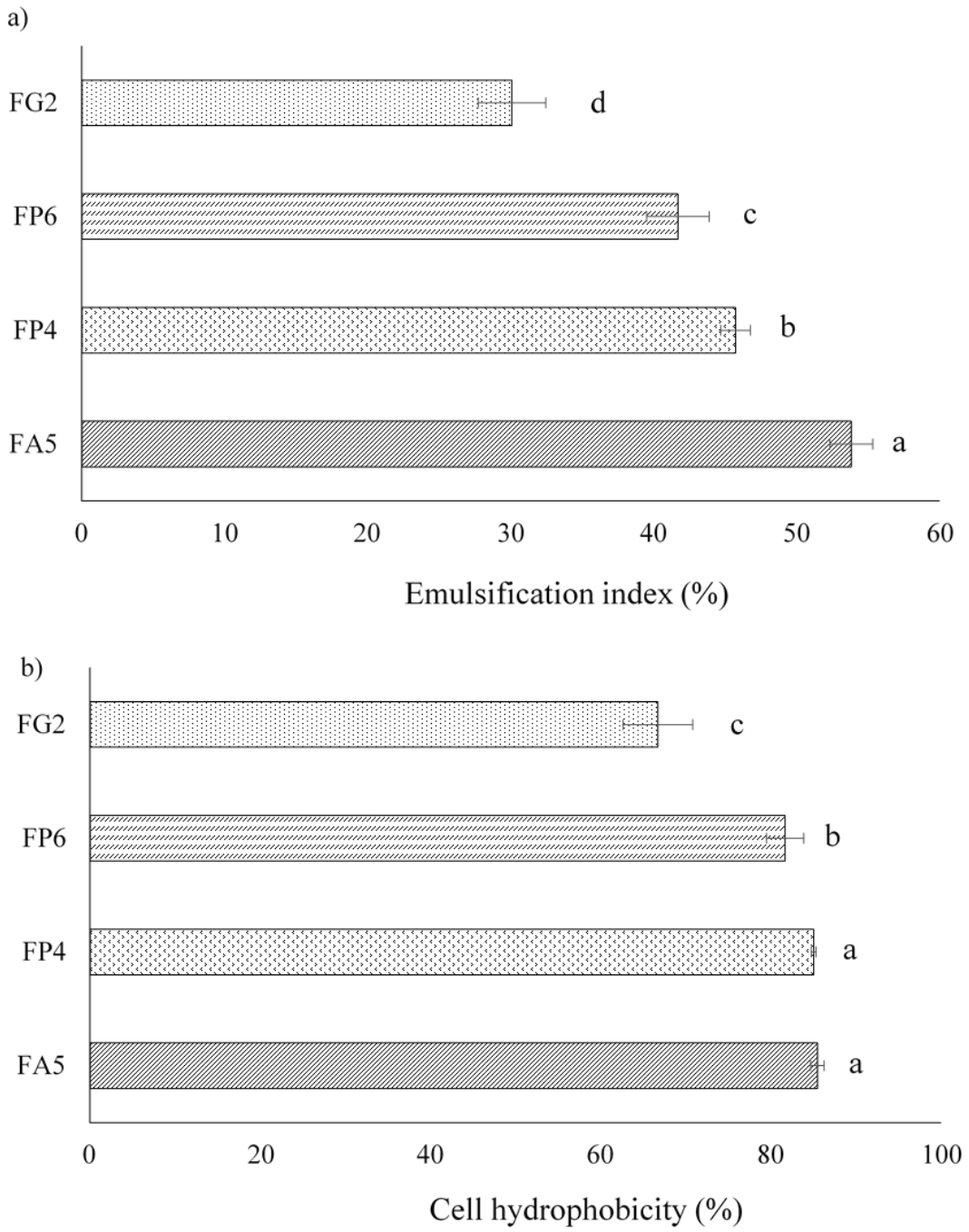

Figure 1. Fungal strains screening for a) Emulsification index and b) Cell hydrophobicity of fungal strains. Alphabet(s) in the label of bars represents statistical difference. Alphabets "a" represents a significantly higher mean compared to the later alphabets.

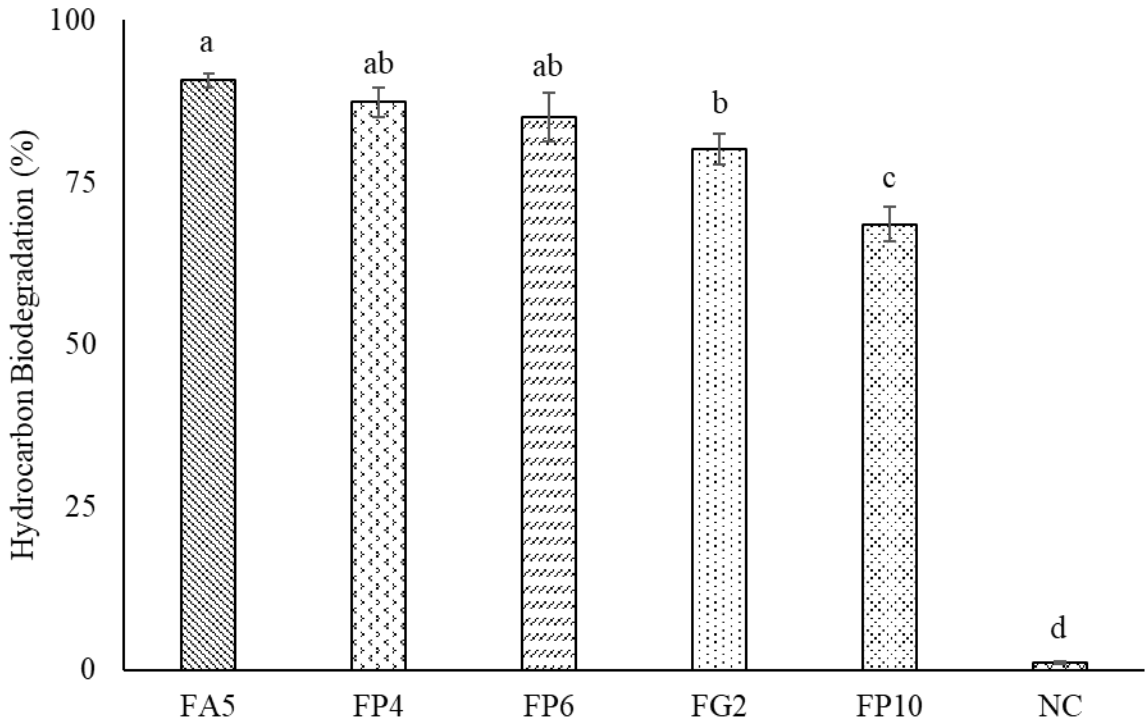

Figure 2. Biodegradation of hydrocarbons. FP10 is a biosurfactant non-producing fungal strain, and NC is the negative control. Alphabet in the label of bars represents statistical difference. Alphabet "a" represents a significantly higher mean compared to the later alphabets.

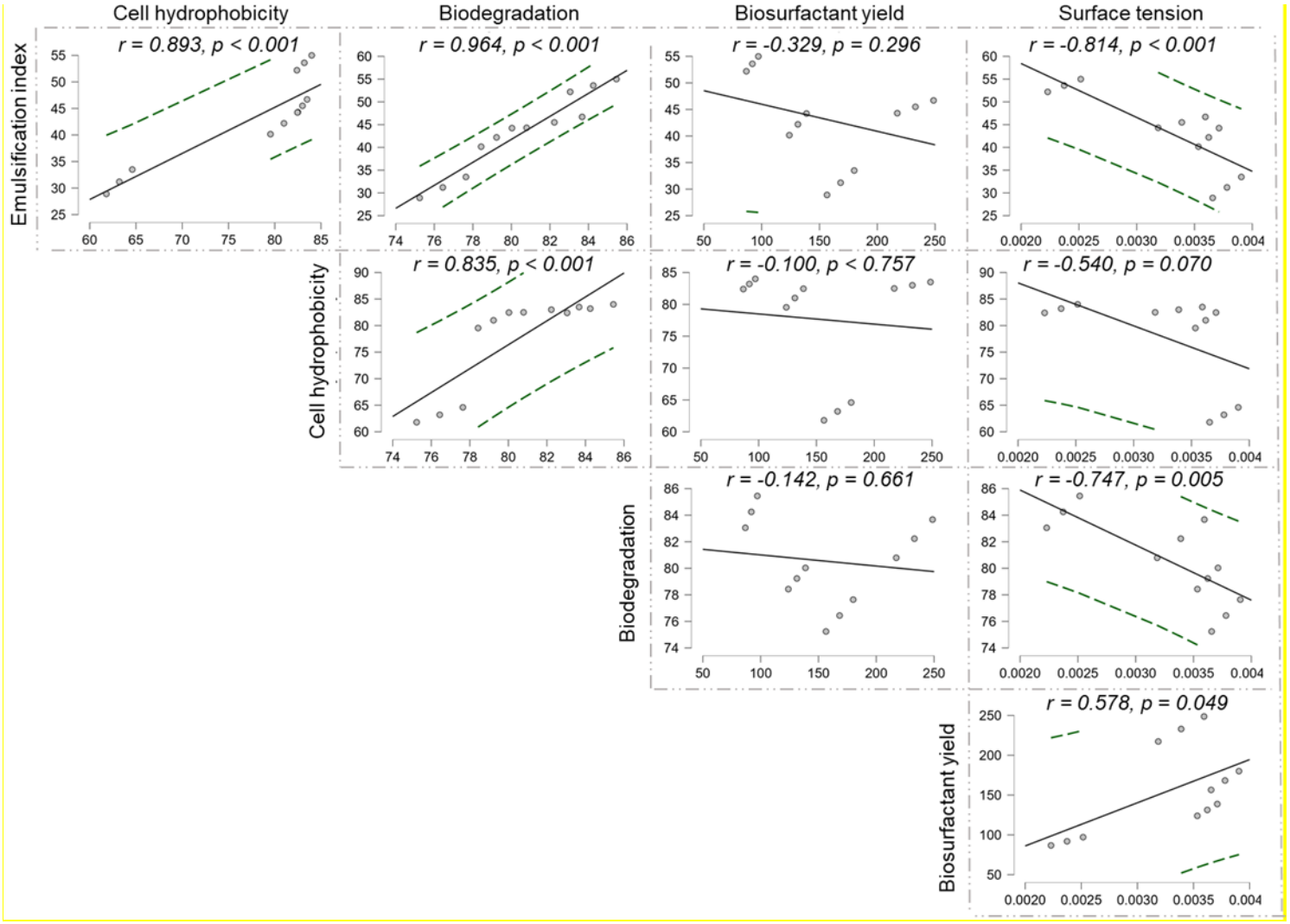

Figure 3. Pearson correlation plots for the studied parameters including Surface tension, Emulsification index, Cell hydrophobicity, Hydrocarbon biodegradation, and Biosurfactant yield. Green colored lines??? are showing the occurrence of values in prediction interval. While, in the legend of plot Pearson correlation coefficient along with probability is presented (n = 12, as only values from fungal strains capable of producing biosurfactant were used to prevent error due to zero/null values).

Table 1. Hydrocarbon utilization by fungal strains

Strain Lab ID	Nomenclature	Substrate utilization		
		Diesel[a]	Crude oil[b]	Spent engine oil[b]
FA5	*Aspergillus niger*	3	3	1
FP4	*Penicillium chrysogenum*	3	3	2
FP6	*Aspergillus terreus*	3	3	3
FP10	*Aspergillus flavus*	3	3	2
FG1	*Candida* sp.	3	3	3
FG2	*Candida* sp.	2	3	1

[a] (Khan *et al.* 2016)
[b] Present study
Score in substrate column marked 3 is for highest, while 1 is marked for least utilization

Table 2. Biosurfactant production, qualitative characterization and quantification for selected strains

Strain Lab ID	Nomenclature	Drop collapse assay	Biosurfactant type	Biosurfactant yield[1*] (mg ml_{50}^{-1})	Surface tension[1*+] (mN m^{-1})
FA5	*A. niger*	Positive	LP	91.95 ±5.18[d]	23.73 ±0.143[a]
FG1	*Candida* sp.	Positive	LP	185.11 ±12.95[b]	41.59 ±0.137[d]
FG2	*Candida* sp.	Positive	LP	168.28 ±11.77[b]	37.81 ±0.124[bc]
FP4	*P. chrysogenum*	Positive	LP	232.98 ±15.69[a]	33.90 ±0.204[b]
FP6	*A. terreus*	Positive	LP	131.35 ±7.41[c]	36.23 ±0.089[bc]
FP10	*A. flavus*	Negative	NA[2]	NA[2]	66.76 ±0.112[e]

[1] Similar small letter in superscript are insignificant with "a" representing highest followed by later alphabets,
[2] LP = Lipoprotein, and NA = Not applicable
* p-value= 0.05, n=3
[+] ST of blank was 70.27 ±0.14 mN m^{-1}