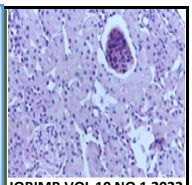
JOBIMB, 2022, Vol 10, No 1, 72-75
https://doi.org/10.54987/jobimb.v10i1.718

HIBISCUS PUBLISHER

JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index



Species of *Pseudomonas* and *Bacillus* Isolated from Refined Oil-contaminated Soil Showed the Potential to Efficiently Degrade Diesel

Munkaila Tirmizhi[1], Abdullahi Adamu Faggo[1]*, Bashar Haruna Gulumbe[2]

[1]Department of Microbiology, Faculty of Science, Bauchi State University Gadau, P.M.B 065, Bauchi, Nigeria.
[2]Department of Microbiology, Faculty of Science, Federal University Birnin-Kebbi, Birnin Kebbi, Kebbi State, Nigeria.

*Corresponding author:
Abdullahi Adamu Faggo,
Department of Microbiology,
Faculty of Science,
Bauchi State University Gadau,
P.M.B 065,
Bauchi,
Nigeria.
Email: abdullahiadamufaggo@gmail.com

HISTORY

Received: 24th May 2022
Received in revised form: 12th June 2022
Accepted: 24th July 2022

KEYWORDS

Diesel
Degradation
Pseudomonas aeruginosa
Pseudomonas putida
Bacillus subtilis

ABSTRACT

The high rise in the exploration and usage of petroleum products have led to widespread contamination in the environment. Thence, the continuous search for microorganisms with the potential to mineralize these pollutants is necessary. This study was conducted to isolate, identify and determine the diesel degradation potential of bacteria from oil-contaminated soil collected from three filling stations in Azare, Katagum LGA, Bauchi State, Nigeria. The diesel degrading bacteria were identified using standard protocols. The isolates were screened spectrophotometrically for their potential to utilize 1% diesel (v/v) as their sole carbon and energy source and the best candidate was used for determining the effect of diesel concentration on its biodegradation. The results revealed the presence of three bacterial species including *Pseudomonas aeruginosa, Pseudomonas putida* and *Bacillus subtilis*. *P. Putida* showed the highest diesel degradation at 120 h with an absorbance of 2.27±0.03 at 600 nm followed by *P. aeruginosa* (2.23±0.03) and *Bacillus subtilis*. The best degradation was observed at 1% diesel concentration (v/v) followed by 2% and the least was recorded at 3% with the absorbance of 2.40±0.00, 2.27±0.03 and 2.20±0.00 respectively. The detection of these potential degraders is crucial in the light of the lingering search for efficient hydrocarbon degraders for efficient bioremediation since their degrative capability could be enhanced for deployment in the bioremediation of diesel-contaminated environments.

INTRODUCTION

Large-scale environmental contamination is a direct result of the dramatic increase in both petroleum exploration and consumption [1]. Soil pollution reports frequently include diesel oil because of the complex hydrocarbon contaminant it is. Diesel oil is a mixture of alkanes and aromatic compounds [2]. Almost 1.7-8 million metric tons of diesel are reported to be released into the water and soil environment annually across the world [3]. The build-up of petroleum products in the environment poses serious risks to human health and the safety of biotic and abiotic components of the ecosystem [4]. For example, both vegetable oil and diesel fuel have been shown to have profound effects on plant roots, halting their development [5]. Unlike the mechanical method, which is very expensive and time-consuming, the best approach for reclamation of contaminated soil is bioremediation which uses microorganisms to degrade the toxic compounds in the environment [6,7]. Numerous isolates of bacteria and fungi have been reported to degrade crude oil from soil polluted with petroleum hydrocarbons and these include *P. aeruginosa, P. putida, Proteus sp., Acinetobacter lwoffi, Alcaligenes eutrophus, Bacillus subtilis, Micrococcus luteus, Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp. and *Candida* spp. [8]. Bioremediation is a low-cost method for removing petroleum hydrocarbons from the environment due to its widespread applicability and its ability to eradicate the contaminant entirely [9]. It is common practice to employ a variety of microbial communities, especially soil bacteria, to aid in the cleanup of a petroleum-contaminated area [10].

Degradation rates of hydrocarbons are highly sensitive to their physical form, chemical make-up and concentration [8]. Biodegradation of diesel has been shown to be sensitive to a variety of environmental conditions, including nutrients, pH, temperature and hydrocarbon concentration. Hydrocarbon concentration in diesel oil degradation has been overlooked

despite its importance. The purpose of this work was to conduct a preliminary screening of diesel degrading bacteria isolated from diesel contaminated soil under varying hydrocarbon concentrations.

MATERIALS AND METHODS

Collection of samples

Soil samples were collected from three (3) different filling stations including, Madobi, A.A Rano, and Katagum petroleum at Azare, Katagum L.G.A, Bauchi State. The samples were collected at each site by digging up the soil with a shovel for about 10cm and transferred directly into clean, sterile containers. Also, a contaminant-free soil sample was collected from the botanical garden of Bauchi State University, Gadau [10].

Isolation and identification of diesel degrading bacteria

We spread 100 µL of serially diluted diesel-contaminated soil samples on nutrient agar plates and incubated them at 37 °C for 24 h. Under the same conditions as described by Palanisamy et al. [11], we sub-cultured the colonies that had formed on the agar plates to obtain the pure colonies. Bacterial isolates were identified using standard biochemical protocols [12].

Screening of diesel degrading bacteria

The bacteria isolated from different diesel-contaminated soil were screened based on their ability to degrade diesel oil supplemented in the Mineral Salt medium (MSM). The MSM contained the following compositions: $NH_4.NO_3$ – 3 g/L, $K_2HPO_4.3H_2O$ - 0.5 g/L, KH_2PO_4 - 0.5 g/L, and trace amounts of $CuSO_4.4H_2O$ - 0.002 g/L,$MgSO_4.7H_2O$ - 0.008 g/L, $FeSO_4.7H_2O$ - 0.002 g/L, $MnSO_4.H_2O$ - 0.002 g/L, and $CaCl_2.2H_2O$ - 0.002 g/L. Briefly, as described by Palanisamy et al [11], the bacterial isolates were grown in Mineral Salt Medium with 1% diesel oil (v/v) as the only carbon source and incubated for 3 days at 37°C. Using a spectrophotometer, the optical density at 600 nm of the growing isolates was measured at set time intervals. The absorbance was measured and recorded after the spectrophotometer had been calibrated to zero with formulations identical to those used in the treatment (except for the addition of bacterial isolates).

Effect of diesel concentration on biodegradation

Substrate for a study on the impact of diesel concentration on the biodegradation process was MSM amended with 1%, 3%, and 5% concentrations of diesel oil. Bacterial strains were inoculated into the flasks, and the containers were then shaken in an incubator at 37 degrees Celsius (120 rpm). The both the tests and controls were both maintained in identical conditions. The impact of concentration on diesel biodegradation was investigated by collecting and analysing residual diesel oil samples at regular 6-hour to 120-hour intervals [11].

RESULTS

Bacterial isolates and their diesel degradation potential

In this study, three (3) bacterial species were identified which include *Pseudomonas putida, Bacillus subtilis* and *P. aeruginosa.* These bacteria were further used in the degradation of diesel which was assessed spectrophotometrically at the wavelength of 600nm. The degradation of 1% diesel (v/v) revealed that *Pseudomonas putida* had the highest degradation potential followed by *Pseudomonas aeruginosa* with the absorbance of 2.27±0.03 and 2.23±0.03 respectively while *Bacillus subtilis* had the lowest degradation potential of 2.00±0.00 after 120 h of incubation (**Table 1**).

Table 1. Degradation potential of diesel degrading bacterial isolates at 1%v/v diesel.

	Time (h)					
Isolates	0	24	48	72	96	120
Control	0.32±0.00	0.43±0.00	0.61±0.00	0.65±0.00	0.85±0.01	1.16±0.09
P. putida	0.34±0.00*	0.65±0.00*	0.86±0.00*	0.97±0.00*	1.88±0.00*	2.27±0.03*
B. subtilis	0.34±0.00*	0.64±0.00*	0.75±0.00*	0.95±0.00*	1.75±0.00*	2.00±0.00*
P. aeruginosa	0.35±0.00*	0.65±0.00*	0.84±0.00*	0.97±0.00*	1.71±0.01*	2.23±0.03*

Note: Results are expressed as mean ± SEM. Results are statistically significant at p≤0.05 as compared with the control group.

Effect of concentration on the biodegradation of diesel (1%, 2%, and 3%)

The best diesel degrading bacteria, *Pseudomonas putida* was used for this study. The highest degradation of diesel was observed at 1% concentration (2.40±0.00) after 120 h followed by 2% v/v (2.27±0.03). However, the least degradation was recorded at the concentration of 3% v/v with an absorbance of 2.20±0.00 (600 nm) optical density at 120 h (**Table 2**). There was a significant difference between the statistical values.

Table 2. Effect of concentration on the degradation of diesel by *Pseudomonas putida.*

Diesel (%)	Time (h)					
	0	24	48	72	96	120
Control	0.45±0.01	0.63±0.01	0.68±0.00	0.83±0.00	0.95±0.03	1.35±0.00
1%	0.59±0.00*	0.86±0.02*	1.65±0.00*	2.23±0.00*	2.30±0.00*	2.40±0.00*
2%	0.55±0.01*	0.78±0.00*	1.54±0.00*	2.00±0.00*	2.13±0.03*	2.27±0.03*
3%	0.52±0.02*	0.79±0.02*	1.53±0.01*	1.99±0.01*	2.10±0.00*	2.20±0.00*

Note: Results are expressed as mean ± SEM. Results are statistically significant at p≤0.05 as compared with the control group.

DISCUSSION

Three bacterial species capable of degrading 1% (v/v) diesel oil were isolated from different diesel-contaminated soil within Azare, Katagum LGA Bauchi State, Nigeria. These isolated bacteria included *P. putida, P. aeruginosa* and *B. subtilis.* These organisms have previously been linked to the degradation of hydrocarbons. In a study by Prakash et al [13], a total of 59 bacteria were isolated from soil contaminated with hydrocarbons, out of which only *Bacillus sp., Pseudomonas sp.,* and *Micrococcus* species could grow in 1% of diesel oil which indicated their capability to utilize and degrade diesel as a carbon source. In a different study, Titah et al [14] isolated *Staphylococcus* and *Micrococcus* species capable of degrading diesel oil from the ship dismantling facility at Tanjungjati, Madura, Indonesia. Geetha et al [15] used the hole-plate diffusion assay technique and found that out of 14 bacterial isolates, 9 were able to grow around the holes and used diesel as a carbon and energy source.

In the present study, the three bacterial species (*P. putida, P. aeruginosa* and *B. subtilis*) isolated were further screened for their potential to degrade diesel oil. The results revealed that *P. putida* had the highest degradation potential followed by *P. aeruginosa* and *B. Subtilis* with the absorbance of 2.27±0.03, 2.23±0.03 and 2.00±0.00 after 120 h of incubation respectively. *P. putida* had been previously reported to degrade various fractions of hydrocarbon [8]. Similarly, Kaczorek and Olszanowski [16] reported that an increase in biodegradation of diesel oil by *P. putida* was observed after the addition of saponins to the system (76%) and found that even without the addition of biosurfactants, its degradation was comparable to the system with the added biosurfactants. In a different study, Shankar et al [17] reported that out of the 49 isolated bacteria isolated from contaminated soil, 32 of them accounting for 65.30 % were able to degrade the various hydrocarbon fractions (including diesel) as the sole carbon source. Kaczorek and Olszanowski [16], on the

other hand, reported an increase in biodegradation of diesel oil by *P. putida* following the addition of saponins to the system (76%). In another study, Titah et al [14] screened thirteen (13) bacterial isolates for the potential to utilize diesel oil in different concentrations ranging from 0 to 15% (v/v). It was revealed that out of the 13 isolates screened, only two belonging to *Staphylococcus* and *Micrococcus* species were found to degrade the diesel compared to other bacteria isolated in the study. However, in a different study, Pirollo et al [18] demonstrated the ability of *P. aeruginosa* to degrade as high as 20% diesel concentration after 144 h of incubation. These findings were attributed to the production of rhamnolipid produced by the isolates.

To determine the effect of diesel concentration on the degradation, the best candidate in this study, *P. putida* was subjected to biodegradation at different diesel concentrations (1-3% (v/v). The results revealed that the degradation of the isolate was observed at 1% (v/v) diesel concentration followed by 2% with 2.40±0.00 and 2.27±0.03 respectively, while the least degradation was recorded at 3% (v/v) after 120 h of incubation. Our findings corroborate the fact that diesel concentration in the medium increases, bacterial growth decreases, and vice versa [14]. This might be because of the toxic effects of high diesel concentrations on the growth of bacteria in the medium, which causes stress and shock [11].

Similar to the findings of the present study, Farag et al [19] investigated the effect of crude oil on the degradation potential of *Pseudomonas* species at 0.2%, 0.5%, 1%, and 1.5% concentrations and found the greatest degradation potential for oil removal when at 0.5 percent (v/v) of crude oil. On the other hand, Palanisamy et al [11] observed the highest degradation of diesel by *Acinetobacter baumannii* at 4% (v/v) but could not tolerate 5% (v/v) of the oil while minimal degradation was recorded at 1% (v/v). However, in a different study, biodegradation of up to 6% diesel has been reported to be aided by the addition of 0.2% w/v glucose and 0.1% w/v yeast extract [20]. Furthermore, Nicolau et al [21] reported the biodegradation of 2-ethylhexyl nitrate which was employed as an additive of diesel fuel, by *M. austroafricanum* , though the results indicated that metabolites accumulated when biodegradation was taking place in the medium

CONCLUSION

In this study, three (3) diesel degrading bacterial species were isolated from contaminated soil and all have shown various degradation capacities. Th three bacterial species included *Pseudomonas aeruginosa, Pseudomonas putida* and *Bacillus subtilis*. *P. putida* was found to be the best candidate based on the screening result and was used in the determination of the effect of diesel concentration on its degradation. The maximum degradation was observed at 1% concentration while the least was recorded at 3% (v/v) suggesting the influence of diesel concentration on its degradation.

ACKNOWLEDGEMENT

The authors sincerely acknowledge Bauchi State University, Gadau for its technical support in the course of this research.

REFERENCES

1. Kawo AH, Faggo AA. Enhanced removal of crude oil in soil by co-culture of *Bacillus subtilis* and *Pseudomonas aeruginosa* isolated from contaminated soil in Kano State, Nigeria. Bayero J Pure Appl Sci 2018;10:423. https://doi.org/10.4314/bajopas.v10i1.83S.
2. Gallego JLR, Loredo J, Llamas JF, Vázquez F, Sánchez J. Bioremediation of diesel-contaminated soils: evaluation of potential in situ techniques by study of bacterial degradation. Biodegradation 2001;12:325–35. https://doi.org/10.1023/A:1014397732435.
3. Dadrasnia A, Agamuthu P. Diesel Fuel Degradation from Contaminated Soil by *Dracaena reflexa* Using Organic Waste Supplementation. J Jpn Pet Inst 2013;56:236–43. https://doi.org/10.1627/jpi.56.236.
4. Tang JC, Wang RG, Niu XW, Wang M, Chu HR, Zhou QX. Characterisation of the rhizoremediation of petroleum-contaminated soil: effect of different influencing factors. Biogeosciences 2010;7:3961–9. https://doi.org/10.5194/bg-7-3961-2010.
5. Hussain I, Puschenreiter M, Gerhard S, Sani SGAS, Khan W, Reichenauer TG. Differentiation between physical and chemical effects of oil presence in freshly spiked soil during rhizoremediation trial. Environ Sci Pollut Res 2019;26:18451–64. https://doi.org/10.1007/s11356-019-04819-6.
6. Pala DM, de Carvalho DD, Pinto JC, Sant'Anna GL. A suitable model to describe bioremediation of a petroleum-contaminated soil. Int Biodeterior Biodegrad 2006;58:254–60. https://doi.org/10.1016/j.ibiod.2006.06.026.
7. Hossain MdF, Akter MstA, Sohan MdSR, Sultana DrN, Reza MA, Hoque KMdF. Bioremediation potential of hydrocarbon degrading bacteria: isolation, characterization, and assessment. Saudi J Biol Sci 2022;29:211–6. https://doi.org/10.1016/j.sjbs.2021.08.069.
8. John RC, Okpokwasili GC. Crude Oil-Degradation and Plasmid Profile of Nitrifying Bacteria Isolated from Oil-Impacted Mangrove Sediment in the Niger Delta of Nigeria. Bull Environ Contam Toxicol 2012;88:1020–6. https://doi.org/10.1007/s00128-012-0609-8.
9. Makkar RS, Rockne KJ. Comparison of Synthetic Surfactants and Biosurfactants in Enhancing Biodegradation of Polycyclic Aromatic Hydrocarbons. Environ Toxicol Chem 2003;22:2280. https://doi.org/10.1897/02-472.
10. Faggo AA, Kawo AH, Gulumbe BH, Ijah UJJ. Assessment of Crude Oil Degradation by Mixed Culture of Bacillus subtilis and Pseudomonas aeruginosa at Different Concentrations. Int J Environ 2020;9:33–50. https://doi.org/10.3126/ije.v9i2.32511.
11. Palanisamy N, Ramya J, Kumar S, Vasanthi N, Chandran P, Khan S. Diesel biodegradation capacities of indigenous bacterial species isolated from diesel contaminated soil. J Environ Health Sci Eng 2014;12:142. https://doi.org/10.1186/s40201-014-0142-2.
12. Chessbrough M. District Laboratory Practice in Tropical Countries. Part 2 Second edition, Cambridge University Press, UK,; 2006.
13. Prakash A, Bisht S, Singh J, Teotia P, Kela R, Kumar V. Biodegradation potential of petroleum hydrocarbons by bacteria and mixed bacterial consortium isolated from contaminated sites n.d.:11.
14. Titah HS, Pratikno H, Moesriati A, Imron MF, Putera RI. Isolation and Screening of Diesel Degrading Bacteria from Ship Dismantling Facility at Tanjungjati, Madura, Indonesia n.d.:11.
15. Geetha SJ, Joshi SJ, Kathrotiya S. Isolation and Characterization of Hydrocarbon Degrading Bacterial Isolate from Oil Contaminated Sites. APCBEE Procedia 2013;5:237–41. https://doi.org/10.1016/j.apcbee.2013.05.041.
16. Kaczorek E, Olszanowski A. Uptake of Hydrocarbon by Pseudomonas fluorescens (P1) and Pseudomonas putida (K1) Strains in the Presence of Surfactants: A Cell Surface Modification. Water Air Soil Pollut 2011;214:451–9. https://doi.org/10.1007/s11270-010-0436-7.
17. Shankar S, Kansrajh C, Dinesh MG, Satyan RS, Kiruthika S, Tharanipriya A. Application of indigenous microbial consortia in bioremediation of oil-contaminated soils. Int J Environ Sci Technol 2014;11:367–76. https://doi.org/10.1007/s13762-013-0366-1.
18. Pirôllo MPS, Mariano AP, Lovaglio RB, Costa SGVAO, Walter V, Hausmann R, et al. Biosurfactant synthesis by *Pseudomonas aeruginosa* LBI isolated from a hydrocarbon-contaminated site. J

Appl Microbiol 2008;105:1484–90. https://doi.org/10.1111/j.1365-2672.2008.03893.x.
19. Farag S, Soliman NA, Abdel-Fattah YR. Statistical optimization of crude oil bio-degradation by a local marine bacterium isolate Pseudomonas sp. sp48. J Genet Eng Biotechnol 2018;16:409–20. https://doi.org/10.1016/j.jgeb.2018.01.001.
20. Kwapisz E, Wszelaka J, Marchut O, Bielecki S. The effect of nitrate and ammonium ions on kinetics of diesel oil degradation by Gordonia alkanivorans S7. Int Biodeterior Biodegrad 2008;61:214–22. https://doi.org/10.1016/j.ibiod.2007.08.002.
21. Nicolau E, Kerhoas L, Lettere M, Jouanneau Y, Marchal R. Biodegradation of 2-Ethylhexyl Nitrate by *Mycobacterium austroafricanum* IFP 2173. Appl Environ Microbiol 2008;74:6187–93. https://doi.org/10.1128/AEM.01142-08.