Ministry of Higher Education and Scientific Research

University of Babylon

College of Science

Biology department

Isolation and identification of Candida species from soil contaminated with petroleum products

Research submitted to the Department of Biology as part of the requirements for obtaining a BSc degree in Biology

By

Noor qassim ali Redha

Supervisor

Assist prof Zahraa Mohammed Al-Taee

2023 A.D **1444 A.H**

بِسْمِ اللهِ الرَّحْمنِ الرَّحِيمِ

(وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلاَّ قَلِيلاً)

صَدق الله العَلي الْعَظِيمِ

سورة الأسراء الأية ﴿٨٥﴾

Dedication

To My Family...

Acknowledgements

My first thanks is for god absolutely, then my parents for all efforts and prayers to me and for struggles that I faced at any time and every one that support me and laugh me specially my best friend

So thanks for all of them

Iam so pleased to present my thanks to everyone who guided , advices and directed me at any time

Finally I especially so thank ful to my kind and hardworking doctor at ever cause she learns me every thing so seriously my lecturer ,Dr. Zahraa AL-Taee

My thanks also for management of the college of science in the university of babylon, biology, All members of this search ....

.

Summary

Gasoline, kerosene, diesel fuels, and oils are all examples of petroleum products. Petroleum hydrocarbons, which are found in all of these products, are the building blocks from which crude oil is extracted. Depending on how much carbon is present in their molecular structure, they can take on a wide variety of properties. Many of these can be problematic when they become established in the soil.

Twenty soil samples, each containing around 100 gm, were collected at a depth of 10 cm using sterile instruments and stored in sterile jars. Hilla Governorate soils were collected from a variety of locations, as were Basra soils from oil fields.

From each sample, 1 gm of soil was taken and added to 9 ml of brain heart infusion media, and incubated at a temperature of 30-37 °C for 24 hours, after which a loop full transfer was taken and plotted on the chromogenic agar medium of Candida and incubated at 30-37 °C for 24 hours. Three types of Candida; *Candida krusei* ; *C. glabrata* , and *C.parapsilosis* were obtained. They were diagnosed on the chromogenic medium and molecularly. Two antibiotics were taken from the Candida antibiotics and the extent of resistance of the obtained isolates was studied. These antibiotics are Nestatine (50mcg/disc) and Fluconazol (10mcg/disc) ,both type of *Candida* was resistant to both antibiotics except *C. parapsilosis* was sensitive to Flc.

Growing on minimal salt medium and adding desal at a concentration of 0.05% is to determine the efficiency of bacteria to grow in environments containing petroleum products by degrade desal. In this test, the optical density is measured every seven days for a period of 21 days, and it is noted from the results a rise in the optical density, which indicates an increase in growth in the culture medium used.

Introduction

Petroleum extraction, storage, and use create PHC-based soil pollution worldwide. These organic heterogeneous pollutants have different carbon and hydrogen atom structures (Haque et al., 2022). Hydrocarbons link halogen, nitrogen, and sulfur. PHC-based pollutants provide health and environmental risks (Koshlaf and Ball, 2017). Physical, chemical, and biological methods cleared PHC-polluted soils. Chemical procedures may produce secondary pollutants, while physical methods with extracting agents increase soil PHC desorption (Lu et al., 2013; Bianco, 2022). Biological treatments are popular for their cheap cost and environmental impact. Bioremediation, which uses efficient microorganisms or microbial consortia to break down complex substances via redox reaction, is promising (Abena et al., 2019; Haque et al., 2022; Koshlaf and Ball, 2017; Mukherjee et al., 2017). Synergistic microbial communities increase soil microbiome substrate and product transfer (Megharaj et al., 2011). Despite breaking down oil and its products, soil homeostasis is affected by microbial community and structure changes (Yi et al., 2022). PHC-contaminated places have many microorganisms. Maintaining synergy to breakdown PHC-based contaminants is difficult (Wang et al., 2022). Simplified bioremediation methods were created. To study the genes, enzymes, and microbes that biodegrade PHC-based contaminants in soils, an efficient microbe was used to treat soil samples spiked with the target standard chemical. Systems biology is an interdisciplinary field that explores biological system assembly and interaction. Pollutant metabolism's complex metabolic and regulatory networks in a cell or microbiome are studied using systems biology (Lu et al., 2019; Mukherjee, 2017). These discoveries are leading to functioning microbial consortia for field application and new ways to find novel genes and regulatory networks. Microorganisms, contaminants, and polluted sites' temperature, pH, and oxygen affect bioremediation

(Fan et al., 2020; Koshlaf and Ball, 2017). Nutrients, surfactants, efficient bacteria, and compounds that strengthen the soil matrix and induce microbial electron shuttling accelerate restoration (Abena et al., 2019; Almansoory, 2019). Soil samples from polluted sites should be researched to create bioremediation technologies to comprehend exogenous microorganisms, soil microbiota, and contaminants. It was removed to clean Tianjin, China's polyether glycol manufacturing soil. Over 40 years, the factory has produced gasoline, polyether polyols, polymer polyols, and ethylene glycol diethyl ether. Petroleum hydrocarbons including n-alkanes, PAHs, and halohydrocarbons poisoned the soil.

Aim of the study

This study aims to identify the types of candida species present in soils contaminated with petroleum products, and to evaluate the efficiency of these isolates in eliminating contamination.

Objective of the study

1- Isolation of Candida from soil contaminated with petroleum products and soil contaminated with crude oil.
2- Diagnosis of Candida using chromogenic agar and using PCR technology.
3- Study the sensitivity of Candida isolates to antibiotics.
4- A study of the efficiency of isolated Candida in the analysis of petroleum products.

Review of literatures

Introduction

Candida species are opportunistic fungi that reside on mucosal surfaces of the human body, such as the mouth, gut, and vagina. There are over 20 different Candida species, with Candida albicans being the most widely studied and well-known. Other species include Candida glabrata, Candida tropicalis, Candida parapsilosis, and Candida krusei. Candida species can exist as commensals in healthy individuals, but given the right conditions, they can also become pathogenic and cause a variety of infections (Fauci,2015).

Pathogenesis

Candida infections are a significant cause of morbidity and mortality, especially in immunocompromised patients, such as those with HIV, cancer, organ transplant, or autoimmune diseases. Candida infections can also occur in patients with underlying medical conditions or those who use immunosuppressive medications, including corticosteroids, chemotherapy, or long-term antibiotics.

The ability of Candida to cause disease depends on its capability to adhere to host tissues, produce virulence factors, such as phospholipase, proteinases, hydrolases, and toxins, and also evade the host immune response. The host immune response to Candida infection involves innate and adaptive immune mechanisms, including neutrophils, macrophages, dendritic cells, T and B lymphocytes, and complement activation. However, in immunocompromised patients, these mechanisms may be impaired, leading to a failure to control infection.

Clinical Manifestations

Candida infections can manifest as local or systemic diseases. Localized infections include oropharyngeal candidiasis (thrush), vaginal candidiasis, and cutaneous candidiasis, among others. Systemic candidiasis, or candidemia, occurs when Candida disseminates into the bloodstream and can have a wide range of clinical manifestations, including fever, sepsis, endocarditis, meningitis, and deep-seated organ involvement.

Diagnosis and Treatment

The diagnosis of Candida infections typically involves a combination of clinical presentation, imaging studies, and laboratory testing, including blood culture, tissue biopsy, and fungal culture. The treatment of Candida infections depends on the severity and location of the disease, as well as the underlying medical conditions of the patient. Antifungal agents such as fluconazole, amphotericin B, and echinocandins are commonly used to treat Candida infections.

Ability of candida to reduce pollution

So far, most research on fungal bioremediation has been conducted on land [5]; marine habitats have received less attention. 6 . The rate of crude oil biodegradation by marine fungus was calculated by measuring the mass loss and gain over time [6]. Fungal isolates from hydrocarbon-contaminated Gulf of Mexico environments have been shown to be capable of degrading n-alkanes and polycyclic aromatic hydrocarbons[7]. In addition, the production of biosurfactants by certain fungus can increase the hydrocarbon bioavailability to other microbial communities, such as bacteria or fungi[8]. The capacity of microbes to produce biosurfactants is the most

important feature of a potential hydrocarbon degrader[8]. Biosurfactants improve the solubility of oily contaminants, making them more accessible as carbon sources for microbes and thereby speeding up their degradation[9]. Micro- biological surfactants have characteristics similar to synthetic surfactants, but they degrade naturally and can be manufactured on-site s[10]. There is a substantial competitive advantage for businesses operating in polluted areas if they can isolate microorganisms with the ability to emulsify and solubilize hydrophobic contaminants ex-situ and in-situ8. Microbes or microbial surfactants are introduced directly into contaminated wells to help reduce oil viscosity, allowing for smoother pipeline movement and more stable fuel water-oil emulsions [11].(41598_2022_)

Oil and its byproducts polluting soil and water is a major and growing environmental risk. Because of their widespread use and the logistics involved in transporting them, petroleum and its compounds have become serious environmental hazards. One barrel of crude oil can contaminate one million barrels of water, so the magnitude of an oil spill can be grasped from this simple statement alone [1]. Pollutant accumulation in animal and plant tissues can cause mortality or mutations [2], so hydrocarbon contamination of soil and water is extremely harmful to all forms of life in the ecosystem. Organic pollutants, such as hydrocarbon discharges from storage tank spills, threaten agricultural soils in the Fez-Meknes area of Morocco. The welfare of people, businesses, and ecosystems are all negatively affected by this pollution. Hydrocarbon pollution can be reduced through the use of biodegradation, which involves the widespread distribution of microorganisms that house specialized degradative machinery. It's widely recognized as a low-cost, effective method of in-situ, self-propelled, environmentally favorable mineralization. Bacteria, yeasts, and fungus are the primary hydrocarbon-degrading microorganisms in polluted environments [3, 4]. According to Walker et al. [6], yeasts are superior

to microbes as a crude oil degrading agent. Yeasts, as stated by Obuekwe et al., Ashraf and Ali [7,8], can use n-alkanes as a special carbon and energy source. Yeasts like Candida lipolytica, Geotrichum sp., Trichosporon mucoides, and Yorrowia lipolytica were found to degrade petroleum compounds when they were separated from polluted water [9,10]. Recent research by Benmessaoud et al. on the biotypology and diversity of soil yeasts in this area provides useful information for gauging the degree to which oil pollution has disrupted local ecosystems. [11]. However, more research into this aspect of petroleum biodegradation is necessary. Therefore, the laboratory of Biotechnology, Conservation and Valorization of Natural Resources in Fez, Morocco, set out to conduct a research to broaden and deepen understanding of biodegradation of soil yeasts, particularly those contaminated by organic pollutants.(sustainability-14)

Patients with weakened immune systems and those on long-term antibiotic therapy are particularly vulnerable to opportunistic infections like candidiasis, which is caused by the Candida species. Antifungal drugs foster resilience. Therefore, the final therapy must take into account the antifungal sensitivity pattern of these organisms. Therefore, the purpose of this research is to characterize those who have candidiasis and to establish a pattern of antifungal susceptibility among Candida species. Among the 87 Candida species isolated, 55.2% were found in men and 44.8% were found in females. In addition, 48.3% of all microorganisms were found to be Candida albicans, making it the most frequently isolated species. Approximately 41.1% of the sputum samples tested positive for Candida species. The susceptibility of these species to Flucytosine and Micafungin ranges from 100% to 98.9%, while sensitivity to Caspofungin, Voriconazole, Amphotericin B, and Fluconazole ranges from 97.7% to 93.1%. Patients with cancer and diabetes mellitus

both had Candida species present. They were also monitored after receiving antibiotics for preventative, trial, and final treatment.(004+-+)

Material and Methods

Sample collection

Twenty soil samples were taken from a depth of approximately 10 cm using sterile tools, as an amount of about 100 ml of soil was taken and kept in sterilized containers. Soil samples were taken from different regions of Hilla Governorate, and soil samples were also taken from the crude oil fields in Basra.

Culture Cultivation

From each sample, 1 gm of soil was taken and added to 9 ml of brain heart infusion media, and incubated at a temperature of 30-37 °C for 24 hours, after which a loop full transfer was taken and plotted on the chromogenic agar medium of Candida and incubated at 30-37 °C for 24 hours.

Antibiotic susceptibility

Two antibiotics were taken from the Candida antibiotics and the extent of resistance of the obtained isolates was studied. These antibiotics are Nestatine (50mcg/disc) and Fluconazol (10mcg/disc).

Identifying the activity of microorganisms in diesel.

Inoculate colonies of different bacteria (previous experiment) into 50 ml mineral salts medium (MSM) .

Components of the culture medium

Minimal salt media that used to test the ability of candida to degrade hydrocarbons content are listed in table (1)

Table (1) MSM content

no.	Per liter	weight
1.	FeSO 4	1 mg
2.	MgSO 4 .7H 2 O	200 mg
3.	Na2 HPO 4	210 mg
4.	NaH 2 PO 4	90 mg
5.	CuSO 4 .5H2 O	5 µg
6.	H 3 Bo3	10 µg
7.	MnSO 4 .5H 2O	10 µg
8.	ZnSO 4 .7H 2 O	70 µg
9.	MoO 3	10 µg
10.	CoSO 4	10 µg
11.	KCl	40 mg
12.	CaCl 2	15 mg

13.	NH 4 Cl	500 mg
14.	NaNO3	2 mg
15.	0.05% (v/v) diesel	

-Incubate at 28°C for 21 days.

-The growth response of each of the above isolated bacteria on diesel can initially determine at 7 days intervals by physical appearance (turbidity) and measuring the optical density (O.D.) at 540 nm.

Molecular identification of candida species

For the purpose of ascertaining the isolated species from the soil contaminated with petroleum products, the PCR test was carried out using the primer ,in table (2) and according to the conditions mentioned in the table (3)

Table (2) Primer sequence 5′→ 3′ (

Gene	**Direction**	**Primer sequence 5′→ 3′**	**PCR product bp**
cand	F	AGCTTGCGTTGATTACGTCCCTGCCC	1000bp *C. glabrata*
	R	TTCACTCGCCGCTACTAAGGCAATCCC	731bp *C. Parapsilosis*
			800bp *C. krusei*

Table(3) PCR condition for amplifying Cand gene

No.	Steps	Temperature (°C)	Time	Cycles
	Initial Denaturation	95	3 min.	
	Denaturation	95	45 sec.	35 cylce
	Annealing	68	45 sec.	
	Extension	72	45 sec.	
	Final Extension	72	5 min.	
	Storage	4		

The mixture of PCR was 2µl of DNA sample , 1.5µl of each forward and reverse primer, and 15µl of green master mix(Promega).

Results and Discussion

Isolation

Twenty soil samples contaminated with crude oil and petroleum products were collected from Basra oil fields and different regions of Babylon governorate, and out of these twenty samples, only three samples gave growth of Candida on the chromogenic agars, and these types of yeasts are *Candida krusei* ; *C. glabrata* , and *C.parapsilosis* ,figure 1

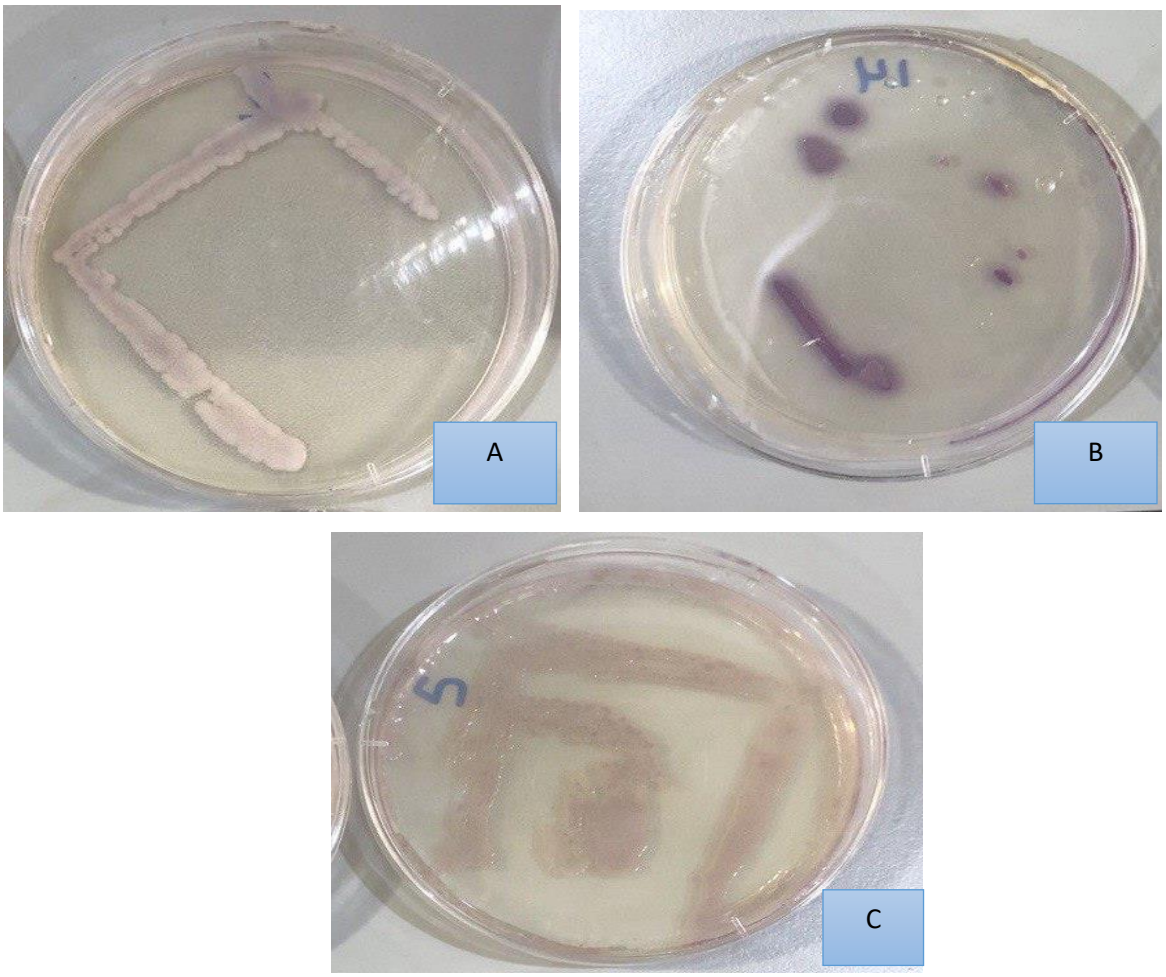

Figure (1) Candida species isolated in this study on Chromogenic agar .(A) *C. glabrata*; (B) *C. krusei*; (C) *C. parapsilosis* .

Three novel yeast strains, Candida parapsilosis SK1, Rhodotorula mucilaginosa SK2, and SK3, capable of decomposing crude oil were isolated and characterized in a study by Benmessaoud and colleagues. Bioremediation of hydrocarbon-contaminated areas will use these yeasts to design a regimen comprising optimization of physico-chemical parameters (Benmessaoud et al.,2022).

Six soil samples were taken from three locations (two from each) in and around the crude oil reserves in Faisaliyyah, Al Sina'iyah, and Ghubairah in the Riyadh region of Saudi Arabia. Four different strains of the fungus Candida were found throughout all soil samples cultured on Potato Dextrose Agar (PDA), for an overall prevalence of 13.33%. Using the growth and physical traits typically associated with mycelia as described in mycological keys, various species of yeast were determined using standard taxonomic keys. The isolated species were identified as Candida parapsilosis, Candida krusei, Candida famata, and Rhodotorula spp. based on physical and microscopic identification (Al-Otibi et al., 2022).

Candida glabrata, and Candida krusei, were the two species of Candida isolated from polluted soil and examined for their ability to biodegrade crude oil(Burghal et al., 2016).

Molecular identification of candida species

In this study, Candida species isolated from soil contaminated with petroleum products were diagnosed using a universal primer Table(1) by using PCR technology, as the product of amplification of this gene in; *C. glabrata* 1000bp and for *C.parapsilosis* 731bp, while for *Candida krusei* the product of amplification is 800bp as in the figure

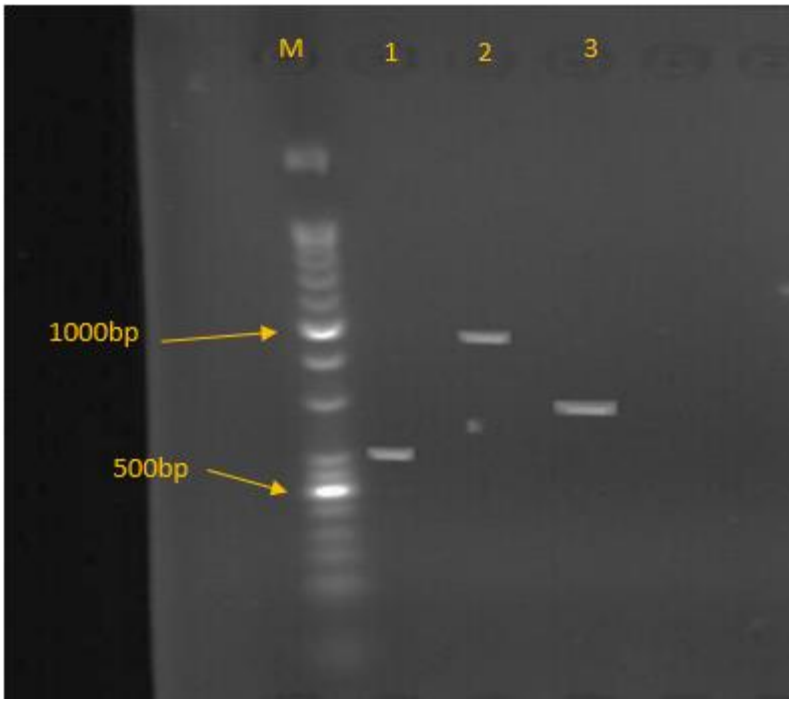

Figure (2) agarose gel electrophoresis of PCR for *Cand* gene ; M: Marker DNA ladder; lane 1 *C. glabrata (1000bp)* ; lane2 *C. parapsilosis (713bp);* lane3*C. krusei(800bp)*;1.5% agarose gel with safe red stain, at 80 V\Cm ,it took sixty minutes.

Yeast bioremediation is an alternate strategy for reducing soil contamination after an oil spill. The focus of this research is on optimizing physicochemical parameters as part of a bioremediation strategy. As such, three novel yeast strains, designated SK1, SK2, and SK3, were isolated from hydrocarbon-tainted materials gathered in the Fez-Meknes area of Morocco. On the basis of their similarities in the ITS region, these isolates were determined to be novel species of Candida parapsilosis (SK1) and Rhodotorula mucilaginosa (SK2 and SK3). Degradation kinetics of petroleum oils are discussed in detail. Gravimetric analysis and gas chromatography coupled to mass spectrometry were used to determine degradation kinetics and biomass production. In 21 days, the strains degraded 68% of the total petroleum hydrocarbon

using just carbon dioxide. When glucose was introduced, the isolates' crude oil consumption accelerated. Our findings point to the viability of using inoculants derived from Candida parapsilosis (SK1) and Rhodotorula mucilaginosa (SK2 and SK3) cells for the biodegradation of crude oil and probably other related aromatic chemicals (Benmessaoud et al ,2022). Around the crude oil reservoirs, six soil samples were taken from three separate locations (two from each). Areas of Riyadh, Saudi Arabia, including Faisaliyyah, Al Sina'iyah, and Ghubairah. The isolated species were identified as Candida parapsilosis, Candida krusei, Candida famata, and Rhodotorula spp. based on physical and microscopic identification (Al-Otibi et al, 2022).

Antibiotic susceptibility

Two antibiotics were taken from the Candida antibiotics and the extent of resistance of the obtained isolates was studied. These antibiotics are Nestatine (Ns)(50mcg/disc) and Fluconazol (Flc) (10mcg/disc), the three types of candida isolated in this study were as follow; For *C. parapsilosis* was sensitive to Flc and the inhibition diameter 30mm and resist Ns with 0mm inhibition; Both *C. glabrata and C. krusei* were resist to both antibiotic with no inhibition zone.

In a study conducted by Richter and colleagues on a group of Candida isolates, they concluded that Susceptibility test results for the 593 isolates revealed that resistance to fluconazole (3.7%), elevated fluconazole MICs (>16 _g/ml) were only observed in *C. glabrata* (15.2% resistant [R], 51.8% susceptible-dose dependent [S-DD]), *C. parapsilosis* (3.3% S-DD), *S. cerevisiae* (11.1% S-DD), and *C. krusei* (50% S-DD, 41.7% R, considered intrinsically fluconazole resistant). Nystatin MICs ranged from 1 to 16 _g/ml, with a MIC inhibiting 90% of isolates (Richter et al;2005) .

revealed that fluconazole had MIC90 values of 8, 64, and 2 g/mL against C. albicans, C. glabrata, and C. parapsilosis; itraconazole, 16, 16, and 0.5; and voriconazole, 8, 16, and 0.25. C. glabrata, the most frequent non-Candida albicans species, has a higher fluconazole, itraconazole, and voriconazole MIC90 than C. albicans and C. parapsilosis. Fluconazole MIC90 64, itraconazole 16, voriconazole 16. C. glabrata is less responsive to azoles than other Candida species, and long-term fluconazole and itraconazole prophylaxis lowered susceptibility. Amphotericin B and caspofungin had MIC90 values of 2, 2, and 0.5 g/mL and 0.5, 0.75, and 0.5, respectively, against C. albicans, C. glabrata, and C. parapsilosis. Other studies revealed substantially lower amphotericin B MIC90 values (0.25, 0.5, and 0.25 g/mL). Many studies found that fluconazole, itraconazole, and voriconazole were effective against 87.5%, 37.5%, and 75% of C. parapsilosis strains.(**Baghdadi et al,2016;** Roy et al,2013; Silva et al,2012; Badiee and Alborzi,2011; Bizerra et al,2014)

Identifying the activity of microorganisms in diesel.

The aim of preparing minimal salt medium and adding desal at a concentration of 0.05% is to determine the efficiency of bacteria to grow in environments containing petroleum products by degrade desal. In this test, the optical density is measured every seven days for a period of 21 days, and it is noted from the results a rise in the optical density, which indicates an increase in growth in the culture medium used table 4, It was also observed that the turbidity and color of the center changed figure

Table (4) The change in optical density over three weeks for different isolates

Candida species	After 7 days	After 14 days	After 21 days
0.05% msm with desal	1.694	-	-
C. krusei	0.347	0.865	1.365
C. parapsilosis	0.103	0.849	1.349
C. glabrata	0.091	0.352	0.852

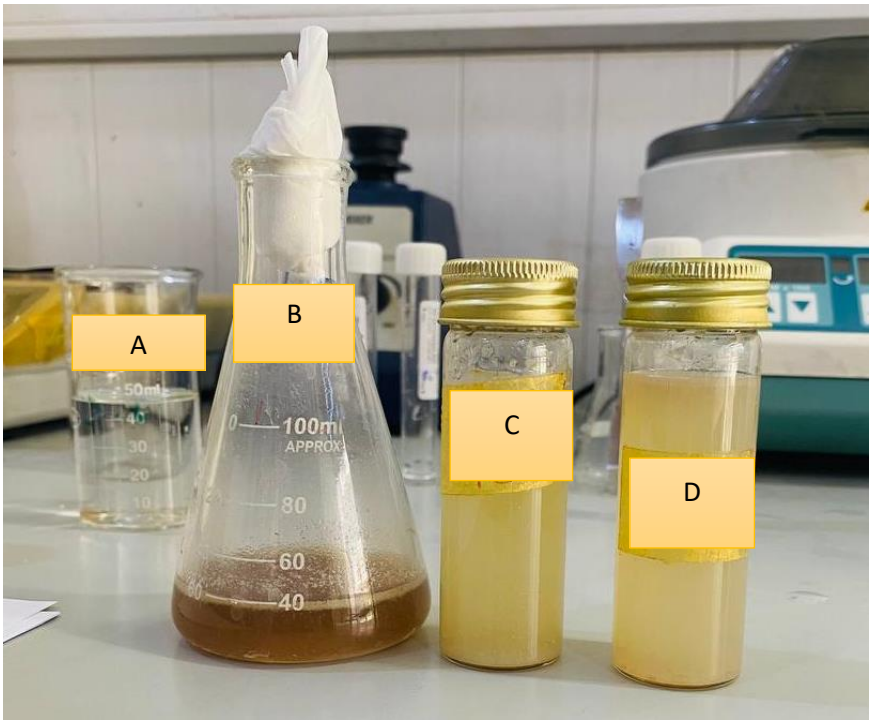

figure (3) Change in turbidity and color ;(A) control ;(B) *C. krusei*; (C)*C. parapsilosis;* (D) *C. glabrata.*

Benmessaoud et al. found that after 4, 15, and 21 days of incubation at 30 _C in Bushnell Has medium containing 3.74% crude oil, the crude oil biodegradation potential of the three investigated strains increased with incubation time and exceeded 60%. Furthermore, it is predicted that Candida parapsilosis contributes to

crude oil degradation at a rate of 68 _ 0.9%, whereas Rhodotorula mucilaginosa degrades it at a rate of 68 _ 1.18% . Furthermore, Rhodotorula mucilaginosa strain degrades at a rate of 15.87 _ 0.1% after 4 days and 56.04 _ 0.07% after 15 days, respectively. When glucose was used as a secondary carbon source, total petroleum hydrocarbon removal increased to 78.6% for Candida parapsilosis and 89.6% for Rhodotorula mucilaginosa, respectively(Benmessaoud et al ,2022)..

Conclusion

1- Some Candida species can grow in soil contaminated with petroleum products.
2- Isolated Candida species from this study had the ability to degrade petroleum pollutant.
3- Candida species were highly resisted to antibiotic that used in this study.

Recommendation

1- Study genes responsible for bioremediation.
2- Study enzymes responsible for bioremediation.

References

Fauci, A.S., 2015. *Harrison's principles of internal medicine*. McGraw-Hill Education.

Mayer FL, Wilson D, Hube B. Candida albicans pathogenicity mechanisms. Virulence. 2013;4(2):119-28.

Richardson JP, Moyes DL. Adaptive immune responses to Candida albicans infection. Virulence. 2015;6(4):327-37.

Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007;20(1):133-63.

Azad K, Tomar D, Gupta S. Identification and management of Candida infections. Clin Microbiol Newsl. 2013;35(4):29-36.

Haque, S., et al., 2022. Functional microbiome strategies for the bioremediation of petroleum-hydrocarbon and heavy metal contaminated soils: a review. Sci. Total Environ. 833, 155222.

Koshlaf, E., Ball, A.S., 2017. Soil bioremediation approaches for petroleum hydrocarbon polluted environments. Aims Microbiol. 3, 25–49.

Bianco, F., et al., 2022. Coupling of desorption of phenanthrene from marine sediments and biodegradation of the sediment washing solution in a novel biochar immobilized–cell reactor. Environ. Pollut. 308, 119621.

Lu, M., et al., 2013. Treatment of petroleum refinery wastewater using a sequential anaerobic–aerobic moving-bed biofilm reactor system based on suspended ceramsite. Water Sci. Technol. 67, 1976–1983.

Mukherjee, A., et al., 2017. Bioinformatic approaches including predictive metagenomic profiling reveal characteristics of bacterial response to petroleum hydrocarbon contamination in diverse environments. Sci. Rep. 7, 1–22.

Abena, M.T.B., et al., 2019. Biodegradation of total petroleum hydrocarbons (TPH) in highly contaminated soils by natural attenuation and bioaugmentation. Chemosphere 234, 864–874.

Megharaj, M., et al., 2011. Bioremediation approaches for organic pollutants: a critical perspective. Environ. Int. 37, 1362–1375.

Yi, M.L., et al., 2022. Structural, metabolic, and functional characteristics of soil microbial communities in response to benzo[a]pyrene stress. J. Hazard. Mater. 431, 128632.

Wang, A., et al., 2022. Synergetic effects of microbial-phytoremediation reshape microbial communities and improve degradation of petroleum contaminants. J. Hazard. Mater. 429, 128396.

Lu, Q.Y., et al., 2019. Benzo(a)pyrene degradation by cytochrome P450 hydroxylase and the functional metabolism network of *Bacillus thuringiensis*. J. Hazard. Mater. 366, 329–337.

Fan, R.J., et al., 2020. Microbial community responses to soil parameters and their effects on petroleum degradation during bio-electrokinetic remediation. Sci. Total Environ. 748, 142463.

Almansoory, A.F., et al., 2019. Biosurfactant produced by the hydrocarbon-degrading bacteria: characterization, activity and applications in removing TPH from contaminated soil. Environ. Technol. Innov. 14, 100347.

Benmessaoud, S.; Anissi, J.; Kara, M.; Assouguem, A.; AL-Huqail, A.A.; Germoush, M.O.; Ullah, R.; Ercisli, S.; Bahhou, J. Isolation and Characterization of Three New Crude Oil Degrading Yeast Strains, Candida parapsilosis SK1, Rhodotorula mucilaginosa SK2 and SK3. Sustainability **2022**, 14, 3465. https://doi.org/10.3390/su14063465

Al-Otibi, F., Al-Zahrani, R.M. & Marraiki, N. The crude oil biodegradation activity of *Candida* strains isolated from oil-reservoirs soils in Saudi Arabia. *Sci Rep* **12**, 10708 (2022). https://doi.org/10.1038/s41598-022-14836-0

Burghal, A.A., Abu-Mejdad, N.M.J.A. and Al-Tamimi, W.H., 2016. Mycodegradation of crude oil by fungal species isolated from petroleum contaminated soil. *International Journal of Innovative Research in Science, Engineering and Technology*, *5*(2), pp.1517-1524.

Richter SS, Galask RP, Messer SA, Hollis RJ, Diekema DJ, Pfaller MA. Antifungal susceptibilities of Candida species causing vulvovaginitis and epidemiology of recurrent cases. J Clin Microbiol. 2005 May;43(5):2155-62. doi: 10.1128/JCM.43.5.2155-2162.2005. PMID: 15872235; PMCID: PMC1153777.

Baghdadi E, Khodavaisy S, Rezaie S, Abolghasem S, Kiasat N, Salehi Z, Sharifynia S, Aala F. Antifungal Susceptibility Patterns of Candida Species Recovered from Endotracheal Tube in an Intensive Care Unit. Adv Med. 2016;2016:9242031. doi: 10.1155/2016/9242031. Epub 2016 Aug 23. PMID: 27642628; PMCID: PMC5011531.

Badiee P., Alborzi A. Susceptibility of clinical Candida species isolates to antifungal agents by E-test, Southern Iran: a five year study. Iranian Journal of Microbiology. 2011;3(4):183–188. - PMC - PubMed

Silva S., Negri M., Henriques M., Oliveira R., Williams D. W., Azeredo J. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiology Reviews. 2012;36(2):288–305. doi: 10.1111/j.1574-6976.2011.00278.x. - DOI - PubMed

Bizerra F. C., Jimenez-Ortigosa C., Souza A. C. R., et al. Breakthrough candidemia due to multidrug-resistant Candida glabrata during prophylaxis with a low dose of micafungin. Antimicrobial Agents and Chemotherapy. 2014;58(4):2438–2440. doi: 10.1128/aac.02189-13. - DOI - PMC - PubMed

Roy R. C., Sharma G. D., Barman S. R., Chanda S. Trend of Candida infection and antifungal resistance in a tertiary care hospital of north east India. Blood. 2013;100:p. 19.

Benmessaoud, S.; Anissi, J.; Kara, M.; Assouguem, A.; AL-Huqail, A.A.; Germoush, M.O.; Ullah, R.; Ercisli, S.; Bahhou, J. Isolation and Characterization of Three New Crude Oil Degrading Yeast Strains, Candida parapsilosis SK1, Rhodotorula mucilaginosa SK2 and SK3. Sustainability **2022**, 14, 3465. https:// doi.org/10.3390/su14063465

Al-Otibi, F., Al-Zahrani, R.M. & Marraiki, N. The crude oil biodegradation activity of *Candida* strains isolated from oil-reservoirs soils in Saudi Arabia. *Sci Rep* **12**, 10708 (2022). https://doi.org/10.1038/s41598-022-14836-0

الخلاصة

البنزين والكيروسين ووقود الديزل والزيوت كلها أمثلة على المنتجات البترولية. الهيدروكربونات البترولية ، الموجودة في جميع هذه المنتجات ، هي اللبنات الأساسية التي يتم استخراج النفط الخام منها. اعتمادًا على كمية الكربون الموجودة في هيكلها الجزيئي ، يمكن أن تأخذ مجموعة متنوعة من الخصائص. يمكن أن يكون العديد من هؤلاء مشكلة عندما يتم ترسيخها في التربة.

تم جمع عشرين عينة تربة ، تحتوي كل منها على حوالي 100 جرام ، على عمق 10 سم باستخدام أدوات معقمة وتم تخزينها في أوعية معقمة. تم جمع تربة محافظة الحلة من عدة مواقع مثل تربة البصرة من حقول النفط.

من كل عينة ، تم أخذ 1 جرام من التربة وإضافتها إلى 9 مل من وسط تسريب القلب بالمخ ، وحضنت عند درجة حرارة 30-37 درجة مئوية لمدة 24 ساعة ، وبعد ذلك تم أخذ حلقة نقل كاملة ورسمها على أجار الكروموجينيك. وسط المبيضات والمحتضنة عند 30-37 درجة مئوية لمدة 24 ساعة. ثلاثة أنواع من المبيضات. المبيضات كروسي تم الحصول على C. glabrata ، و C. parapsilosis. تم تشخيصهم على الوسط اللوني وجزيئيًا. تم أخذ اثنين من المضادات الحيوية من المضادات الحيوية المبيضات ودراسة مدى مقاومة العزلات التي تم الحصول عليها. هذه المضادات الحيوية هي Nestatine (50mcg / disc) و Fluconazol (10mcg / disc) ، وكلا النوعين من المبيضات كان مقاومًا للمضادات الحيوية باستثناء C. parapsilosis كان حساسًا لـ Flc.

إن النمو على وسط ملح ضئيل وإضافة تحلية بتركيز 0.05٪ لتحديد كفاءة البكتيريا في النمو في البيئات التي تحتوي على منتجات بترولية عن طريق تحلية المياه. في هذا الاختبار ، يتم قياس الكثافة الضوئية كل سبعة أيام لمدة 21 يومًا ، ويلاحظ من النتائج ارتفاع الكثافة الضوئية ، مما يشير إلى زيادة النمو في وسط الاستزراع المستخدم.

وزارة التعليم العالي والبحث العلمي

جانعة بابل

كلية العلوم

قسم علوم الحياة

عزل وتشخيص انواع الكانديدا المختلفة من التربة الملوثة بالمنتجات النفطية

بحث مقدم الى قسم علوم الحياة كجزء من متطلبات نيل شهادة البكلوريوس تخصص علوم حياة

بواسطة

نور قاسم علي رضا

باشراف

أ.م.د. زهراء محمد الطائي

2023 م 1444هـ