International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Bacterial Diversity from Bottoms Tanks

Pichl G[1], Pucci G N[2]

Microbiology- Biochemistry department- CEIMA- Universidad Nacional de la Patagonia San Juan Bosco, Argentina

Abstract: ***Bioremediation of crude oil contaminated soil is an effective process that makes use of ubiquitously microorganisms distributed in the bottom tank samples. In this study, 20 crude oil degradating native bacteria were isolated from a bottom tank sludge sample from oil industry after a treatment with solvent 1:1:1 (Bottom tank sludge:diesel oil:water). Bacterial count and identification were done by Sherlock MIDI method, finding Arthrobacter oxydan, Bacillus sp., Bacillus-simplex, Bacillus sphaericus, Paenibacillus polymyxa, Staphylococcus cohnii cohnii. All microorganism has grown in presence of hexadecane, ciclohexane, kerosene and diesel oil.***

Keywords: bottom tank sludge, bacteria, degradation

1. Introduction

Bioremediation is considered a low-cost and environmental friendly solution to remediate (Remove) petroleum from contaminated soil and is usually used in Patagonia Argentina, specially by means of biopiles. However, this procedure can be affected by other components found in the sludge, such as sulphur compounds, so it is important to evaluate the effect of these on oil biodegradation in order to prevent negative impacts. Studies of biodegradation processes have demonstrated that various species belonging to different genera, such as *Achromobacter*, *Bacillus*, *Kocuria*, *Micrococcus*, *Rhodococcus* and *Pseudomonas* among other genera are capable of utilizing TPH as a sole carbon source [2, 8]. These genera are capable of degrading crude oil in pure and mixed cultures with natural or constructed bacterial consortiums. Removal of petroleum hydrocarbon pollutants from environment by applying ubiquitously consortium microorganisms is ecofriendly and economic [8]. Anthropogenic activities such as offshore and onshore petroleum industry activities produce bottoms tank sludges with petroleum hydrocarbon [10]. This product is stocked and recently is treated by wash with hot water and solvent. This produces three currents, oil, water and sediment. Crude oil is a mixture of variety of simple and complex hydrocarbons, which are degraded by several indigenous microorganisms [8].
The aim of the study was search and identify bacteria after treatment of bottom tank sludge and study their capacity to used hydrocarbons as carbons energy.

2. Material and methods

Samples

Samples were taken from oil tank bottoms located in San Jorge Gulf basin. Sludge Oil tank bottoms were subject to a separation treatment and washing procedure. Hydrocarbon concentration was determined by Soxhlet extractor using trichloriethane as the extraction solvent. The extracted hydrocarbons were quantified on a mass difference basis [1]. The extracted hydrocarbons were separated into class fractions by silica gel column chromatography. Aliphatic, aromatic and asphaltic oil fractions were eluted with 250 mL of hexane, 150 mL of benzene and 150 mL of chloroform methanol 1:1 respectively [1].

Enumeration of aerobic and degrading bacteria

Several dilutions of the bacterial suspension were plated on R2A [9] (yeast extract,0.5g; proteose peptone, 0.5 g; casamino acids, 0.5 g; glucose, 0.5 g; soluble starch, 0.5 g; K_2HPO_4, 0.3 g; $MgSO_4$ $7H_2O$, 0.05 g; sodium pyruvate, 0.3 g; agar, 15 g, suspended in distilled water), and incubated at 28°C for 28 days in the dark, and on MBMPGO (Pucci and Pucci 2003) (NaCl 5 g, K_2PO_4H 0.5 g, $NH_4PO_4H_2$ 0.5 g, $(NH_4)_2SO_4$ 1 g, Mg SO_4 0.2 g, KNO_3 3 g, $FeSO_4$ 0.05 g, suspended in 1L of distilled water). The surface of the agar plate was coated with 30 µL of a 1:1 mixture of petroleum diesel oil and dried for 15 minutes at 65 °C and incubated for 28 days at 28 °C.

Bacterial identification

Bacterial identification by FAMEs determination Fatty acids were determined as methyl esters from whole-cell hydrolysates according to the procedure of the SHERLOCK microbial identification system (MIDI Inc., Newark, Del.). Data acquisition and data analysis were controlled by ChemStation software (version 10.01; Agilent) and the Sherlock software package (version 6.0; MIDI).

OD density

Optical density: bacterial communities' growth was determined by optical density (OD) at 600nm.

3. Results

Sludge tank characterization showed high TPH concentrations (52, 72%), enumerations of heterotrophic and hydrocarbon degrading bacteria in oily sludge confirmed the presence of native microbiota 3.2 x 10^3 cfu/ml in R2A and 3.5 x10^3 cfu/ml on MM, after treatment in sediment sample was 4.1x10^3 cfu/ml R2A and 2.91x10^3 cfu/ml in BDH which is indicative of microorganism viability and the biodegradation potential of the system (Pucci and Pucci 2003). A total of 20 bacteria were isolated from sediment after treatment with diesel oil and water. The bacillus genus was the predominant (Table 1), because this genus can sporulate and support hydrocarbon concentration. The identifications of the strain, table 1shows growth in different hydrocarbons types.

4. Discussion

Species of Bacillus genus have been isolated from diverse habitats and *some Bacillus e.j. Bacillus subtilis,* B. *atrophaeus* , had been successfully used for degradation of crude oil, especially *n*-alkanes [4], and this can be produced by biosurfactants [3] which can stimulate crude oil degradation by enhancing the bioavailability of hydrocarbons. Based on genotypic analysis and chemotaxonomic data, several spore-forming species in the Bacillus rRNA were reclassified into the following novel taxa like as *Lysinibacillus* [2]. The fatty acid profile is characterized by high proportions of saturated branched fatty acids, in such amounts as 15:0 17:0 found between strains and its closest relatives and these characteristics could be used to differentiate the novel isolated specimen from related species [2]. This work report *L. sphaericus* as a microorganism with the ability to degrade petroleum hydrocarbons in Patagonia sludge oil. The grown in medium with petroleum distillation fraction mixtures as the sole source of carbon and energy results in the field demonstrate the potential for biotechnological applications in contaminated soils in our zone, its ability to produce biosurfactants, and its capacity to persist in the environment as it is a sporulated microorganism[6]. The recent sequencing of *L. sphaericus* Ot4b31 and CBAM5 genome [7] analysis identified proteins that might participate in peripheral pathways for degradation of benzoate, amino benzoate, toluene, and other aromatic compounds, which might be an indication of the ability of these genera to biodegrade TPH. The results show that hydrocarbon biodegradation of oil sludge by the native microbiota on the sediment is feasible.

References

[1] Acuña A, Pucci GN, Morales MJ, Pucci OH. Biodegradation of petroleum and its derivatives by the bacterial community in the soil of the Argentinean Patagonia. Revista Sociedad Venezolana de Microbiología. Sociedad Venezolana de Microbiología. 2010,30:29-36.

[2] Ahmed, I., Yokota, A., Yamazoe, A. & Fujiwara, T. Proposal of *Lysinibacillus boronitolerans gen. nov. sp. nov*., and transfer of *Bacillus fusiformis* to *Lysinibacillus fusiformis comb. nov. and Bacillus sphaericus to Lysinibacillus sphaericus* comb. nov. Int J Syst Evol Microbiol 2007,57: 1117–1125.

[3] Becerra, L., & Horna, M. (2016). Isolation of biosurfactant producing microorganisms and lipases from wastewaters from slaughterhouses and soils contaminated with hydrocarbons. *Scientia Agropecuaria*,2016, *7*(1): 23-31.

[4] Das K, A.K. Mukherjee. Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East India. Bioresour. Technol., 98 (2007), pp. 1339-1345

[5] Hamed, S., Maaroufi, A., Ghram, A., & Zouhai, B. Isolation of four hydrocarbon effluent-degrading *Bacillaceae* species and evaluation of their ability to grow under high-temperature or high-salinity conditions. African Journal of Biotechnology, 2013, 12 (14), 1636–1643.

[6] Manchola, L., & Dussán, J. *Lysinibacillus sphaericus* and *Geobacillus* sp biodegradation of petroleum hydrocarbons and biosurfactant production. Remediation Journal, 2014,25(1), 85-100.

[7] Peña-Montenegro, T. D., & Dussán, J. Genome sequence and description of the heavy metal tolerant bacterium *Lysinibacillus sphaericus* strain OT4b. 31. Standards in genomic sciences, 2013,*9*(1), 42.

[8] Pucci GN, Tiedemann MC, Acuña A & Pucci O. Change in Bacterial diversity after oil spill in Argentina. The Importance of Biological Interactions in the Study of Biodiversity. Editorial Intech. Croacia. ISBN 978-953-307-751-2. Chapter 6. Pág. 91-108. Septiembre de 2011.

[9] Reasoner, D. J., & Geldreich, E. E. A new medium for the enumeration and subculture of bacteria from potable water. Appl. Environ. Microbiol., 1985,*49*(1), 1-7.

[10] Sajna, K.V., Sukumaran, R.K., Gottumukkala, L.D., Pandey, A.. Crude oil biodegradation aided by biosurfactants from *Pseudozyma sp*. NII 08165 or its culture broth. Bioresour. Technol. 2015,191, 133–139.

Table 1: Bacterial identifications and their OD value at 600 in 20 days

Microorganism	Hexadecane	Ciclohexane	Kerosene	Diesel oil
Arthrobacter-oxydans	0,33	0,63	0,35	0,23
Bacillus-GC group 22	0,13	0,53	0,39	0,42
Bacillus sp.	0,34	0,25	0,81	0,85
Bacillus sp.	0,17	0,47	0,38	0,39
Bacillus sp.	0,04	0,12	0,27	0,64
Bacillus sp.	0,18	0,2	0,06	0,25
Bacillus sp.	0,15	0,24	0,24	0,30
Bacillus sp.	0,25	0,12	0,33	0,33
Bacillus sp.	0,17	0,18	0,14	0,54
Bacillus-GC group 22	0,2	0,33	0,28	0,38
Bacillus-GC group 22	0,21	0,28	0,29	0,21
Bacillus-simplex	0,23	0,63	0,3	0,42
Lysinibacillus sphaericus	0,17	0,41	0,41	0,37
Lysinibacillus sphaericus	0,21	0,67	0,51	0,42
Lysinibacillus sphaericus	0,42	0,46	0,37	0,47
Lysinibacillus sphaericus	0,34	0,49	0,38	0,25
Lysinibacillus sphaericus	0,08	0,53	0,38	0,44

Paenibacillus-polymyxa	0,15	0,53	0,18	0,6
Sin identificación	0,07	0,08	0,12	0,25
Staphylococcus-cohnii-cohnii	0,06	0,36	0,46	0,48

Author Profile

Gabriel Pilch is Graduate in Sanitation and environmental protection and woks in oil company.

Dra. Graciela Natalia Pucci Professor of General Microbiology and Environmental Microbiology at the National University of Patagonia San Juan Bosco. Director of the centers of studies and research in applied microbiology (CEIMA)