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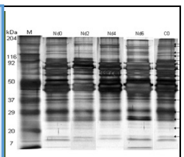

SDS-PAGE of N-nitrosodiethylamine (NDEA) treated protein samples of chicken egg embryos

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A Mini Review on the Biodegradation of Petroleum Hydrocarbon Pollutants by the Genus *Rhodococcus*

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ABSTRACT

The genus *Rhodococcu*s is a very diverse group of Eubacteria that are commonly found in many environmental niches such as soils and seawaters likewise. Owed to their remarkable range of varied catabolic gene and robust cellular physiology, this genus is competent to degrade a great number of organic compounds including some of the most difficult compounds such as petroleum hydrocarbons. This review discusses two interrelated topics, which are the petroleum hydrocarbons pollution in the environment and the utilization of the *Rhodococcus* genus in microbial remediation of petroleum hydrocarbons and its derivatives. The first part focuses on the application of hydrocarbon components in standard routine, sources of pollution and the toxicity of these compounds. On the second fraction of this assessment, the biochemistry, physiology and genetic versatility of the genus *Rhodococcus* will be reviewed with emphasizing their significance in environmental biotechnology and bioremediation of petroleum hydrocarbons.

INTRODUCTION

Crude oil and other petroleum derivatives produced from fossil fuels are refined into petroleum products that have been used for many different purposes. They are also one of the principal sources of energy for industry and daily life. One of the examples is biofuels, which generally used in petroleum-based fuels such as gasoline and diesel. Other examples of the petroleum-based product are the heating oil and propane. Heating oil is commonly used for power plants generation, and heating applications such as boilers and furnaces to heat homes and buildings [1]. In spite of this, the release of these hydrocarbons components into the environment whether through accidents or improper discharge is reported to be the main sources of pollution that occur in both soil and aqueous systems [2]. As a result of this pollution, the potential for hydrocarbons accumulation within animal and plant tissues may rise and may instigate toxic effects and even death [3].
Consequently, the requirement for removal of these hazardous compounds is essential. Several removal methods have been proposed ranging from the physical, chemical and biological method. Bioremediation technology emerged as among the best technologies offered at present due to several valuable qualities such as cost-efficient, non-invasive, and utilize natural resources such as microorganisms and plants as the bioremediation agent [4–6].

Petroleum pollution

Petroleum and its derivatives, albeit not categorised as xenobiotic, can be considered as one of the major substances that cause contamination. Petroleum hydrocarbon contamination of water bodies such as lakes and estuaries have detrimental effects on aquatic biota and water quality [7]. The petroleum industry is a major industrial concern and widely considered among the most severe polluter from 130 industrial branches in the whole world. It has contributed to the largest scale environmental pollutant and the most dangerous contaminant in biosphere due to the wide use of petroleum products that has grown tremendously in the last century [5,8].
Since the last two centuries, amounts of disposed of oils in the environments have been intensified, and these issues have received increasing attention and concern around the world. According to Gallego et al. [9], among the oil pollutants, diesel oil is frequently reported to pollute soil, ground water and

marine environment. The cause of this pollution involves the seepage of underground storage tanks and tanker trucks, accidental spills during transportation and pipeline leakage [10]. Leakage of storage tanks originates from corrosion of old bare steel tanks and the connecting pipes or lines, improper connection and incompatibility between pipes and their fittings, or shifting of the piping after installation, and pump failures.
Another major source of oil pollution ensues from a variety of anthropogenic activities that includes manufacturing, machinery-based production and illegal disposal of petroleum products [11]. This rising issue regarding leakage and contamination of the surrounding environment might be the main reason for the losses of millions of dollars yearly to the petroleum industries.

The fate of petroleum hydrocarbons may occur differently in different systems. For instance, in the soil system, petroleum that spilt onto the soil will break down into many different compounds. Some of this compound may evaporate, and some may dissolve in ground water, far from the release point. Some compounds will stay in, and the attachment of oil particles in the soil may cause the toxic components to stay in the soil for long periods of time, while others will be broken down by organisms found in the soil [12]. Oil pollution is likely to remain a significant hazard to the organism, ecosystem and industry for many more years even after the spill occur [13]. This is because hydrocarbons can persist for ages in one environment [14].

Toxicity of petroleum hydrocarbons
Petroleum hydrocarbons are highly complex compounds chemically and physically that rich in carbon atoms that can pose a risk to human health and ecological receptors if discharged into the natural environment. The large quantities of hydrocarbon present in the environment must be removed from the contaminated sites because of their toxicity [15] and their potential to disturb the structure and functioning of the natural ecosystem [16]. Petroleum hydrocarbons, containing toxic components, become stable and recalcitrant in the subsurface. These properties make their efficient removal considerably difficult.

The compounds in different petroleum fractions affect the human body in different ways. BTEX (benzene, toluene, ethylbenzene, and xylene) compounds can affect the human central nervous system, and several reported cases can cause death if exposed to high concentration [17]. Inhalation of high concentration toluene of about 100 part per million in a short period can cause fatigue, headache, nausea and drowsiness. Once the exposure is stopped, the symptom will go away. However, exposure for a long period can damage the central nervous system permanently [18]. Hexane, a six-carbon short-length alkane can also affect the central nervous system although in a dissimilar fashion. The main symptoms that associated with hexane toxicity are the numbness in the feet and legs and severe reports may cause a particular type of paralysis known as peripheral neuropathy [19].

Ingestion of such petroleum hydrocarbons like gasoline, kerosene or diesel will cause throat and stomach irritation, depression of the central nervous system, breathing difficulties and related symptoms such as pneumonia. Furthermore, some tissues and organs such as blood, liver, spleen, kidneys, lungs and development of foetus will also be affected by some of the petroleum compounds. Certain hydrocarbon compounds can be irritating to the skin and eyes. However, other hydrocarbon compounds such as some mineral oil, are not very toxic and are used in foods [18].

In the marine environment, the direct or indirect contact of petroleum hydrocarbons and its derivatives to the aquatic organism can act as a mediator in the generation of free radicals in fish which also increase in antioxidant defence in an animal after exposure [20]. Crab, shellfish and mussels can be tainted from the small oil spill in shallow shore areas [21]. According to the similar report, the first larvae stage and fish eggs will be damaged and cannot achieve the next larval stage even with the exposure at a low concentration (0.1 mg/L diesel oil). The untreated contamination of hydrocarbon will accumulate and damage the ecosystem. The accumulation of hydrocarbon in animal and plant tissues will produce a mutant young and eventually death of progeny [22].

Microbial degradation of petroleum hydrocarbons
Bioremediation technologies employ the participation of microbial species to break down the noxious waste to a less harmful subsequent product thus purifying the site. Microbial flora and fauna have their particular fundamental role in the natural ecosystem. Owed to their capability to rapidly and adeptly purify contaminated sites, this microorganism has the competence to thrive in constricting settings such as industrially polluted soils and water. Indirectly, this will help in protecting the environment and human well-being [23].

Recent years have shown an increase in the microbial remediation reports as this approach is seen as the most sustainable method due to their rare prospects in the termination the pollutants [24]. Bacterial species have been seen as the most prevalent microorganisms in petroleum hydrocarbon degradation as they play the most part in the degradation of the hydrocarbon pollutants [6,10,25]. One of the engaging bacterial groups in petroleum hydrocarbons degradation is from the genus *Rhodococcus* because of their superior qualities.

***Rhodococcus* spp.**
The genus of *Rhodococcus* is a very diverse group. A frequent microbial inhabitants of the marine, freshwater and soils community, *Rhodococcus* is Gram-positive bacteria belonging to the family Nocardiaceae, in the suborder of the Corynebacterineae [26,27]. Other well-known families of this suborder include the Mycobacteriaceae (*Mycobacterium* genus) and Corynebacteriaceae (*Corynebacterium* genus) [27].

After Zopf first proposed the genus *Rhodococcus* in 1891, there are many environmental isolates that have been formally placed in this genus [28]. The name Rhodo (derived from the Greek term, rhodon, meaning red or rose) and coccus (a New Latin term derived from Greek, kokkos, meaning grain or seed) have persisted. Although the name of the genus indicates that the cells of these bacteria are likely to be cocci, the basic phenotypic characteristic of rhodococci is pleomorphic Gram-positive cell. The cell may grow as short rod-shaped or cocci or multinucleated filaments [29,30]. The colonies form on agar plates observed to be small and ‘‘dry.’’ Some of this genus produce pale red and orange pigmentation, not only on minimal agar but also on a richer nutrient medium.

The members of this genus are autochtonous when they are very persistence in the environment even though they show a slow growth rate. *Rhodococcus* also indigenous in many polluted sites, therefore it is very suitable to be used as bioremediator [31]. Besides, the absence of catabolic repression in *Rhodococcus* ensures that the pollutants like hydrocarbons would be degraded even in the presence of more easily assimilable carbon sources [32]. *Rhodococcus* strains are efficient degraders of pollutant as they can degrade many organic compounds, able to produce surfactants, and their environmental persistence makes them ideal candidates for enhancing the bioremediation of contaminated sites. The importance of *Rhodococcus* spp. Is reflected in increasing reports of their biodegradative capabilities in the literature. The rhodococci appear to survive in many toxic substrates and solvents, which are highly toxic for other organisms. They are capable of using all of these compounds as a source of carbon and energy in the study by Lee et al.[33], the investigated *Rhodococcus* strain can efficiently mineralize 3,5-dichloroaniline of vinclozolin, a very toxic metabolite. Other toxic compounds that can be tolerated by this genus are dodecane, bis(2-ethylhexyl) phthalate, and toluene, up to 5% v/v. They can tolerate up to 50% v/v of ethanol, butanol, and dimethylformamide [34].

The robustness of this genus has been observed to tolerate up to 80% (v/v) toluene and 50% (v/v) ethanol [35] which clearly cannot be endured by most bacterial genera. There are also rhodococci from contaminated groundwater have been observed to be tolerant of up to 1 g/L of benzene [31]. This evidence and observation indicated the rhodococci might be important in mediating the environment in which the concentration of pollutants is particularly high. *Rhodococcus* possesses a very versatile metabolic capability. Therefore, it has an ability to degrade a wide range of xenobiotic compound including some of the most complex compounds that are considered recalcitrant and toxic. They appear to have adopted a hyper-recombination evolutionary strategy that relies upon the acquisition and storage of many genes to deploy as recombination substrate upon adaptation [36].

In general, the genus of Rhodococcus appears proficient of surviving in harsh environments. LeBlanc et al. [37] observed that there are many *Rhodococcus* cell that survive desiccation for long periods and this provides scope for deploying them for remediation in dry environments. The studies by Alvarez [38] also revealed that *Rhodococcus opacus* possesses protection mechanisms to withstand desiccation. This involves a wide range of physiological responses, including the ability of the rhodococci cells to tightly regulate its energy-producing system, among others. This species not only can survive under dry conditions, but they also able to withstand and show metabolic respond in the presence of increased hexadecane vapour. However, they exhibit limited growth on the hydrocarbon and under conditions of low water availability.

Besides the practicality of this genus, *Rhodococcus* genus does include several pathogenic species. One of the most common examples is *Rhodococcus equi. Rhodococcus equi* causes chronic bronchopneumonia and enteritis in horses and infects immunosuppressed human [39]. Aside from *Rhodococcus equi*, most Rhodococcus species are non-pathogenic with the exception of the well-characterized plant pathogen *Rhodococcus fascians* [40].

With regards to the petroleum hydrocarbons degradation, several common features of the *Rhodococcus* genus can be highlighted that contributed to the excellence of the genus to degrade this recalcitrant compound. Firstly, the degree of alkane compounds mineralization is quite expansive due to the presence of multiple alkane hydroxylase systems in their cells [41–43]. Secondly, several strains of *Rhodococcus* species that able to degrade ranges of aromatic compounds were also reported [44–46] indicating a full potential of total degradation of petroleum hydrocarbons, which composed mostly of both aliphatics and aromatics. Last but not least, a number of Rhodococcus species was able to produce surface active agents or biosurfactants [47–49] that assist in increasing the rate of petroleum hydrocarbons degradation. **Table 1** shows the list of hydrocarbon-degrading *Rhodococcus* species.

Table 1: Hydrocarbon-degrading strains from the genus *Rhodococcus*.

Strain	Comments	Reference(s)
Rhodococcus spp.	Groups of *Rhodococcus* spp. isolated from both Arctic and Antarctic regions were able to grow on selective media supplemented with different hydrocarbons	[50]
Rhodococcus sp.	Strain was able to degrade anthracene (53%), phenanthrene (31%), pyrene (13%) and fluoranthene (5%) as sole carbon source, but negative results were observed in naphthalene and chrysene	[51]
Rhodococcus sp.	Isolated from oil-contaminated soil from Newfoundland and utilize numerous aromatic compounds as both carbon and energy	[52]
Rhodococcus sp. 5/14	Strain was reported to degrade hexane, eicosane and pristine at -2°C but viable count reduced when temperature increase to 37°C	[53]
Rhodococcus sp. B11/B15	Both psychrotolerant strain isolated from the Terra Nova Bay, Ross Sea, Antarctica were able to degrade commercial diesel oil as their sole organic substrate. Both strains also showed the ability to utilize C_{28} *n*-paraffin octacosane and polychlorinated biphenyls (PCBs) at 20°C	[54]
Rhodococcus sp. DCB-p0610	The phenol-degrading bacterium was tested in a continuous degradation of phenolic substances through immobilization on granular activated carbon and Ca-alginate	[55]
Rhodococcus sp. DK17	Strain shows ability to utilize *o*-xylene as sole carbon source, but displays negative growth on *m*- and *p*-xylene. Mutant strain was made from the strain to test the loss of oxygenases	[45]
Rhodococcus sp. DM1-21	Strain was able to degrade the middle-chain alkanes, crude oil and hydrocarbon vapours	[56]
Rhodococcus sp. EC1	Cyclohexane-degrading strain that optimally degrade the substrate at 25-35°C and pH 6-8. The strain was also reported to be able to mineralize alcohols and PAHs	[57]
Rhodococcus sp. MK1	The hydrocarbon-degrading capability of the strain owed to the founding of numerous routes of oxidative genes for aliphatic and aromatic compounds in the strain's draft genome	[58]
Rhodococcus sp. Q15	A psychrotrophic strain that able to mineralize short alkane-chain at 5°C, and middle- and long-chain alkanes when temperature increased to 20°C	[59]
Rhodococcus sp. RHA1	Potent polychlorinated biphenyl-degrading actinomycetes	[60]
Rhodococcus sp. UKMP-5M	The strain was isolated from petroleum-contaminated sites. The strain was tested for phenol-degrading ability with positive outcomes. The optimized growth condition and phenol mineralization for the bacterium were 30°C, pH 7.5, glucose concentration of 2 g/L, 0.4 g/L of ammonium sulphate as nitrogen source, and 0.9 g/L of phenol	[61]
Rhodococcus aetherivorans IFP 2017	Isolated from petroleum hydrocarbon mixture. Strain have wide mineralization capacities towards hydrocarbon components including	[62]

	the more recalcitrant ones (BTEX)	
Rhodococcus baikonurensis EN3	The actinomycete was isolated from hydrocarbon-contaminated sites. Strain was tested for the effect of mycolic acid (2-hexyl-3-hydroxydecanoic acid) on diesel oil biodegradation. Mycolic acid enhances the biodegradation of diesel in all tested concentration	[63]
Rhodococcus equi	Several strains from this species was isolated and tested for the competency to degrade hexadecane. Study showed a positive interfacial uptake of hexadecane due to the rhodococci cell flocculation	[64]
Rhodococcus erythropolis M1	Used as co-culture along with *Pseudomonas fluorescens* p1 to remove chlorophenols derivatives such as 2-chlorophenol, phenol and *ρ*-cresol	[65]
Rhodococcus erythropolis PR4	The most prominent example of hydrocarbon-degrading strain in topical study. The whole genome of the strain was sequenced and numerous related genes in regards to hydrocarbon degradation was found	[66–69]
Rhodococcus erythropolis T7-2	The psychrotolerant strain was isolated from oil-polluted seabed mud of Bohai Sea, China. In the study, addition of nutrient to the artificial seawater increases the biodegradation rate from 12.61% to 75% within 1 week	[70]
Rhodococcus erythropolis UPV-1	Phenol-degrading strain tested for potential immobilization in Biolite in a packed-bed reactor	[71]
Rhodococcus fascians	The strain was reported to degrade diesel in sand column. The optimal degradation of the substrate by the isolate was at pH 8 with flow rate of air of 30 ml/min. Diesel degradation was also reported to increase when larger inoculum size was used while no growth inhibition occurred up to 5% of diesel	[72]
Rhodococcus pyridinivorans NT2	Study was conducted to optimize the 4-nitrotoluene biodegradation condition by the strain using modelling and kinetics study	[73]
Rhodococcus ruber 219	Previously assigned as Rhodococcus sp. 219, the newly assigned strain was able to degrade tetrahydrofuran, 2,5-dimethyltetrahydrofuran and tetrahydropyran	[74]
Rhodococcus ruber AC239	Potential producer of surface active agent due to the attainment of 63% emulsifying index for a diesel-water binary system	[75]
Rhodococcus wratislaviensis IFP 2016	Isolated from a consortium containing hydrocarbon mixture. Although reported to be able to degrade wide range of hydrocarbons, the presence of BTEX had a detrimental effect on ethyl *tert*-butyl ether (ETBE) and methyl *tert*-butyl ether (MTBE) mineralization, while presence of octane shows higher percentage of MTBE degradation	[62]

CONCLUSIONS

The wide range of hydrocarbon pollutants mineralized by the *Rhodococcus* species proved that this exceptional genus is a potential hydrocarbon scavenger for the practical use in microbial remediation of hydrocarbon substrates. The genus aptitude in catabolizing the noxious waste is largely owed to their substantial physiological and biotechnological characteristics, large genomes with numerus sets of genes encoding arrays of catabolic pathways, and their availability in most environmental niche. Although the genus' uniqueness is considered as significant for hydrocarbon removal, the actual potential of this genus in the uncontrollable environment cannot be predicted. For example, the expressions of vital catabolic genes during restricting condition may be overlapping (aliphatics catabolic pathways and benzoate degradation) between one another thus causing repression of the genes and overall degradation. Hence, a more detailed information and data in regard to the physiological, biochemical and molecular mechanism of the rhodococci cells in needed to improve our understanding on the principal mechanisms and function of this distinctive genus.

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REFERENCES

1. U.S. Energy Information Administration (EIA). Use of oil - energy explained, your guide to understanding energy [Internet]. [cited 2017 Dec 29]. Available from: https://www.eia.gov/energyexplained/index.cfm?page=oil_use
2. Holliger C, Gaspard S, Glod G, Heijman C, Schumacher W, Schwarzenbach RP, et al. Contaminated environments in the subsurface and bioremediation: organic contaminants. FEMS Microbiol Rev. 1997 Jul 1;20(3–4):517–23.
3. Alvarez PJ, Vogel TM. Substrate interactions of benzene, toluene, and para-xylene during microbial degradation by pure cultures and mixed culture aquifer slurries. Appl Environ Microbiol. 1991;57(10):2981–2985.
4. April TM, Foght JM, Currah RS. Hydrocarbon-degrading filamentous fungi isolated from flare pit soils in northern and western Canada. Can J Microbiol. 1999;46(1):38–49.
5. Ulrici W. Contaminated soil areas, different countries and contaminants, monitoring of contaminants. Biotechnol Set Second Ed. 2000;5–41.
6. Leahy JG, Colwell RR. Microbial degradation of hydrocarbons in the environment. Microbiol Rev. 1990;54(3):305–315.
7. National Research Council (US) Committee. Oil in the Sea III: Inputs, Fates, and Effects. Washington (DC): National Academies Press (US); 2003.
8. Mearns AJ. Cleaning oiled shores: putting bioremediation to the test. Spill Sci Technol Bull. 1997;4(4):209–217.
9. 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 Sep 1;12(5):325–35.
10. Aislabie JM, Balks MR, Foght JM, Waterhouse EJ. Hydrocarbon spills on Antarctic soils: effects and management. Environ Sci Technol. 2004 Mar 1;38(5):1265–74.
11. Mukherji S, Jagadevan S, Mohapatra G, Vijay A. Biodegradation of diesel oil by an Arabian Sea sediment culture isolated from the vicinity of an oil field. Bioresour Technol. 2004 Dec;95(3):281–6.
12. Norris RD. Handbook of bioremediation. CRC Press; 1993.
13. McKew BA, Coulon F, Osborn AM, Timmis KN, McGenity TJ. Determining the identity and roles of oil-metabolizing marine bacteria from the Thames estuary, UK. Environ Microbiol. 2007 Jan;9(1):165–76.
14. Kanaly RA, Harayama S. Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria. J Bacteriol. 2000;182(8):2059–2067.
15. Cavalca L, Di Gennaro P, Colombo M, Andreoni V, Bernasconi S, Ronco I, et al. Distribution of catabolic pathways in some hydrocarbon-degrading bacteria from a subsurface polluted soil. Res Microbiol. 2000;151(10):877–887.
16. Çavaş T, Ergene-Gözükara S. Micronucleus test in fish cells: a bioassay for *in situ* monitoring of genotoxic pollution in the marine environment. Environ Mol Mutagen. 2005;46(1):64–70.
17. Pohanish RP. Sittig's handbook of toxic and hazardous chemicals and carcinogens. William Andrew; 2017. 3564 p.

18. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for total petroleum hydrocarbons (TPH). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 1999.
19. Sendur OF, Turan Y, Bal S, Gurgan A. Toxic neuropathy due to *n*-hexane: report of three cases. Inhal Toxicol. 2009;21(3):210–214.
20. Achuba FI, Osakwe SA. Petroleum – induced free radical toxicity in African catfish (*Clarias gariepinus*). Fish Physiol Biochem. 2003 May 1;29(2):97.
21. King AJ, Readman JW, Zhou JL. Determination of polycyclic aromatic hydrocarbons in water by solid-phase microextraction–gas chromatography–mass spectrometry. Anal Chim Acta. 2004;523(2):259–267.
22. Abdel-Shafy HI, Mansour MSM. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egypt J Pet. 2016 Mar 1;25(1):107–23.
23. Krastanov A, Alexieva Z, Yemendzhiev H. Microbial degradation of phenol and phenolic derivatives. Eng Life Sci. 2013 Jan 1;13(1):76–87.
24. Das N, Chandran P. Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnol Res Int. 2011;1–13.
25. Floodgate GD. Microbial degradation of oil. Mar Pollut Bull. 1972 Mar 1;3(3):41–3.
26. Stackebrandt E, Rainey FA, Ward-Rainey NL. Proposal for a new hierarchic classification system, actinobacteria classis nov. Int J Syst Evol Microbiol. 1997;47(2):479–91.
27. Goodfellow M. The Family Nocardiaceae. In: The Prokaryotes: Actinobacteria. Berlin: Springer; 2014. p. 595–650.
28. Gürtler V, Mayall BC, Seviour R. Can whole genome analysis refine the taxonomy of the genus *Rhodococcus*? FEMS Microbiol Rev. 2004;28(3):377–403.
29. Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG. The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria. Springer Science & Business Media; 2013. 1276 p.
30. Whitman WB, Goodfellow M, Kämpfer P, Busse H-J, Trujillo ME, Ludwig W, et al. Bergey's Manual of Systematic Bacteriology: Volume 5: The Actinobacteria. Springer Science & Business Media; 2012. 2102 p.
31. Warhurst AM, Fewson CA. Biotransformations catalyzed by the genus *Rhodococcus*. Crit Rev Biotechnol. 1994;14(1):29–73.
32. Quek E, Ting Y-P, Tan HM. *Rhodococcus* sp. F92 immobilized on polyurethane foam shows ability to degrade various petroleum products. Bioresour Technol. 2006;97(1):32–38.
33. Lee J-B, Sohn H-Y, Shin K-S, Kim J-S, Jo M-S, Jeon C-P, et al. Microbial biodegradation and toxicity of vinclozolin and its toxic metabolite 3, 5-dichloroaniline. J Microbiol Biotechnol. 2008;18(2):343–349.
34. de Carvalho CC, da Fonseca MMR. Solvent toxicity in organic-aqueous systems analysed by multivariate analysis. Bioprocess Biosyst Eng. 2004;26(6):361–375.
35. de Carvalho CC, Fatal V, Alves SS, Da Fonseca MMR. Adaptation of *Rhodococcus erythropolis* cells to high concentrations of toluene. Appl Microbiol Biotechnol. 2007;76(6):1423.
36. Larkin MJ, Kulakov LA, Allen CC. Biodegradation and *Rhodococcus* – masters of catabolic versatility. Curr Opin Biotechnol. 2005 Jun 1;16(3):282–90.
37. LeBlanc JC, Gonçalves ER, Mohn WW. Global response to desiccation stress in the soil actinomycete *Rhodococcus jostii* RHA1. Appl Environ Microbiol. 2008;74(9):2627–2636.
38. Alvarez HM. Relationship between *β*-oxidation pathway and the hydrocarbon-degrading profile in actinomycetes bacteria. Int Biodeterior Biodegrad. 2003;52(1):35–42.
39. Kedlaya I, Ing MB, Wong SS. *Rhodococcus equi* infections in immunocompetent hosts: case report and review. Clin Infect Dis. 2001;32(3):e39–e46.
40. Goethals K, Vereecke D, Jaziri M, Van Montagu M, Holsters M. Leafy gall formation by *Rhodococcus fascians*. Annu Rev Phytopathol. 2001;39(1):27–52.
41. Aggarwal RK, Dawar C, Phanindranath R, Mutnuri L, Dayal AM. Draft genome sequence of a versatile hydrocarbon-degrading bacterium, *Rhodococcus pyridinivorans* strain KG-16, collected from oil fields in India. Genome Announc. 2016 Feb 11;4(1).
42. Nie Y, Chi C-Q, Fang H, Liang J-L, Lu S-L, Lai G-L, et al. Diverse alkane hydroxylase genes in microorganisms and environments. Sci Rep. 2014;4.
43. Whyte LG, Smits THM, Labbé D, Witholt B, Greer CW, van Beilen JB. Gene cloning and characterization of multiple alkane hydroxylase systems in *Rhodococcus* strains Q15 and NRRL B-16531. Appl Environ Microbiol. 2002 Dec;68(12):5933–42.
44. Rast HG, Engelhardt G, Wallnöfer PR. Degradation of aromatic compounds in the actinomycete-genus *Rhodococcus*. FEMS Microbiol Lett. 1980 Jan 1;7(1):1–6.
45. Kim D, Kim Y-S, Kim S-K, Kim SW, Zylstra GJ, Kim YM, et al. Monocyclic aromatic hydrocarbon degradation by *Rhodococcus* sp. strain DK17. Appl Environ Microbiol. 2002 Jul;68(7):3270–8.
46. Egorova DO, Korsakova ES, Demakov VA, Plotnikova EG. Degradation of aromatic hydrocarbons by the *Rhodococcus wratislaviensis* KT112-7 isolated from waste products of a salt-mining plant. Appl Biochem Microbiol. 2013 May 1;49(3):244–55.
47. Kuyukina MS, Ivshina IB, Philp JC, Christofi N, Dunbar SA, Ritchkova MI. Recovery of *Rhodococcus* biosurfactants using methyl tertiary-butyl ether extraction. J Microbiol Methods. 2001 Aug;46(2):149–56.
48. Peng F, Liu Z, Wang L, Shao Z. An oil-degrading bacterium: *Rhodococcus erythropolis* strain 3C-9 and its biosurfactants. J Appl Microbiol. 2007 Jun 1;102(6):1603–11.
49. Pacheco GJ, Ciapina EMP, Gomes E de B, Pereira Junior N. Biosurfactant production by *Rhodococcus erythropolis* and its application to oil removal. Braz J Microbiol. 2010 Oct;41(3):685–93.
50. Malavenda R, Rizzo C, Michaud L, Gerçe B, Bruni V, Syldatk C, et al. Biosurfactant production by Arctic and Antarctic bacteria growing on hydrocarbons. Polar Biol. 2015 Oct 1;38(10):1565–74.
51. Dean-Ross D, Moody JD, Freeman JP, Doerge DR, Cerniglia CE. Metabolism of anthracene by a *Rhodococcus* species. FEMS Microbiol Lett. 2001 Oct 16;204(1):205–11.
52. Whalen MY, Armstrong SM, Patel TR. Characterization of a *Rhodococcus* species that utilizes numerous aromatics. Soil Biol Biochem. 1993 Jun 1;25(6):759–62.
53. Bej AK, Saul D, Aislabie J. Cold-tolerant alkane-degrading *Rhodococcus* species from Antarctica. Polar Biol. 2000 Jan 1;23(2):100–5.
54. De Domenico M, Giudice AL, Michaud L, Saitta M, Bruni V. Diesel oil and PCB-degrading psychrotrophic bacteria isolated from Antarctic seawaters (Terra Nova Bay, Ross Sea). Polar Res. 2004 Dec 1;23(2):141–6.
55. Pai S-L, Hsu Y-L, Chong N-M, Sheu C-S, Chen C-H. Continuous degradation of phenol by *Rhodococcus* sp. immobilized on granular activated carbon and in calcium alginate. Bioresour Technol. 1995 Jan 1;51(1):37–42.
56. Ruberto L, Vazquez S, Balbo A, Mac Cormack WP. Psychrotolerant hydrocarbon-degrading *Rhodococcus* strains isolated from polluted Antarctic soils. Antarct Sci. 2005 Mar 1;17:47–56.
57. Lee E-H, Cho K-S. Characterization of cyclohexane and hexane degradation by *Rhodococcus* sp. EC1. Chemosphere. 2008;71(9):1738–1744.
58. Kis ÁE, Laczi K, Zsíros S, Kós P, Tengölics R, Bounedjoum N, et al. Characterization of the *Rhodococcus* sp. MK1 strain and its pilot application for bioremediation of diesel oil-contaminated soil. Acta Microbiol Immunol Hung. 2017 Dec 1;64(4):463–82.
59. Whyte LG, Hawari J, Zhou E, Bourbonnière L, Inniss WE, Greer CW. Biodegradation of variable-chain-length alkanes at low temperatures by a psychrotrophic *Rhodococcus* sp. Appl Environ Microbiol. 1998 Jul;64(7):2578–84.
60. McLeod MP, Warren RL, Hsiao WWL, Araki N, Myhre M, Fernandes C, et al. The complete genome of *Rhodococcus* sp. RHA1 provides insights into a catabolic powerhouse. Proc Natl Acad Sci. 2006 Oct 17;103(42):15582–7.
61. Suhaila YN, Rosfarizan M, Ahmad SA, Abdul Latif I, Ariff AB. Nutrients and culture conditions requirements for the degradation of phenol by *Rhodococcus* UKMP-5M. J Environ Biol. 2013 May;34(3):635–43.
62. Auffret M, Labbé D, Thouand G, Greer CW, Fayolle-Guichard F. Degradation of a mixture of hydrocarbons, gasoline, and diesel oil additives by *Rhodococcus aetherivorans* and *Rhodococcus*

wratislaviensis. Appl Environ Microbiol. 2009 Dec 15;75(24):7774–82.
63. Lee M, Kim MK, Singleton I, Goodfellow M, Lee S-T. Enhanced biodegradation of diesel oil by a newly identified *Rhodococcus baikonurensis* EN3 in the presence of mycolic acid. J Appl Microbiol. 2006 Feb 1;100(2):325–33.
64. Bouchez-Naïtali M, Blanchet D, Bardin V, Vandecasteele JP. Evidence for interfacial uptake in hexadecane degradation by *Rhodococcus equi*: the importance of cell flocculation. Microbiol Read Engl. 2001 Sep;147(Pt 9):2537–43.
65. Goswami M, Shivaraman N, Singh RP. Microbial metabolism of 2-chlorophenol, phenol and *ρ*-cresol by *Rhodococcus erythropolis* M1 in co-culture with *Pseudomonas fluorescens* P1. Microbiol Res. 2005 Apr 25;160(2):101–9.
66. Urai M, Yoshizaki H, Anzai H, Ogihara J, Iwabuchi N, Harayama S, et al. Structural analysis of an acidic, fatty acid ester-bonded extracellular polysaccharide produced by a pristane-assimilating marine bacterium, *Rhodococcus erythropolis* PR4. Carbohydr Res. 2007 May 21;342(7):933–42.
67. Laczi K, Kis Á, Horváth B, Maróti G, Hegedüs B, Perei K, et al. Metabolic responses of *Rhodococcus erythropolis* PR4 grown on diesel oil and various hydrocarbons. Appl Microbiol Biotechnol. 2015 Nov;99(22):9745–59.
68. Bosello M, Zeyadi M, Kraas FI, Linne U, Xie X, Marahiel MA. Structural characterization of the heterobactin siderophores from *Rhodococcus erythropolis* PR4 and elucidation of their biosynthetic machinery. J Nat Prod. 2013;76(12):2282–2290.
69. Sekine M, Tanikawa S, Omata S, Saito M, Fujisawa T, Tsukatani N, et al. Sequence analysis of three plasmids harboured in *Rhodococcus erythropolis* strain PR4. Environ Microbiol. 2006 Feb;8(2):334–46.
70. Huang L, Ma T, Li D, Liang F, Liu R-L, Li G. Optimization of nutrient component for diesel oil degradation by *Rhodococcus erythropolis*. Mar Pollut Bull. 2008 Oct;56(10):1714–8.
71. Begoña Prieto M, Hidalgo A, Serra JL, Llama MJ. Degradation of phenol by *Rhodococcus erythropolis* UPV-1 immobilized on Biolite in a packed-bed reactor. J Biotechnol. 2002 Jul 17;97(1):1–11.
72. Moon J-H, Koo J-R, Yun H-S. Biodegradation of diesel by *Rhodococcus fascians* in sand column. KSBB J. 2011;26(1):1–6.
73. Kundu D, Hazra C, Chaudhari A. Enhanced degradation of 4-nitrotoluene by *Rhodococcus pyridinivorans* NT2: multivariate optimization of process parameters and evaluation of biokinetic parameters. Environ Eng Sci. 2016 Jul 19;33(9):705–18.
74. Bock C, Kroppenstedt RM, Diekmann H. Degradation and bioconversion of aliphatic and aromatic hydrocarbons by *Rhodococcus ruber* 219. Appl Microbiol Biotechnol. 1996;45(3):408–410.
75. Bicca FC, Fleck LC, Ayub MAZ. Production of biosurfactant by hydrocarbon degrading *Rhodococcus ruber* and *Rhodococcus erythropolis*. Rev Microbiol. 1999 Jul;30(3):231–6.