Enrichment, Isolation and phylogenetic identification of fluoranthene and nonylphenol-degrading bacteria

By

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DECLARATION

I, **LEHLOHONOLO BENEDICT QHANYA** (South African ID number: [redacted]), hereby certify that the dissertation submitted by me for the degree MASTER OF HEALTH SCIENCES IN BIOMEDICAL TECHNOLOGY, is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology (Free State). I hereby declare, that this research project has not been previously submitted before to any university or faculty for the attainment of any qualification. I further waive copyright of the dissertation in favour of the Central University of Technology (Free State).

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Dedication

This thesis is dedicated to my late

Grandmother

Puleng Elizabeth Qhanya

Mother

Selloane Jennifer Qhanya

and

Uncle

Tshepiso Palfrey Qhanya

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LIST OF ABBREVIATIONS

%	Percentage
µl	Micro-liter
16S rRNA	16 Subunit ribosomal ribonucleic acid
APEs	Alkylphenol ethoxylates
ATSDR	Agency for toxic substances and disease registry
BLAST	Basic local alignment search tool
BPA	Bisphenol-A
$CaCl_2$	Calcium chloride
CO_2	Carbon dioxide
CSIR	Council for scientific and industrial research
$CuSO_4$	Copper sulfate
CYP	Cytochrome P450 monooxygenases
DBP	Dibutyl phthalate
DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene
DDT	*o,p*'-dichlorodiphenyl-trichloroethane
DEHP	Diethylhexyl phthalate
DES	Diethylstilbestrol
DMSO	Dimethyl sulfoxide

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DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease enzyme
E. coli	*Escherichia coli*
e.g	For example
EDCs	Endocrine disrupting chemicals
ER	Estrogen receptor
$FeCl_3.6H_2O$	Iron(III) chloride hexahydrate
g	Gram
g/l	Grams per liter
gDNA	Genomic DNA
GEN	Genistein
H_3BO_3	Boric acid
IARC	International agency of research of cancer
IPCS	International programme on chemical safety
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogen phosphate
KI	Potassium iodide
LB	Luria broth
MEGA	Molecular evolutionary genetics analysis

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mg/l	Milli-gram per liter
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
Min	Minutes
ml	Milli-liters
mM	Milli-molar
MM	Minimal medium
$MnSO_4.H_2O$	Manganese sulfate
$Na_2HPO4.2H_2O$	Potassium phosphate monobasic
$Na_2MO_4.2H_2O$	Sodium molybdate dihydrate
NCBI	National center for biotechnology information
ng/L	Nanogram/liter
NH_4Cl	Ammonium chloride
NIEHS	National institute of environmental health sciences
NPEOS	Nonylphenol polyethoxylates
O_2	Oxygen
^oC	Degree celsius
PAHs	Polycyclic aromatic hydrocarbons
PCBs	Polychlorinated biphenyls
PCR	Polymerase chain reaction

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PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
ppb	Parts per billion
PVC	Polyvinyl chloride
RNase	Ribonuclease enzyme
rpm	Revolution per minute
TBBPA	Tetrabromobisphenol A
USEPA	United States environmental protection agency
WWT	Waste water treatment
$ZnSO_4.7H_2O$	Zinc sulfate heptahydrate

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CHAPTER 1

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) and Endocrine disrupting chemicals (EDCs) are man-made chemicals that cause cancer and alter the function of endocrine systems in both humans and wildlife, respectively. PAHs and EDCs are considered as one of the priority pollutants and world-wide research is on-going to develop bioremediation strategies to remove these toxic xenobiotics from environment. Understanding indigenous microorganisms is important to design efficient bioremediation strategies. However, much of the information available on PAHs and EDCs has been generated from developed regions. In this direction, recent studies revealed presence of different PAHs and EDCs in South African natural resources. However, to date, study on analysis of microorganisms capability to utilize/degrade EDCs has not been reported and studies on PAHs are scares from South Africa. Soil samples collected at the different coal-fired power stations in and around Mpumalanga province, South Africa was used for enriching microorganisms. Enrichment method employed for isolating fluoranthene (as a model compound for PAHs) or nonylphenol (as a model compound for EDCs) degrading microorganisms. Identification of microorganisms was carried out using 16S rRNA gene analysis. Phylogenetic analysis of isolates was carried out using MEGA5. For each substrate, six pure and distinct bacterial cultures were successfully enriched. Pseudomonas dominated the strains enriched on nonylphenol, with 5 of the 6 isolates belonging to this genus. All four of these isolates however belong to different species. Highest diversity observed when fluoranthene was used as a carbon source. Strains of *Pseudomonas*, *Stenotrophomonas*, *Cupravidus* and *Ochrobactrum* were isolated using fluoranthene as a carbon source. Study results are the beginning of identification of microorganisms capable of degrading carcinogenic and endocrine disruptors and pave the way for exploring PAHs and EDCs degrading

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microorganisms from South Africa. An article on EDCs utilization organisms and their capability to degrade nonylphenol is submitted to South African Journal of Sciences. Here the details:

- Qhanya LB et al. (2016) Isolation and characterization of endocrine disruptor nonylphenol-using bacteria from South Africa. SAJS-2016-0287 (under review).

Apart from my Masters study, I also supervised four B. Tech student projects and managed to publish an article with students. Furthermore, I also worked on few bioinformatics projects and earned co-authorship in two manuscripts listed below:

- Parvez M, **Qhanya LB**, Mthakathi NT, Kgosiemang IKR, Bamal HD, Pagadala NS, Xie T, Yang H, Chen H, Theron CW, Monyaki R, Raselemane SC, Salewe V, Mongale BL, Matowane RG, Abdalla SMH, Booi WI, van Wyk M, Olivier D, Boucher CE, Nelson DR, Tuszynski JA, Blackburn JM, Yu J-H, Mashele SS, Chen W, Syed K. (2016) Molecular evolutionary dynamics of cytochrome P450 monooxygenases across kingdoms: Special focus on mycobacterial P450s. Scientific Reports | 6:33099 | DOI: 10.1038/srep33099.
- **Qhanya LB**, Matowane G, Chen W, Sun Y, Letsimo EM, Parvez M, Yu J-H, Mashele SS, Syed K. (2015) Genome-wide annotation and comparative analysis of cytochrome P450 monooxygenases in basidiomycete biotrophic plant pathogens. PLoS ONE 10(11): e0142100. doi:10.1371/journal.pone.0142100.
- Sello MM, Jafta N, Nelson DR, Chen W, Yu J-H, Parvez M, Kgosiemang IKR, Monyaki R, Raselemane SC, **Qhanya LB**, Mthakathi NT, Mashele SS, Syed K. (2015) Diversity and evolution of cytochrome P450 monooxygenases in Oomycetes. Scientific Reports 07/2015; 5. DOI:10.1038/srep11572 · (Discovered novel P450 fusion protein).

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In addition to the above credits, I was featured on national TV and in newspapers for discovering a novel drug target. I also presented work at both national and international (Canada) conferences.

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CHAPTER 2

INTRODUCTION AND LITERATURE REVIEW

2.1. Introduction to environmental pollution

Pollution has enormous effects on earth's environment. Chapman *et al.*, (2003) defines pollution as contamination that may result in an adverse biological alteration of the natural environment. Water, air and soil pollution have been categorised as the major types of pollution (Chapman *et al.*, 2003). Pollution can be caused by both natural and manmade factors. Mines, industries, farms and urban settlements are the main contributors to heavily polluted water bodies like the rivers and lakes (Cai *et al.*, 2016). Accumulation of heavy metals and metalloids such as arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn) in soil is also regarded as pollution (Bortey-Sam *et al.*, 2015). Natural disasters such as volcanic ash also contribute to the ever growing concerns of environmental pollution (WHO, 2010).

Pollution is a worldwide problem and its potential to influence the physiology of human populations is great. Exposure to air pollution is heavily linked to cause lung cancer and more adverse effects on human (Yang *et al.*, 2016). Prenatal growth is one of the detrimental effects pollutants have on human growth (Schell *et al.*, 2006).

Environmental pollution is one of the greatest problems this world is currently facing. The fact of the matter is that with every year passing this is increasing and may get to the irreversible stage. Among quite a number of different classes of chemical compounds, polycyclic aromatic hydrocarbons (PAHs) and endocrine disrupting chemicals (EDCs) considered one of the most hazardous environmental pollutants.

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2.2. Polycyclic Aromatic Hydrocarbons (PAHs)

2.2.1. Introduction to PAHs

PAHs are a collection and diverse group of chemicals that are characterised by benzene compounds that are fused together i.e. two or more aromatic rings (Haritash & Kaushik, 2009; Bosetti *et al.*, 2007). A lot of these PAHs are present in the environment due to the incomplete combustion of organic matter. They originate from both human and natural activities. Human activities (Anthropogenic) includes coal-fired electricity generating power stations, burning of wood, coal, household garbage and fossil fuel (vehicle exhausts) amongst a few. Other sources are naturally enthused like forest fires, volcano eruptions, oil seeps and agricultural burning (Haritash & Kaushik, 2009; Samanta *et al.*, 2002). PAHs are also ubiquitous environmental pollutants that are very persistent under normal natural conditions and do not degrade easily. PAHs do not easily dissolve in water and are known to have low water solubility and are highly lipophilic (Sun *et al.*, 2010).

According to US Environmental Protection Agency (USEPA) and International Agency of Research of Cancer (IARC), 16 PAHs have been identified and are known to be highly toxic pollutants. Even though about 100s of these PAH compounds have been identified in nature only few are shown to have detrimental effects on humans and living organisms (Figure 2.1) (Sun *et al.*, 2010; Skupińska *et al.*, 2004). Many of these compounds are suspected to be mutagenic and/or carcinogenic (Peng *et al.*, 2008).

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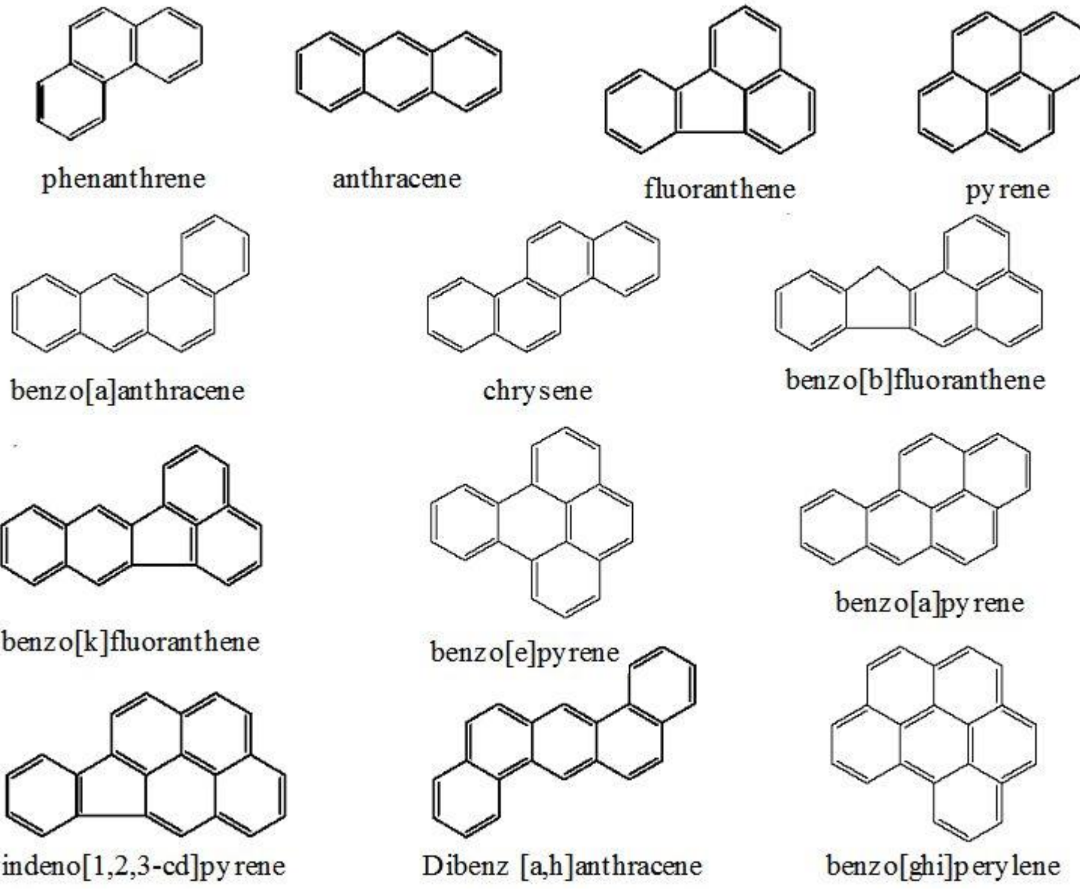

Figure 2.1. Chemical structures of model PAHs (Maigari & Maigari, 2015).

2.2.2. Source and nature of PAHs

PAHs are released into the environment due to incomplete combustion activities. Incomplete combustion is thought to occur when the temperature is low without access air. Generally, PAHs are formed from incomplete burning of coal, crude oil, gas, wood, burning of refuse, or other organic compounds, such as tobacco smoke and braai-meat. They occur in nature as a complex mixture (e.g combustion products such as dust/smoke) and mostly not as separate compounds unless manufactured for a specific purpose e.g research activity (Liu *et al.*, 2008; Baek *et al.*, 1991). About 51% of PAHs are caused by anthropogenic actions from coal-fired electricity generating power station and domestic house warming. Not only human activities contribute to the overall PAHs in the air but also natural activities e.g volcanic eruption,

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forest fires. Only a handful PAHs are used in the production of dyes (clothing manufactures), pesticides and plastics (Skupińska *et al*., 2004).

PAHs can occur in the air, attached to dust particle or as soil sediment. As reported by Skupińska *et al*., (2004) 89% of PAHs tend to accumulate mostly in the humus layer of soil either as wet or dry depository. Dust and sludge used as fertilizer and compost are good sources of soil contaminated with PAHs.

Considerable quantities of PAHs can be found in some foods depending on the mode of cooking, preservation and storage. They have also been found in a wide range of meat, fishes, vegetables and fruits. Contamination of food by PAHs comprises of number of sources, including environment; food processing techniques and methods of analysis. Human exposure can be through consumption of vegetables that having taken up PAHs through ambient air and soil. Compared to inhalation, food ingestion is a major path of exposure (Okedeyi *et al*., 2013; Liu *et al*., 2008). Leafy vegetables take up PAHs through atmosphere and contaminated soil as their main source (Diggs *et al*., 2011).

An increase in molecular weight tends to increase the persistence of these chemicals. PAHs containing fewer than four rings (Low molecular weight) are linked with aquatic animals having effects ranging from reproductive abnormalities and mortality rates whereas higher molecular weight (containing four or more rings) are reputable for their carcinogenic and mutagenic properties (Sun *et al*., 2010). Only in few and rare encounters does one find PAHs alone in nature since the majority occur as a mixture of PAHs; this tendency enhances the potency of carcinogenic PAHs in the environment.

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2.2.3. Effects of PAHs on living organisms

Properties of PAHs play a critical role in their environmental fate. Depending on the substance these can either dissolve very easily in water or can easily evaporate into the air (Guillen *et al.*, 2007). Studies have shown that PAHs can be harmful to the health of living organisms. Some of the PAHs have been heavily linked with causing tumours in laboratory animals, either through eating, breathing or through skin contact. These include benzo[*b*]flouranthene, benzo[*a*]pyrene, benz[*a*]anthrancene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, chrysene and dibenz[*a,h*]anthracene. Studies on people exposed for a longer periods of time to a mixture of PAHs have shown that there is potential for cancer development. IARC and EPA have determined that benz[*a*]anthracene and benzo[*a*]pyrene are probable carcinogenic to humans (ATSDR, 1995). It has already been emphasised that PAHs are thought to be toxic and have carcinogenic and/or mutagenic properties (Samanta *et al.*, 2002). In metropolitan areas, studies have revealed that there's a significant increase in morbidity and mortality from cardiovascular and respiratory diseases associated with exposure to these particulate matter (Perera *et al.*, 1992). Extensive studies conducted in Europe (Poland and Czech Republic) shown that the population exposed to environmental pollution have increased levels of PAH DNA adducts. Furthermore, population in Poland showed several genotoxicity markers like chromosome aberrations, sister chromatid exchanges and *ras* oncogene overexpression (Kyrtopoulos *et al.*, 2001; Perera *et al.*, 1992). DNA damage occurs when exogenous PAHs (those having carcinogenic activity) modifies the DNA often by oxidation through radicals. Endogenous damage to human DNA is more abundant than that caused by exogenous agents (Farmer *et al.*, 2003).

Naphthalene, a well-known micro pollutant in potable water is an example of PAHs with a well-documented toxicity. In humans an acute poising can lead to haemolytic anaemia because it covalently binds to molecules in the kidneys, livers and lungs (Samanta *et al.*,

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2002). On the other hand there is sufficient evidence that Benzo(*a*) pyrene, benzo(*b*) flouranthene, benzo(*a*)anthracene, benzo(*k*)flouranthene, dibenz(*a*,*h*)anthracene and indeno(1,2,3-*c*,*d*)pyrene are carcinogenic to mammals (Mastrangelo *et al*., 1997).

2.3. Endocrine disrupting chemicals (EDCs)

2.3.1. Introduction to EDCs

International Programme on Chemical Safety (IPCS) and U.S Environmental Protection Agency (EPA) has defined Endocrine disrupting chemicals as "exogenous substance or mixture that alters function(s) of the endocrine system and consequently cause adverse health effect in an intact organism, or its progeny, or (sub) population" (Baker, 2001). These chemicals are termed disruptors because they can alter the normal functioning of the endocrine system, that is, they interfere in the complex communication system between chemical signals and their target responsible for regulating internal functioning of the body. Interference/mimic actions of endocrine system could result in developmental deficit in a wide scale of living organisms; invertebrate, aquatic species and mammals (Roger *et al*., 2013; Schug *et al*., 2011; NIEHS, 2010). Convincing evidence exist that these chemicals can be classified as pollutants with adverse effect on animals (including humans and wild life).

Endocrine system functions in the controlling and coordinating of various body functions. Hormones produced by endocrine organs and glands such as testes, ovaries, adrenal, pituitary, thyroid, and pancreas secret release into the blood to act as the body's chemical messengers where they directly communicate and coordinate with other tissues throughout the body. Endocrine system is a complex system where hormones work with other systems such as nervous, reproductive, kidney, gut and the liver, to help control and maintain various functions in the body such as reproduction, growth and development, homeostasis and body energy levels. List of chemicals known to act as EDCs are shown in Figure 2.2.

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Compound	Structure	Description
Estradiol		Endogenous Estrogen
DDT		Pesticide
BPA		Plastics Component
GEN		Phytoestrogen
DBP		Phthalate
DEHP		Phthalate

Figure 2.2. Chemical structures and uses of common endocrine disruptors (Patisaul & Adewale, 2009)

2.3.2. Source and nature of EDCs

EDCs are ubiquitous in our environment. They can be found from many different sources, including industrial chemicals, pharmaceutical, pesticides, insecticide and household's products (Figure 2.3) (Kitamura *et al.*, 2005). A number of these chemicals have also been found in the drinking water as a result of manufacturing plants effluent been discharged into the streams in addition to agricultural run-off (Figure 2.3). Some appears in the personal hygiene products or from containers of food or maybe beverages (Sellin *et al.*, 2009; Bonefeld-Jorgensen *et al.*, 2007). Bisphenol-A (BPA), is well-studied EDCs. Klecka *et al.*, (2009) reported up to 12 parts per billion (ppb) of BPA in the effluent in the North America,

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whereas 43 ppb was in the European water. Thus concludes that an exposure to these chemicals varies with the lifestyle and the geographic region (Roger *et al*., 2013). Over hundreds EDCs are manufactured for an absolutely unrelated purposes (Figure 2.3). Pesticide and herbicides such as *o,p'*-dichlorodiphenyl-trichloroethane (DDT), dieldrin, chlordane and endosulfan; polychlorinated biphenyls (PCBs) and dioxins; and BPA (used in epoxy resins). Other derivatives of BPA such as tetrabromobisphenol A (2,2-bis-(3,5-dibromo-4-hydroxyphenyl)propane) are used by numerous products throughout the world as a flame retardant, as a nontoxic flame retardant (Kitamura et al., 2005; Kitamura *et al*., 2002; Baker., 2001).

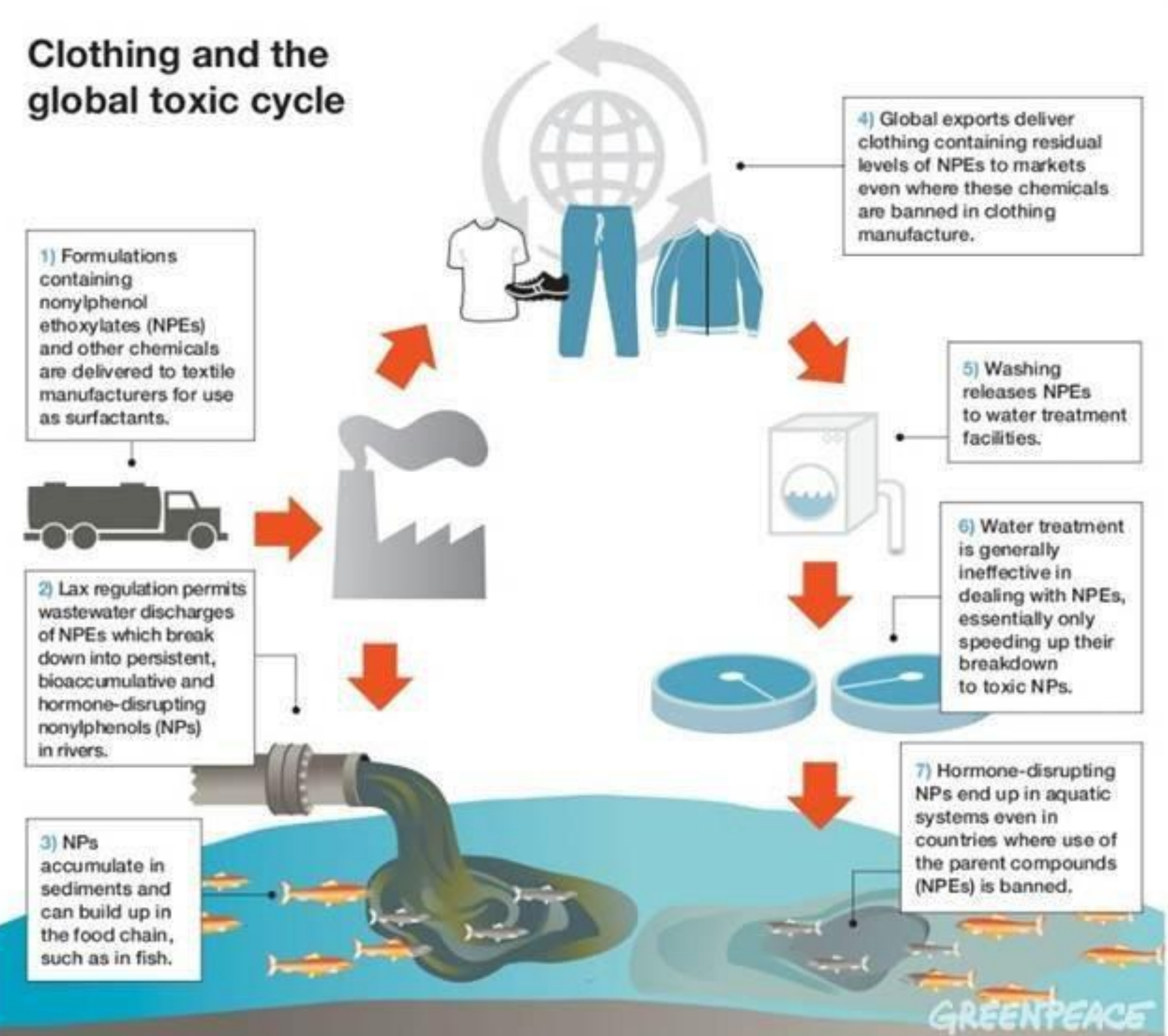

Figure 2.3. Endocrine disrupting chemicals global toxic cycle (taken from http://www.individualoperator.com/2016_04_01_archive.html)

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These can also disrupt hormones. If discharged into the water, nonylphenol is highly toxic to aquatic creatures (Brittgow, 2014).

Some EDCs are highly resistant to breaking down in the environment making them potentially hazardous over an extended period of time hence the term "persistent organic pollutants". Between 1940 and 1970, pharmaceutical diethylstilbestrol (DES) was used to prevent miscarriage in women with high risk pregnancies. Later it was observed that daughters who were exposed to DES through their mothers have developed a rare form of vaginal cancer and few non-cancerous changes in both sexes of offspring's (Schug *et al*., 2011). Animal models were then used to predict the long term effects of these chemicals (NIEHS, 2010). The effects of exposure to EDCs are permanent and irreversible. This indicates a transgenerational exposure, that is, if the mother is exposed to endocrine disrupting chemicals before producing any offspring this chemicals can have an effect on the offspring due to the persistence of these chemicals in body fat, directly *via* egg laying (birds) or pregnancy and lactation (mammals) (Tanabe, 2002; Damstra, 2002; Colborn *et al*., 1993).

Nonylphenol plyethoxylates non-ionic surfactants that widely used worldwide in both industry and households are often seen as persistent pollutants in natural aquatic environment which possess a threat to fish species and in the raw municipal waste water (Liu *et al*., 2006). Estrone, 17β-estradiol (natural estrones) and 17α-ethynylestradiol (synthetic estrone) are compounds documented to have estrogenic activities in waste water treatment and sewage runoffs from livestock and agricultures. An occurrence in trace level (ng/L) of estrogens in wastewater and receiving waters has been recognised (Chang *et al*., 2011). This further indicates the persistence of these chemicals in the environment.

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2.3.3. Effects of EDCs on living organisms

In the past years there has been a great level of concern which has intensified within the last few years about the adverse effect linked with exposure to EDCs following reports about the reproduction health of both humans and wildlife. The deleterious health effects have been observed over the years and a lot of papers have been published addressing the effects of EDCs on living organisms (Bernanke & Köhler, 2009; Baker, 2001; Colborn *et al*., 1993).

Endocrine disrupting chemicals have been reported for decades from as early as 1950s to have had some adverse effect on the invertebrates, fishes and, wildlife populations. Some reported effects include: (1) females snails exposed to tributylin resulted to masculinisation (imposex: imposition of male sex organ, including penis and vas deferens onto females) that lead to decline and extinction of their population in a localized area (Matthiessen & Gibbs, 1998); (2) Alligators of Lake Apopka, Florida with impaired sexual development and function. Effects linked to DDT, following the pesticide spill in 1980 (Guillette *et al*., 1994); (3) reproductive failure reported by Sumpter & Jobling (1995) in various fish species associated with sewage effluents paper industry and industrial chemicals; Exposure to DDE linked with egg-shell thinning in bald eagles that saw a decline in numbers in Europe and North America (Baker, 2001; Cooke, 1973). To name just a few reported cases over past the decades on wildlife and fishes.

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Pituitary Gland
Growth Hormone
Oxytocin
Pineal Gland
Melatonin
Hypothalamus
Oxytocin
Thyroid
Calcitonin
Thyroxine
Parathyroid
PTH
Adrenal Gland
Corticosteroids
Pancreas
Insulin
Testes
Testosterone
Ovaries
Estrogen
Progesterone

Figure 2.4. Endocrine organs targeted by EDCs (taken from Schug *et al*., 2011).

Figure 2.4 illustrates all major endocrine organs that are vulnerable to EDCs. The pancreas and the thyroid gland and EDCs are also known to impact hormone-dependent metabolic systems and brain function. In humans it might take years or decades to assess the effects of the exposure to EDCs because of individual needs to attain a certain age in order to assess sexual maturity and fertility. In male reproductive health, EDCs have been hugely

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linked to disrupting reproductive function, which is displayed as reduced semen quality and infertility; development of fetal is altered, that is abnormalities in the urogenital tract (Figure 2.5) (Diamanti-Kandarakis *et al.*, 2009). Industrially produced EDCs phthalate is responsible for phthalate syndrome in males. Which suppresses the fetal androgen action, which is the driving key to male reproductive organ development and phthalate is also linked to lowering testosterone and its derivatives by interfering with the uptake of steroid hormone. Other pesticides are also able to block the androgen receptor, thus producing the effects that are those of phthalate. Androgen action is necessary for the production of sperm. Overall, reduced fertility later in life is aided by EDC disrupting androgen action during fetal development (Sharpe, 2010). Men aged 10-27 years exposed showed slightly positive or no difference in semen quality as was the conclusion from the study done by Mocarelli *et al.*, (2008). Pesticides exposure and reduced semen quality have been heavily linked in some occupational studies closing the gap between EDCs and reduced male reproductive health (Schug *et al.*, 2011).

EDCs can also alter the female reproductive health and function, this was clearly demonstrated in the used of DES on pregnant women, with their daughters later shown to have rare cervicovaginal cancers, they also seem to reach menopause early and decrease in fertility (Figure 2.5) (Goldberg & Falcone, 1999, Hatch *et al.*, 2006). Research on DES in human and animals have revealed the susceptibility of female fetus to environmentally induced reproductive abnormalities also reproductive deformation may only appear decades later after exposure. Puberty starts later in childhood life, this transition period were individual's move from non-reproductive to reproductive state. In females, this leads to oestradiol secretion from the ovaries. The onset of puberty is thought to be determined by a number of factors including: nutrition, ethnicity, psychosocial and socio-economic conditions. But in the past decades, a trend of earlier age reaching puberty has been noted,

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particularly in the Europe and US (Parent *et al*., 2003). As noted by Schug *et al*., (2011), that exposure to DES can impart a hormonal imprint on the developing uterus thus causing an increase in estrogen-responsive gene expression. One hypothesis to explain this change in the timing of puberty was exposure to EDCs in the pre-pubertal period. While there has been a number of studies' suggesting that there has not been a conclusive link between early puberty and environmental agents (Fowler *et al*., 2012).

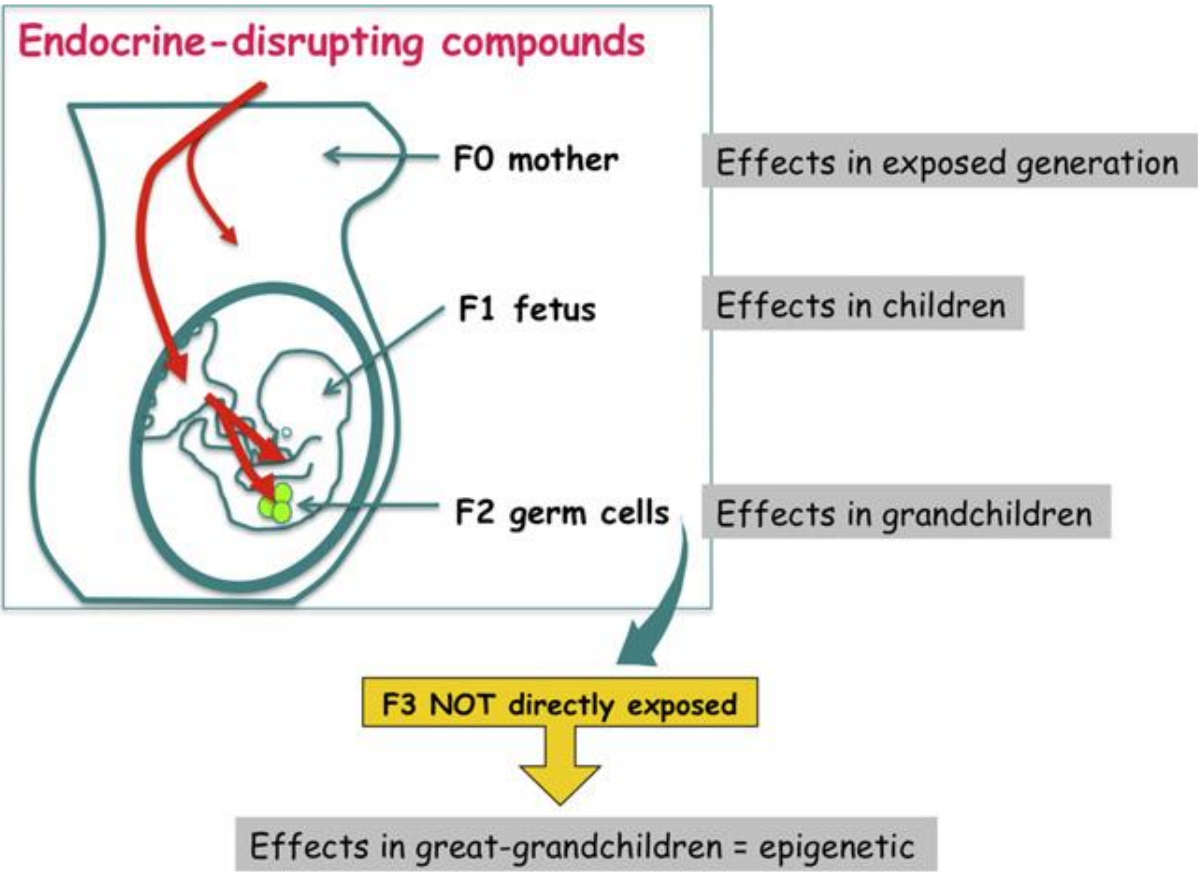

Figure 2.5. Schematic representation of effects of EDCs across generations (taken from Fowler *et al*., 2012). If the mother is exposed to EDCs directly her fertility may be affected and, if she is pregnant, her foetus will be directly exposed (F1, children). However, the germ cells in the foetus will also be exposed and this may result in disturbance of both the F1 directly and of the F2 in the form of the F1 germ cells for a true trans-generational effect.

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2.3.4. Mechanisms of EDCs on the Endocrine system

EDCs can only interfere with hormone signalling because of their structure and activities (Figure 2.6). An ordinary chemical structure of a chemical does not make it an endocrine disruptor.

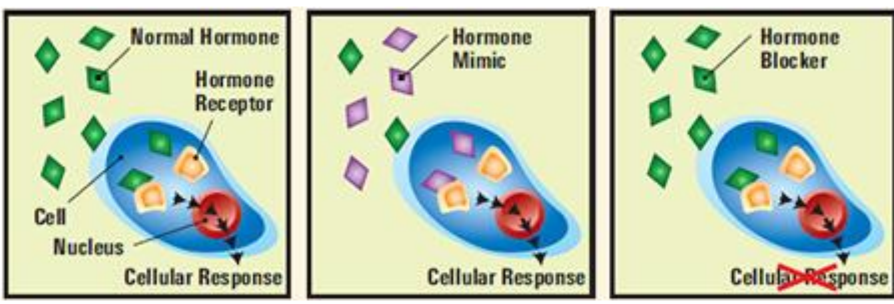

Figure 2.6. Mechanisms of EDCs. When absorbed in the body, an endocrine disruptor can decrease or increase normal hormone levels (left), mimic the body's natural hormones (middle), or alter the natural production of hormones (right) (taken from NIEHS, 2010).

With the complexity of the endocrine system, possibilities of EDCs interfering or altering endocrine functions are countless. The interference with the system include: (1) mimicking the effects of a natural hormones (e.g. sex hormones; estrogen and androgen) by binding to their natural receptors – as agonists or as antagonists by blocking the natural hormone from binding to the hormone receptor (Figure 2.6); (2) by reacting directly/indirectly with the breakdown and synthesis of natural hormones; (3) by changing the functioning and production of hormones (Schug *et al.*, 2011; Baker, 2001).

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2.4. Bioremediation of EDCs and PAHs

Various environmental pollutants are posing as threats to humans and the ecosystem. These pollutants only enter the ecosystem through soil, water and air. A major contributor to soil contamination reported in recent years are the industries and agricultural activities (Udeigwe *et al*., 2011; Ha *et al*., 2014). Air and water pollution is propelled mostly through anthropogenic activities, which are the burning of the fossil fuels and forest fires etc. Water pollution can either be from industries directly dumping effluent into the stream or runoff waste water treatments. Main environmental pollutants include PAHs, EDCs and lastly heavy metals. There has been a lot of research done to remove these pollutants from our environments. Bioremediation has been one of the methods that standout. Which is the process of employing living microorganisms in degrading and transforming hazardous compounds to less hazardous/non-hazardous form, it's efficient and costs little compared to chemical and physical remediation technologies available (Arun *et al*., 2008; Librando & Pappalardo, 2013; Chen *et al*., 2015). The use of microorganisms (algae, bacteria or fungi) to degrade these molecules (PAHs and EDCs) is mostly used. A large number of studies conducted across the world have shown that microbes have the ability to breakdown various organic compounds by biotransformation or mineralization. To devise an effective bioremediation system one has to take into consideration a number of limiting factors ranging from pH, temperature, oxygen, type of microbial population and availability of nutrients (Haritash & Kaushik, 2009). As of 14 October 2016, 38517 publications were found in PubMed using the word "bioremediation" indicating the importance on use of microbes to remove the pollutants from the environment.

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2.5. EDCs and PAHs in South Africa

Studies on EDCs from South Africa in particular are very scarce. A report presented by the Water Research Commission of South Africa revealed the presence of EDCs in South African water (Burger & Nel, 2008). In addition to this report, studies conducted in a few places within South Africa also revealed the presence of EDCs. DDT, DDE, and phthalate esters have been found in Limpopo (Fatoki *et al.*, 2010; Aneck-Hahn *et al.*, 2009 & 2007); Estrone, estradiol, and estriol (steroids hormones) in the Western Cape (Swart & Pool, 2007) and in Kwazulu Natal (Manickum & John, 2014); p-nonylphenol, diethylhexyl phthalate and dibutyl phthalate in Gauteng (Mahomed, 2008) and lastly DDT, chlordane, hexachlorobenzene, heptachlor and endosulfan in the Eastern Cape (Fatoki & Awofolu, 2004). In addition, quite a large number of EDCs were found in upstream and downstream sections of wastewater treatment plants (Olujimi *et al.*, 2012; Olujimi *et al.*, 2010).

Despite a great deal of evidence of contamination of our natural resource with toxic chemicals, studies on isolation and further utilization of indigenous microorganisms capable of degrading these toxic chemicals are scarce. So far only two microbes that can degrade PAHs, *Pseudomonad* and *Alcaligenes* spp are the only studied organisms isolated from soil and mine drainage in South Africa (Tikilili & Nkhalambayausi-Chirwa, 2011). However, the detoxification ability of the isolates has not been studied. In addition to the above, to date, studies on the analysis of the capabilities of microorganisms to utilize/degrade EDCs have not been reported from South Africa.

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2.6. Rational and aims and objectives of the study

It has been found that soil and water particularly from mines in and around South Africa is contaminated with hazardous chemicals and compounds that are possible cancer causing agents and endocrine disruptors. About 15 polycyclic aromatic hydrocarbons (PAHs) have been identified in soils in three areas at South African coal-fired power plants (Okedeyi *et al.*, 2013). The PAHs ranged from phenanthrene, anthracene, flouranthene and pyrene etc. (Okedeyi *et al.*, 2013). The river water in Vaal Triangle area in South Africa, is amongst few places where these compounds are found (Moja *et al.*, 2013). Furthermore these compounds are detected as late as at wastewater treatments plants, which the process itself is not designed to remove these compounds.

Thorough understanding of indigenous microorganisms and their capabilities is necessary to design efficient bioremediation strategy. Considering the contamination of our natural resources with toxic compounds and the apparent lack of studies on the isolation and utilization of indigenous microbiome for their capability towards degrading the toxic compounds, it is very critical that the abundant microbiome should be explored due to their possible potential in the degradation of both type of toxic compounds i.e. carcinogenic and endocrine disrupting chemicals.

The proposed master study is aimed to (i) Isolate microorganism(s) capable of utilising carcinogenic chemical fluoranthene and endocrine disrupting chemical nonylphenol as a carbon source and (ii) Identification and phylogenetic analysis of isolated microorganisms.

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CHAPTER 3

ENRICHEMENT AND ISOLATION OF FLUORANTHENE AND NONYLPHENOL UTILISING MICROORGANISMS

3.1. Introduction

For few years new techniques have been devised to increase the number of microbes that can be cultivated in the laboratory. Staley and Konopka (1985) were the scientists who expressed their frustrations about uncultivable microbes in laboratories from different natural environments, on their paper titled "The Great Plate Count, Anomaly". Which compared direct microscopic counts to number of colonies that have grown on plates inoculated which the same sample (Nichols, 2007; Schloss and Handelsman, 2004; Sait *et al*., 2002; Amann *et al*. 1995). Several studies have documented success by altering a variety of traditional cultivation methods, including substrate, incubation period, pH, and O_2/CO_2 concentration. Study by Mitsui *et al*. (1997) revealed a different bacteria from soil, when comparison studies were performed between diluted nutrient broth and nutrient rich broth. A lot has been achieved with the slightest change of existing methods (Overmann, 2006; Joseph *et al*., 2003; Janssen *et al*., 2002; Sait *et al*., 2002).

In recent years, the most preferred method of isolating microorganisms from the environment is the enrichment culture technique. The essence of this process is to provide suitable conditions for the growth of microorganisms capable of metabolising desired compounds. By providing the compound as a sole carbon source, microorganisms that can utilize the provided compound can be enriched (Gaskin & Bentham, 2005; Bastiaens *et al*., 2001). These organic compounds can easily be broken down as a source of carbon and energy by variety microorganisms. Only those microorganisms that can use the compounds in the media will grow, while others will not, because they unable to use the compounds as a sole carbon source and energy.

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Hence, in this study, enrichment culture technique was followed to isolate microorganisms capable of utilizing fluoranthene and nonylphenol as a sole source of carbon.

3.2. Materials and methods

3.2.1. Soil sample collection and preparation

Soil samples were aseptically collected from different coal-fired power stations in and around the Mpumalanga province, South Africa. The selected sampling areas with GPS coordinate ware represented Figure 3.1. Soil samples (5g) were resuspended in 30 ml of DNase-free and RNase-free water. The samples were vigorously vortexed for 5 min, followed by incubation on a rotary shaker for 1 hour at room temperate at 100 rpm. After incubation, the soil was allowed to settle out of solution (30 min), and the supernatants were collected and used for isolation of microorganisms.

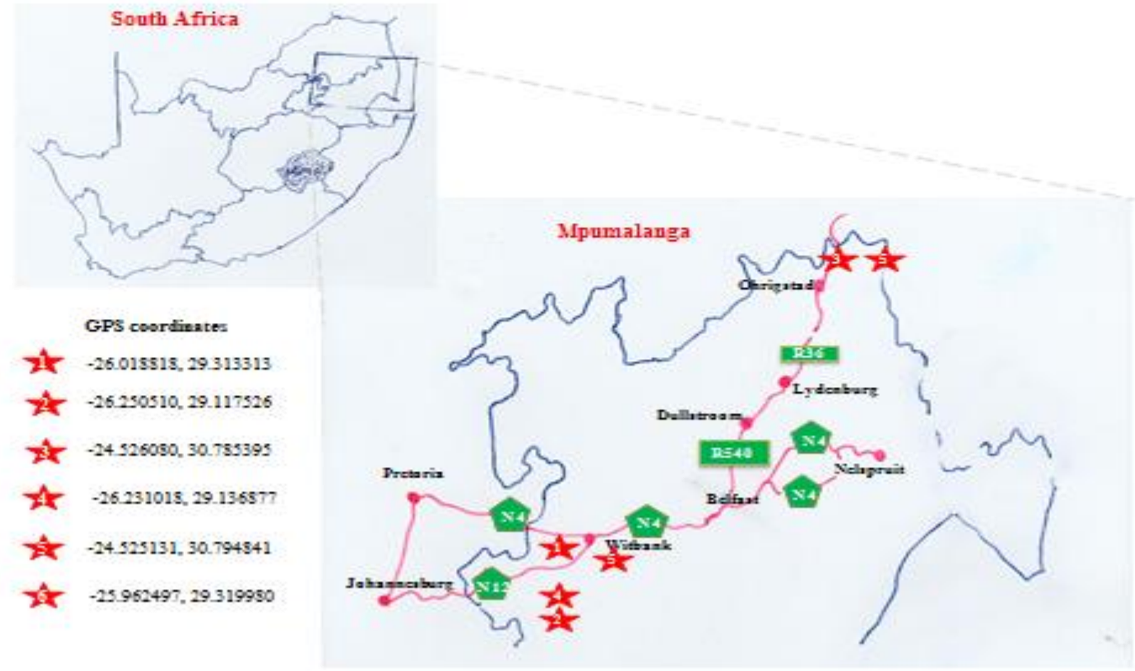

Figure 3.1. Schematic representation of soil sample collection areas in Mpumalanga, South Africa. The numbers 1 to 6 in stars indicates the areas collected soil samples. The sampling areas GPS co-ordinates are listed in the figure.

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3.2.2. Media preparation

All chemicals and reagents used in this study were purchased form Sigma-Aldrich, unless otherwise stated. Minimal medium (Boldrin *et al*., 1993; Zhang *et al*., 2004) with the addition of trace element solution (Zeng *et al*., 2010), was used for isolation of microorganisms. The minimal medium consisted of 8.5 g/l $Na_2HPO_4.2H_2O$; 3.0 g/l KH_2PO_4; 0.5 g/l NaCl; 1.0 g/l NH_4Cl; 0.5 g/l $MgSO_4.7H_2O$; 14.2 mg/l $CaCl_2$ and 0.15 g/l KCL. The minimal medium was supplemented with 10 ml of trace element solution (Zeng *et al*., 2010), consisting of 0.4 mg/l $CuSO_4$; 1.0 mg/l KI; 4.0 mg/l $MnSO_4.H_2O$; 4.0 mg/l $ZnSO_4.7H_2O$; 5.0 mg/l H_3BO_3; 1.2 mg/l $Na_2MO_4.2H_2O$ and 2.0 mg/l $FeCl_3.6H_2O$; per litre of medium. Technical grade Nonylphenol (Catalog number: 290858) and Fluoranthene (Catalog number: 423947) was added as a sole source of carbon to a final concentration of 5 mM.

3.2.3. Enrichment procedure

Supernatant (1 ml) from the soil samples was used to inoculate 100 ml of minimal medium in a 500 ml of conical flask, supplemented with nonylphenol and fluoranthene as sole carbon sources. Controls were set up to contain medium and nonylphenol and fluoranthene, without inoculation of soil samples. After four weeks of incubation at 37°C at 100 rpm, 1 ml of culture was used to inoculate fresh minimal medium (100 ml) with nonylphenol and fluoranthene as sole carbon sources. This serial enrichment of bacterial isolates was repeated until a single, homogenous culture was obtained. Aliquots (100 µl) of cultures were spread on minimal medium agar plates with nonylphenol (5 mM) and fluoranthene (5 mM) as sole carbon sources, to monitor the growth of microorganisms at 37 C. The minimal medium plates with nonylphenol were prepared as described elsewhere (Porter and Hay, 2007). In addition, bacterial growth was also analysed by measuring the absorbance at 600 nm. Fresh inoculum was prepared by inoculating a homogeneous colony in a 15ml LB broth. All isolates were stored as a liquid cultures containing 20% glycerol at -80°C.

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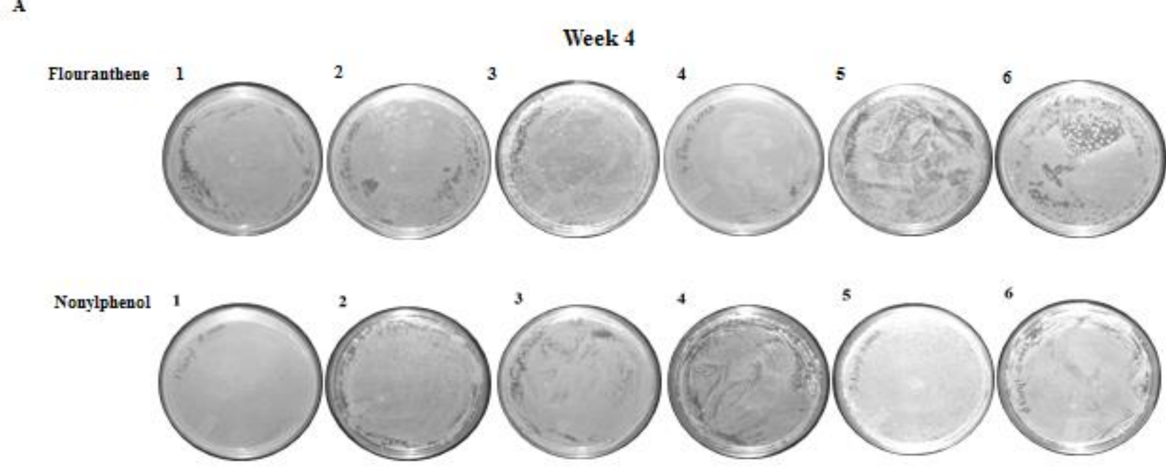
3.3 Results and discussion

To isolate microorganisms capable of utilizing nonylphenol and fluoranthene as the sole source of carbon standard enrichment culture technique were followed. Soil samples collected from six different places (Figure 3.1) were inoculated into minimal medium supplemented with nonylphenol and fluoranthene as carbon source. After 4 weeks of incubation, growth of bacteria was observed on minimal medium plates supplemented with nonylphenol as a carbon source (Figure 3.2). The initial bacterial growth on plates was non-homogenous, suggesting the presence of more than one type of species. After three successive serial culturing, a homogenous population of bacteria was observed on minimal medium plates (Figure 3.3). This indicated that successive serial culturing resulted in the enrichment of single type bacteria that are capable of utilizing nonylphenol and fluoranthene as sole source of carbon (Figure 3.2). In this study, twelve bacteria were isolated from the six different soil samples.



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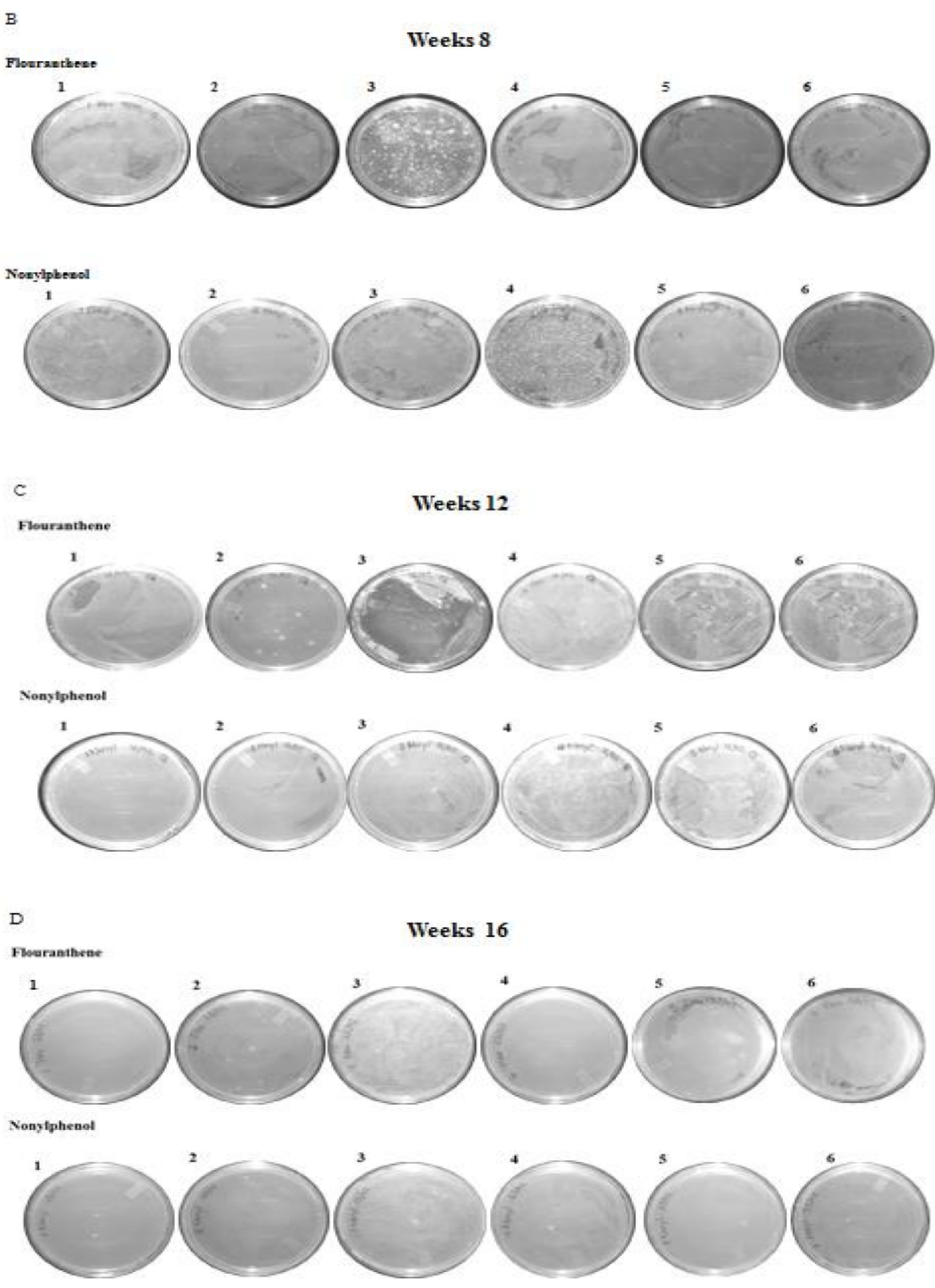

Figure 3.2. Analysis of growth of microorganisms on minimal medium plate supplemented with fluoranthene and nonylphenol as sole carbon source. Panels A to D represent samples spread on plates at indicated time-period.

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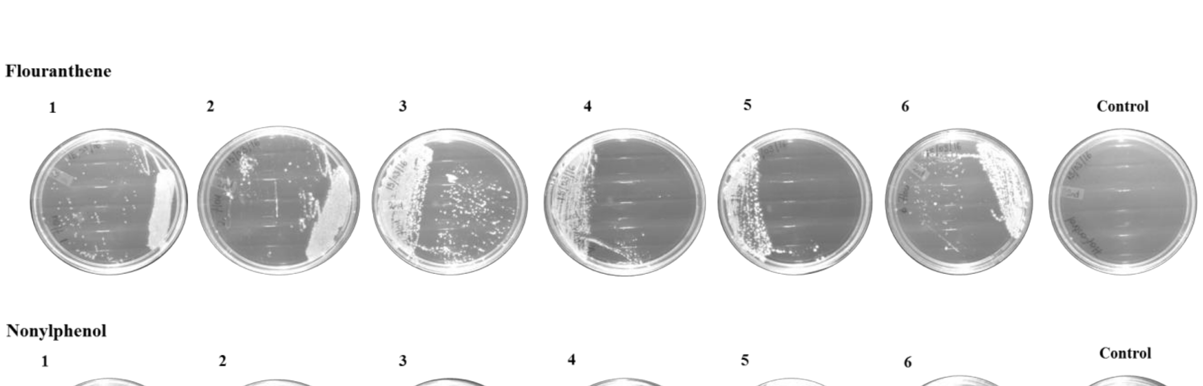

Figure 3.3. Isolation of homogenous bacteria capable of using fluoranthene and nonylphenol as carbon source.

3.4. Conclusion

It is evident from the study that enriching and isolating microorganism from soil can be achieved. The indigenous microorganisms found in the soils of South Africa do have the capabilities of utilizing fluoranthene and nonylphenol. In the present study enrichment culture technique resulted in isolation of bacterial species.

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CHAPTER 4

IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF BACTERIAL ISOLATES

4.1. Introduction

The prerequisite for the functional understanding of bacteria in the soil environment is often based on studying bacterial genetics and physiology. The applications of molecular methods have been in use since the early 1990s, ever since, these ecological methods have assisted in the investigation of cultivation-independent microbial communities from soil (Janssen, 2006). Through this, new diagnostic and quantitative methods have been developed to target functional genes and phylogenetically informative genes, or RNAs (Liesack & Dunfield, 2002; Gray & Head, 2001). The most useful and influential biomarkers have proven to be 16S rRNA genes in bacteria (Janssen, 2006; Rappé & Giovannoni, 2003; Pace *et al.,* 1997; Woese, 1987).

16S rRNA genes are widely used in studying bacterial identification due to the essentiality of the gene and consist of highly conserved regions which have endured slow evolution (Janssen, 2006; Rappé & Giovannoni, 2003; Pace *et al.,* 1997; Woese, 1987). This highly conserved region serves as templates for designing specific PCR primers or specific nucleotide probes (Lane, 1991; Giovannoni *et al.,* 1990). PCR aids in the analysis of 16S rRNA genes by amplifying target sequences. PCR primers for 16S rRNA amplification are widely available and this has helped to provide better understanding of bacterial diversity (Marchesi *et al.,* 1998; Wheeler *et al.,* 1996; Lane, 1991). Solely based on the application of molecular approaches bacterial diversity can be investigated from the natural environment without cultivation of any culture (Santos & Ochman, 2004; Marchesi *et al.,* 1998; Amann *et al.,* 1995).

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In this chapter the aim is to identify the bacterial species isolated in previous chapter using 16S rRNA gene.

4.2. Materials and methods

4.2.1. Isolation of genomic DNA and PCR amplification of 16S rRNA gene

Genomic DNA (gDNA) from bacterial isolates were extracted using the ZR Fungal/Bacterial DNA MiniPrep kit (Cat. No. D6005, Inqaba Biotec, South Africa) according to the manufacturer's protocol. The gDNA was visualised using Agarose-gel electrophoresis, and gDNA concentration was measured using SimpliNano microvolume spectrophotometer (catalog number: GE29-0617-12, Sigma-Aldrich Co. LLC. USA). The isolated gDNA was used for PCR amplification of 16S rRNA gene. 16S rRNA gene was PCR-amplified using primers 63f and 1387r as described elsewhere (Table 4.1) (Marchesi *et al*., 1998). 16S rRNA gene was amplified using the KAPA HiFi HotStart PCR kit (catalog number: KK2501, KAPA Biosystems, USA) according to manufacturer's instructions. PCR conditions for amplification of 16S rRNA gene are listed in Table 4.2. The PCR products were run on 0.8% Agarose-gel and were purified using the Wizard® SV Gel and PCR Clean-Up System (Catalog number: A9281, Promega, USA).

Table 4.1. 16S rRNA gene amplification primer sequence (Marchesi *et al*., 1998).

Primers	Sequences
63f	5'-CAGGCCTAACACATGCAAGTC-3'
1387r	5'-GGGCGGWGTGTACAAGGC-3'

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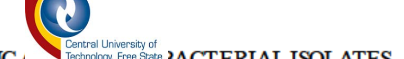

Table 4.2. PCR conditions for amplification of 16S rRNA gene.

Step	Temperature	During	Cycles
Initial denaturation	95°C	3 min	1
Denaturation	98°C	20 sec	25
Annealing	60 - 75°C	15 sec	
Extension	72°C	45 sec	
Final extension	72°C	1 min	1

4.2.2. 16S rRNA gene sequencing

Samples were prepared for sequencing using the BigDye™ Terminator V3.1 Cycle Sequencing Kit (Catalog number: 4337455, Thermo Fischer Scientific, USA). The aforementioned primers 63f and 1387r (Marchesi *et al.*, 1998) were used for sequencing. The sequencing reactions were performed according to the parameters described by the manufacturer (Table 4.2). Sequencing reactions were purified using the EDTA-Ethanol method described by the manufacturer, and submitted for sequencing using a 3130xl Genetic Analyzer (Applied Biosystems, USA). Consensus sequences were derived from the sequences obtained from the forward and reverse primer reactions for each product, using Geneious® R9 9.1.2. Software.

4.2.3. Phylogenetic analysis

16S rRNA gene sequences of bacterial isolates were subjected to BLAST analysis at NCBI against 16S ribosomal RNA sequences (Bacteria and Archaea) to identify closest homologs. Among the resulting hits, the 16S rRNA sequences with 100% or 99% identity homologs were selected. Based on the obtained bacterial species, the type strains belonging to each species was selected, and its 16S rRNA sequences were retrieved from elsewhere (http://www.bacterio.net/). The *Escherichia coli* ATCC 11775 type strain 16S rRNA gene

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sequence (also retrieved from http://www.bacterio.net/) was used as an out-group. Phylogenetic analysis was carried out using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei, 1993). Initial tree(s) for the heuristic search were obtained by applying the Neighbour-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA5 (Tamura *et al.,* 2011). Phylogenetic analysis included the isolate 16S rRNA gene sequence, hit homologs and Type strain 16S rRNA gene sequences. The phylogenetic tree was presented with branch lengths, and the bacterial isolates identified in this study were highlighted with bold font.

4.2.4. 16S rRNA gene sequences accession numbers

GebBank accession numbers for 16S rRNA gene sequences of bacterial isolates were obtained from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). The GenBank assigned 16S rRNA gene accession numbers for 12 isolates are listed in respective tables.

4.3. Results and Discussion

4.3.1. 16S rRNA gene amplification and sequencing

In order to identify the enriched bacterial isolates, 16S rRNA gene sequence-based phylogenetic analysis was carried out. The 16S rRNA genes from the gDNA of bacterial isolates were PCR amplified using the 63f and 1387r primer set as described elsewhere (Marchesi *et al.,* 1998). Analysis of the PCR amplified products on Agarose gel showed prominent DNA bands with approximate sizes of ≥1200 base pairs (Figure 4.1). This indicates specific amplification of 16S rRNA gene. The amplified 16S rRNA gene was gel purified and subjected to sequence analysis using the same primers used for its amplification. Sequence analysis was performed using both the forward and reverse primers, yielding a consensus sequence of 300-500 overlapping base pairs between the sequences.

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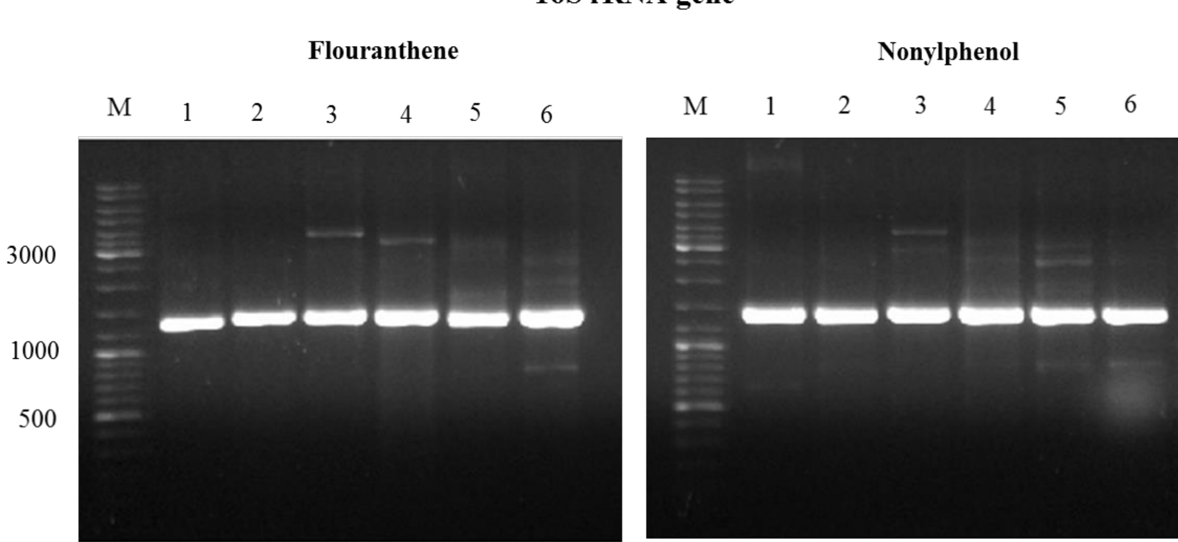

Figure 4.1. Agarose gel electrophoresis analysis of 16S rRNAs genes amplified from twelve bacterial isolates. PCR amplified products were run on 1% Agarose gel. Lane M, indicates DNA Ladder (O'GeneRuler DNA Ladder Mix 100-10000 base pair, from ThermoFisher, Catalog number SM1173). Markers with high intensity were indicated with their size. Lanes 1 to 6 indicate the respective bacterial isolates with either fluoranthene or nonylphenol.

4.3.2. Identification of nonylphenol degrading bacteria

16S rRNA sequence of isolates 1 and 2 showed 100% identity to *Pseudomonas* species, while isolates 5 and 6 also had 99% identity to *Pseudomonas* species (Table 4.3). Isolate 3 showed 99% identity to *Stenotrophomonas* species and Isolate 4 showed 99% identity to *Enterobacter* species. This indicates most of the isolates belong to *Pseudomonas* (Table 4.3). Phylogenetic analysis of isolates based on 16S rRNA gene sequences compared to the 16S rRNA gene sequences of hit species, highlighted the differential alignment of bacterial isolates with different species (Figure 4.2). Based on the phylogenetic alignment the six bacterial isolates were named as shown in Table 4.3. Furthermore, homology analysis (percent identity) of 16S rRNA gene sequences among bacterial isolates (Table 4.3) revealed that isolates 3 and 4 have low percent identity compared to other isolates, clearly reinforcing

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that they in fact belong to different bacterial genera. Species assigned to *Pseudomonas* on the other hand showed high percent identity (Table 4.3), demonstrating that they belong to the same genus.

Nonylphenol degradation by the bacterial species identified in this study is reinforced by literature. Species belonging to the genus *Pseudomonas* have been shown to degrade EDCs such as di-n-butyl phthalate (Liao *et al.,* 2010), p-nonylphenol (Chakraborty & Dutta, 2006) and polyethoxylated nonylphenols (Ruiz et al., 2013; John & White, 1998). Bacterial species belonging *to Stenotrophomonas* were previously found to be capable of using either nonylphenol or octylphenol as sole carbon source (Toyama *et al.,* 2011). For species belonging to the well-known human-pathogenic and plant association, *Enterobacter*, degradation of EDCs has been reported particularly for bisphenol A (Badiefar *et al.,* 2015), polychlorinated biphenyls (Jia *et al.,* 2008), endosulfan (Abrahan & Silambarasan *et al.,* 2015), dibutyl phthalate (Fang *et al.,* 2010) and nonylphenol (Kageyama & Morooka, 2005).

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Table 4.3. Information regarding nonylphenol degrading bacterial isolates identification.

Sample number	**16S rRNA sequenced gene size (base pair)**	**GenBank accession numbers**	**NCBI Blast hit results**			**Name assigned to the bacterial isolate**
			Dominant bacteria genus	Percent identity	Query cover percentage	
1	1242	KX364074	*Pseudomonas*	100	100	*Pseudomonas nitroreducens* strain LBQSKN1
2	1239	KX364075	*Pseudomonas*	100	100	*Pseudomonas putida* strain LBQSKN2
3	1196	KX364076	*Stenotrophomonas*	99	100	*Stenotrophomonas* sp. LBQSKN3
4	1240	KX364077	*Enterobacter*	99	100	*Enterobacter asburiae* strain LBQSKN4
5	1245	KX364078	*Pseudomonas*	99	100	*Pseudomonas* sp. LBQSKN5
6	1237	KX364079	*Pseudomonas*	99	100	*Pseudomonas* sp. LBQSKN6

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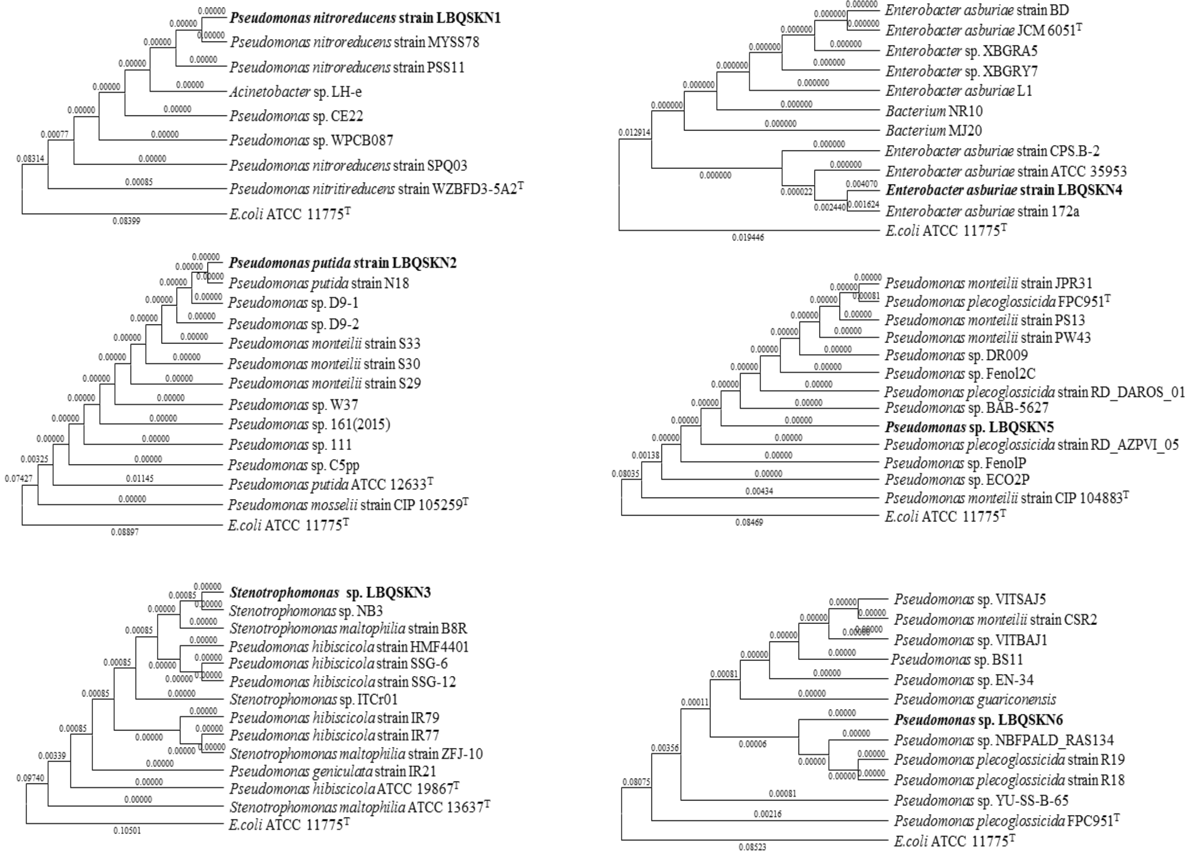

Figure 4.3. Phylogenetic analysis of nonylphenol degrading bacterial isolates 16S rRNA gene sequences. Phylogenetic analysis was performed as described in the materials and methods. 16S rRNA genes sequences of the type strains belonging to the same genus and an out-group bacterial species (*E. coli*) were also included in the analysis. Superscript letter "T" next to strain name indicates the type strain. Each bacterial isolate was named based on its alignment to the homolog bacterial species. Branch lengths are also shown in the tree. Bacterial species isolated and named in this study are highlighted with bold font.

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4.3.3. Identification of fluoranthene degrading bacteria

Phylogenetic analysis of fluoranthene degrading isolates is based on 16S rRNA gene highlighted the alignment of bacterial isolates with different species (Figure 4.3 and Table 4.4). 16S rRNA sequences of isolates 2 and 6 showed 100% identity to *Pseudomonas* species, while isolates 3 and 4 showed 100% identity to *Stenotrophomonas* species (Table 4.4.) Isolate 1 showed 99% identity to *Ochrobactrum* species and isolate 5 showed 100% identity *Cupravidus* species (Table 4.4). Based on the Phylogenetic alignment and percent identity to the homolog species, the six bacterial isolates were named as shown in Table 4.4.

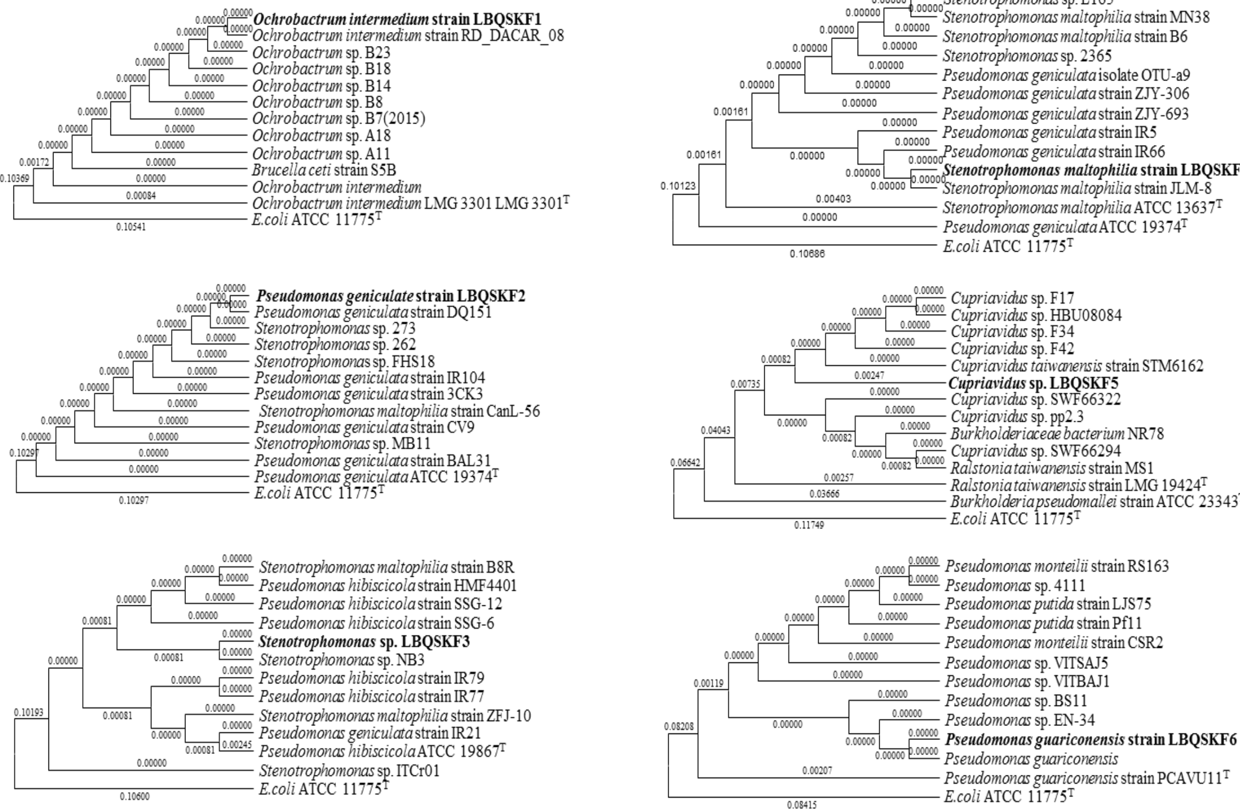

Figure 4.4. Phylogenetic analysis of fluoranthene degrading bacterial isolates 16S rRNA gene sequences. Phylogenetic analysis was performed as described in the materials and methods. Type strains belong the same genus and an out group bacterial species (*E. coli*) 16S rRNA genes sequences were also included in the analysis. Superscript letter “T” next to strain

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name indicates it's a type strain. Bacterial isolate was named based on its alignment to the homolog bacterial species. The branch lengths were also shown in the tree. Bacterial species isolated and named in this study is highlighted with bold font.

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Table 4.4. Information regarding fluoranthene degrading bacterial isolates identification.

Sample number	16S rRNA sequenced gene size (base pair)	GenBank accession numbers	NCBI Blast hit results			Name assigned to the bacterial isolate
			Dominant bacteria genus	Percent identity	Query cover percentage	
1	1185	KX364068	*Ochrobactrum*	99	100	*Ochrobactrum intermedium* strain LBQSKF1
2	1249	KX364069	*Pseudomonas*	100	100	*Pseudomonas geniculata* strain LBQSKF2
3	1240	KX364070	*Stenotrophomonas*	100	99	*Stenotrophomonas* sp. LBQSKF3
4	1259	KX364071	*Stenotrophomonas*	100	99	*Stenotrophomonas maltophilia* strain LBQSKF4
5	1230	KX364072	*Cupravidus*	100	100	Cupravidus sp. LBQSKF5
6	1236	KX364073	*Pseudomonas*	100	100	*Pseudomonas guariconensis* strain LBQSKF6

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The prevalence of *Pseudomonas* species enriched in this study for both substrates is not surprising, as the hydrophobic carbon degradation abilities of this organism have been demonstrated previously (Sharma *et al.,* 2015; Kumar *et al.,* 2011; van Beilen & Funhoff, 2007). *Pseudomonas* has been widely and globally been either isolated from polluted soils and/or considered for bioremediation studies based on degradation properties and biosurfactant production. Such studies have been undertaken from Asia (Sharma *et al.,* 2015; Lin *et al.,* 2013) and Europe to South America (Silva *et al*. 2014); and from the Arctic ocean (Dong *et al*., 2015) to the Antarctic soil (Ma *et al*., 2006). PAH degradation by *Pseudomonas* has in particular been well characterized (Dong *et al.,* 2015; Ma *et al.,* 2013; 2012; Zhang et al., 2011; Ma *et al.,* 2006).

Degradation of PAHs using *Stenotrophomonas* (Mangwani *et al.,* 2014), in particular *Stenotrophomonas maltophilia* (Singh *et al.,* 2015; Juhasz *et al.,* 2000; Boonchan *et al.,* 1998) has been investigated. The PAH degradation ability of *Stenotrophomonas maltophilia* in the presence of various surfactants was investigated (Boonchan *et al*., 1998), with promising results obtained for pyrene degradation using a biosurfactant-producing strain (Singh *et al.,* 2015).

A strain of *Ochrobactrum intermedium* was enriched on fluoranthene (Figure 4.4). PAH degradation has been observed by strains of *Ochrobactrum*, particularly halotrophic strains (Arulazhagan & Vasudevan, 2011; Ghosal *et al.,* 2010; Yirui *et al.,* 2009). The degradation of various types of hydrocarbons by these bacteria have been demonstrated (Bacosa *et al.,* 2012), particularly for substituted aromatic compounds (Berezina *et al.,* 2015; Chang *et al.,* 2011), as well as PAHs (Reddy et al., 2015; Jones *et al.,* 2014). A fascinating study demonstrated that a diverse range of starting hydrocarbon substrates, including PAHs, can in fact be used for PHB production, representing a great opportunity for coupling bioremediation to the production of valuable material (Reddy *et al.,* 2015). Metabolite

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profiling of 2, 4-dichlorophenoxyacetic acid biotransformation by a strain of *Cupriavidus* demonstrated metabolites indicating the degradation of the substituent group, followed by ring hydroxylation to the corresponding catechol (Chang *et al.,* 2011). Taken together, the above results help to explain the ability of *Cupravidus* to degrade PAHs (Berezina *et al.,* 2015; Reddy *et al.,* 2015; Chang *et al.,* 2011).

4.4. Conclusion

In this chapter, bacterial isolates were successfully identified using 16S rRNA gene sequences. 16S rRNA gene was amplified and sequenced. The sequence was subjected to phylogenetic analysis and all bacterial isolates were named based on their 16S rRNA gene percentage identity and phylogenetic alignment with homology species. 16S rRNA gene accession number were obtained from GenBank and presented in the chapter.

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4.6. APPENDIX

Bacterial isolates 16S ribosomal RNA gene sequences

>1 *Ochrobactrum intermedium* strain LBQSKF1

CGTGGGACGTACCATTTGCTACGGAATAACTCAGGGAAACTTGTGCTAATACCGTATGAGCCCGAAAGGGGAAAGATTTATCGGCAAATGATCG
GCCCGCGTTGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCATAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGA
GACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATGCCGCGTGAGTGATGAAGGCC
CTAGGGTTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGAGAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGA
AGGGGGCTAGCGTTGTTCGGATTTACTGGGCGTAAAGCGCACGTAGGCGGGCTAATAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACT
GCCTTTGATACTGTTAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACACCAGTGGC
GAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGAT
GAATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAACATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAA
GGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACATCCCGATCGCGGT
TAGTGGAGACACTATCCTTCAGTTCGGCTGGATCGGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCG
CAACGAGCGCAACCCTCGCCCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAAGGTGGGGATGACG
TCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGGTGGTGACAGTGGGCAGCGAGCACGCGAGTGTGAGCTAATCTCCAA
AAGCCATCTCAGTTCGGATTGCACTCTGCAACTCGAGTGCATGAAGTGGAATCGCTA

>2 *Pseudomonas geniculate* strain LBQSKF2

GAGTGGCGGACGGGTGAGGAATACATCGGAATCTACTCTGTCGTGGGGGATAACGTAGGGAAACTTACGCTAATACCGCATACGACCTACGGGT
GAAAGCAGGGGACCTTCGGGCCTTGCGCGATTGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCAAGGCGACGATCCGT
AGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGC
AAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGCTGGCTAATACCCGGTT
GGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGC
GTAAAGCGTGCGTAGGTGGTCGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGATACTGGGCGACTAGAGTGTGGTA
GAGGGTAGCGGAATTCCTGGTGTAGCAGTGAAATGCGTAGAGATCAGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACATGACACTG
AGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGC
AGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGG
AGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGACATGTCGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTC
GAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCA
CGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTAC
ACACGTACTACAATGGTAGGGACAGAGGGCTGCAAGCCGGCGACGGTAAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTGGAGTCTGCAAC
TCGACTCCATGAAGTCGGAATCGCTA

>3 *Stenotrophomonas* sp. LBQSKF3

GGGTGAGGAATACATCGGAATCTACTCTGTCGTGGGGGATAACGTAGGGAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGG
ATCTTCGGACCTTGCGCGATTGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCAAGGCGACGATCCGTAGCTGGTCTGA
GAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCC
AGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCATCTGGCTAATACCCGGGTGGGATGACGGT
ACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGC
GTAGGTGGTCGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGATACTGGGCGACTAGAATGTGGTAGAGGGTAGCGG
AATTCCTGGTGTAGCAGTGAAATGCGTAGAGATCAGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAACATTGACACTGAGGCACGAAA
GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGCAGTATCGAAG
CTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGTATGTGGT
TTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGACATGTCGAGAACTTTCCAGAGATGGATCGGTGCCTTCGGGAACTCGAACACAGGT
GCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTAGTTGCCAGCACGTAATGGTG
GGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTACTA
CAATGGTAGGGACAGAGGGCTGCAAGCCGGCGACGGTGAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTGGAGTCTGCAACTCGACTCCAT
GAAGTCGGAATCGCTAGT

>4 *Stenotrophomonas maltophilia* strain LBQSKF4

TTGGGTGGCGAGTGGCGGACGGGTGAGGAATACATCGGAATCTACTCTGTCGTGGGGGATAACGTAGGGAAACTTACGCTAATACCGCATACGA
CCTACGGGTGAAAGCAGGGGATCTTCGGACCTTGCGCGATTGAATGAGCCGATGTCGGATTAGCTAGTTGGCGGGGTAAAGGCCCACCAAGGCG
ACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGAC
AATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGCCGGCTAA
TACCTGGTTGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAA
TTACTGGGCGTAAAGCGTGCGTAGGTGGTCGTTTAAGTCCGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGATACTGGGCGACTAG
AGTGTGGTAGAGGGTAGCGGAATTCCTGGTGTAGCAGTGAAATGCGTAGAGATCAGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAAC

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ACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAA
TTTGGCACGCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCA
CAAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGACATGTCGAGAACTTTCCAGAGATGGATTGGTGCCT
TCGGGAACTCGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCTTA
GTTGCCAGCACGTAATGGTGGGAACTCTAAGGAGACCGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGG
CCAGGGCTACACACGTACTACAATGGTAGGGACAGAGGGCTGCAAGCCGGCGACGGTAAGCCAATCCCAGAAACCCTATCTCAGTCCGGATTGG
AGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTA

>5 *Cupriavidus* sp. LBQSKF5

GGCGACGGGTGAGTAATACATCGGAACGTGCCCTGTCGTGGGGGATAACTAGTCGAAAGATTAGCTAATACCGCATACGACCTGAGGGTGAAAG
CGGGGGACCGTAAGGCCTCGCGCGATAGGAGCGGCCGATGTCTGATTAGCTAGTTGGTGGGGTAAGAGCCTACCAAGGCGACGATCAGTAGCTG
GTCTGAGAGGACGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGCAACCC
TGATCCAGCAATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTTGTCCGGAAAGAAATGGCCGGGTTAATACCTGGGGTCGATG
ACGGTACCGGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAG
CGTGCGCAGGCGGTTTGATAAGACAGGCGTGAAATCCCCGAGCTCAACTTGGGAATGGCGCTTGTGACTGTCAGGCTAGAGTATGTCAGAGGGG
GGTAGAATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGGAATACCGATGGCGAAGGCAGCCCCCTGGGACGTCACTGACGCTCATGCA
CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTAGTTGTTGGGGATTCATTTCTTCAGTAACG
TAGCTAACGCGTGAAGTTGACCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATGATGT
GGATTAATTCGATGCAACGCGAAAAACCTTACCTACCCTTGACATGCCACTAACGAAGCAGAGATGCATCAGGTGCCCGAAAGGGAAAGTGGAC
ACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCTCTAGTTGCTACGAAAGG
GCACTCTAGAGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGGCCCTTATGGGTAGGGCTTCACACGTCATAC
AATGGTGCGTACAGAGGGTTGCCAACCCGCGAGGGGGAGCTAATCCCAGAAAACGCATCGTAGTCCGGATCGTAGTCTGCAACTCGACTACGTG
AAGCTGGA

>6 *Pseudomonas guariconensis* strain LBQSKF6

GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGTGGGGG
ATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGA
GAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCC
AGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTT
ACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGC
GTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCGAGCTAGAGTACGGTAGAGGGTGGTGG
AATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAA
GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGC
TAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTT
TAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTG
CTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGG
GCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTAC
AATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCTCACAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTG
AAGTCGGAATCGCT

>7 *Pseudomonas nitroreducens* strain LBQSKN1

GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGG
ACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCCGTAACTGGTCTGA
GAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCC
AGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTT
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GTAGGTGGTTTGGTAAGATGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCATAACTGCCTGACTAGAGTACGGTAGAGGGTGGTGG
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GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGC
TAACGCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTT
TAATTCGAAGCAACGCGAAGAACCTTACCTGGCCTTGACATGTCCGGAATCTTGCAGAGATGCGAGAGTGCCTTCGGGAATCGGAACACAGGTG
CTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGG
GCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTAC
AATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTG
AAGTCGGAATCGCTAGTAAT

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>8 *Pseudomonas putida* strain LBQSKN2

GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGG
ACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGA
GAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATA TTGGACAATGGGCGAAAGCCTGATCC
AGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGCTAATACCTTGCTGTTTTGACGTT
ACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGC
GTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCGAGCTAGAGTACGGTAGAGGGTGGTGG
AATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAA
GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGC
TAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTT
TAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTG
CTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCAC GTTATGGTGG
GCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTAC
AATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCTCACAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTG
AAGTCGGAATCGCTAGT

>9 *Stenotrophomonas* sp. LBQSKN3

CGTAGGGAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGCAGGGGATCTTCGGACCTTGCGCGATTGAATGAGCCGATGTCGGATTA
GCTAGTTGGCGGGGTAAAGGCCCACCAAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACT
CCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGC
CCTTTTGTTGGGAAAGAAATCCATCTGGCTAATACCCGGGTGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGC
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CCTGGGAACTGCAGTGGATACTGGGCGACTAGAATGTGGTAGAGGGTAGC GGAATTCCTGGTGTAGCAGTGAAATGCGTAGAGATCAGGAGGAA
CATCCATGGCGAAGGCAGCTACCTGGACCAACATTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC
CCTAAACGATGCGAACTGGATGTTGGGTGCAATTTGGCACGCAGTATCGAAGCTAACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGA
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GGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTACTACAATGGTAGGGACAGAGGGCTGCAAGCCGGCGACGGTGAGCC
AATCCCAGAAACCCTATCTCAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCT

>10 *Enterobacter asburiae* strain LBQSKN4

GGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAG
GGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACAATCCCTAGCTGGT
CTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTG
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GCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGG
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GAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCG
GAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGT
GGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGAGACA
GGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGC
CGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGC
TACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCC
ATGAAGTCGGAATCGCTA

>11 *Pseudomonas* sp. LBQSKN5

GCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGA
AAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGGCGACGATCCGTAA
CTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAA
GCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGT
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GGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGA
GGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAG
TGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAG
CATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTG
ACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACG
TTATGGTGGGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACAC

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ACGTGCTACAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCTCACAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTC
GACTGCGTGAAGTCGGAATCGCT

>12 *Pseudomonas* sp. LBQSKN6

GGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGTGGGGG
ATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGA
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GTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCGAGCTAGAGTACGGTAGAGGGTGGTGG
AATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAA
GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAG
CTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
TTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGT
GCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTG
GGCACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCTGGGCTACACACGTGCTA
CAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCTCACAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGT
GAAGTCGGAATCGCT

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CHAPTER 5

CONCLUSION AND FUTURE WORK

PAHs and EDCs distribution, their effects towards living organisms and microorganisms capable of degrading PAHs and ECDs, and the mechanisms of degradation have been thoroughly documented by the developed world. Information on these matters is however lacking from Africa. In this direction, our study is first of its kind from South Africa, in which we successfully enriched and isolated indigenous bacterial strains capable of using nonylphenol and fluoranthene as sole carbon source. The area where soil samples were collected, previously reported to be polluted with PAHs, resulted in the isolation of bacterial species capable of degradation of the both fluoranthene and nonylphenol, suggesting that these organisms have the capability to degrade a variety of xenobiotic chemicals.

Further investigation on the capacity of the isolates to degrade different EDCs and PAHs are currently under investigation. The results presented in this study will lead to isolation and characterization of microorganisms from different parts of South Africa capable of degrading different PAHs and EDCs, and thus enrich PAHs and EDCs-related information from Africa.

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RESEARCH OUTPUTS

Research articles

Isolation and characterization of endocrine disruptor nonylphenol-using bacteria from South Africa

Journal:	*South African Journal of Science*
Manuscript ID	SAJS-2016-0287
Manuscript Type:	Research Article
Significance of the Work:	First report on endocrine disruptor nonylphenol-using bacteria from South Africa, Study will pave the way for further exploration of endocrine disruptors degrading microbes from South Africa, Six bacterial species capable of using nonylphenol as carbon source isolated, Phylogenetic analysis of isolates were carriedout using 16S rRNA gene sequence, HPLC analysis revealed degradation of Nonylphenol by all six bacterial isolates
Keywords:	Bacteria, Biodegradation, Endocrine disrupting chemicals, Nonylphenol, Phylogenetic analysis

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Molecular evolutionary dynamics of cytochrome P450 monooxygenases across kingdoms: Special focus on mycobacterial P450s

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RESEARCH ARTICLE

Genome-Wide Annotation and Comparative Analysis of Cytochrome P450 Monooxygenases in Basidiomycete Biotrophic Plant Pathogens

Lehlohonolo Benedict Qhanya[1☯], Godfrey Matowane[1☯], Wanping Chen[2☯], Yuxin Sun[2], Elizabeth Mpholoseng Letsimo[1], Mohammad Parvez[1], Jae-Hyuk Yu[3], Samson Sitheni Mashele[1], Khajamohiddin Syed[1]*

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Diversity and evolution of cytochrome P450 monooxygenases in Oomycetes

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Mopeli Marshal Sello[1], Norventia Jafta[1], David R Nelson[2], Wanping Chen[3], Jae-Hyuk Yu[4], Mohammad Parvez[1], Ipeleng Kopano Rosinah Kgosiemang[1], Richie Monyaki[1], Seiso Caiphus Raselemane[1], Lehlohonolo Benedict Qhanya[1], Ntsane Trevor Mthakathi[1], Samson Sitheni Mashele[1] & Khajamohiddin Syed[1]

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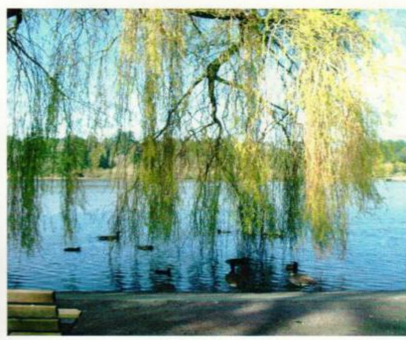
Conference Attendance

13th International Symposium on Cytochrome P450 Biodiversity and Biotechnology

22-26 July, 2016
Vancouver, B.C., Canada



Program and Abstracts

P 84-S2

Genome-wide annotation and comparative analysis of cytochrome P450 monooxygenases in basidiomycete biotrophic plant pathogens

Qhanya, Lehlohonolo Benedict;[1] Matowane, Godfrey;[1] Mashele, Samson Sitheni;[1] Chen, Wanping;[2] Yu, Jae-Hyuk;[3] Syed, Khajamohiddin[1]

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Fungi are an exceptional source of diverse and novel cytochrome P450 monooxygenases (P450s), heme-thiolate proteins, with catalytic versatility. The present study demonstrated the presence of unique P450 family patterns in basidiomycete biotrophic plant pathogens that could possibly have originated from the adaptation of these species to different ecological niches (host influence). Systematic analysis of P450s in basidiomycete biotrophic plant pathogens belonging to three different orders, revealed the presence of numerous putative P450s ranging from 267 to 14. Analysis of P450 families revealed the presence of 41 new P450 families and 27 new P450 subfamilies in these biotrophic plant pathogens. Order-level comparison of P450 families between biotrophic plant pathogens revealed the presence of unique P450 family patterns in these organisms, possibly reflecting the characteristics of their order. Comparison of P450 families with basidiomycete non-pathogens confirmed that biotrophic plant pathogens harbour the unique P450 families. The CYP63, CYP5037, CYP5136, CYP5137, CYP5221, CYP5233 and CYP5341 P450 families were expanded in the biotrophic plant pathogens. The present study initiates our understanding of P450 family patterns in basidiomycete biotrophic plant pathogens.

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DMP 21

Genome-wide annotation and comparative analysis of cytochrome P450 monooxygenases in basidiomycete biotrophic plant pathogens

L.B. Qhanya[1], G. Matowane[1], W. Chen[2], Y. Sun[2], E.M. Letsimo[1], M. Parvez[1], J.H. Yu[3], S.S. Mashele[1] and K. Syed[1]

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Fungi are an exceptional source of diverse and novel cytochrome P450 monooxygenases (P450s), heme-thiolate proteins, with catalytic versatility. Agaricomycotina saprophytes have yielded most of the available information on basidiomycete P450s. This resulted in observing similar P450 family types in basidiomycetes with few differences in P450 families among Agaricomycotina saprophytes. The present study demonstrated the presence of unique P450 family patterns in basidiomycete biotrophic plant pathogens that could possibly have originated from the adaptation of these species to different ecological niches (host influence). Systematic analysis of P450s in basidiomycete biotrophic plant pathogens belonging to three different orders, Agaricomycotina (*Armillaria mellea*), Pucciniomycotina (*Melampsora laricis-populina, M. lini, Mixia osmundae* and *Puccinia graminis*) and Ustilaginomycotina (*Ustilago maydis, Sporisorium reilianum* and *Tilletiaria anomala*), revealed the presence of numerous putative P450s ranging from 267 (*A. mellea*) to 14 (*M. osmundae*). Analysis of P450 families revealed the presence of 41 new P450 families and 27 new P450 subfamilies in these biotrophic plant pathogens. Order-level comparison of P450 families between biotrophic plant pathogens revealed the presence of unique P450 family patterns in these organisms, possibly reflecting the characteristics of their order. Further comparison of P450 families with basidiomycete non-pathogens confirmed that biotrophic plant pathogens harbour the unique P450 families in their genomes. The CYP63, CYP5037, CYP5136, CYP5137 and CYP5341 P450 families were expanded in *A. mellea* when compared to other Agaricomycotina saprophytes and the CYP5221 and CYP5233 P450 families in *P. graminis* and *M. laricis-populina*. The present study revealed that expansion of these P450 families is due to paralogous evolution of member P450s. The presence of unique P450 families in these organisms serves as evidence of how a host/ecological niche can influence shaping the P450 content of an organism. The present study initiates our understanding of P450 family patterns in basidiomycete biotrophic plant pathogens.

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Media coverage

pressreader

CUT's groundbreaking discovery

New discovery may help fight aquatic animal infection

The New Age (Free State) · 17 Jun 2015 · News

leading: Research team leader Dr Khajamohiddin Syed and faculty of environmental science acting dean professor Sam Mashele.

pioneers: The CUT science students who helped discover the drug that may help fight aquatic animal infection.

Acting dean of the faculty Prof Sam Mashele said some of the deadliest pathogens in the world were found in the fish farming industry.

He said the discovery was made by researchers from the unit for drug discovery in the institution.

"For many years researchers across the world have been trying to understand these micro- organisms in order to control the disease and develop novel drugs against these pathogens, and CUT researchers are leading the way in finding solutions that will bring an end to this socio-economic challenge facing aquatic farming," Mashele said.

He said microorganisms were widely known in the scientific world and they continued to wreak havoc on the aqua farming sector worldwide.

"They are considered the deadliest of pathogens, causing diminished production of aquatic food," Mashele said.

The team of researchers who discovered the drug were led by Dr Khajamohiddin Syed.

"We collaborated with highly acclaimed international scientists such as professors David Nelson from the University of Tennessee in the US, ae-Hyuk Yu from the University of Wisconsin-Madison and Dr Wanping Chen from Huazhong Agricultural University in China," Mashele said.

"The university researchers are investigating solutions that would sustain the aquatic resources while helping to increase high production levels of aqua farming for commercial purposes, food security and poverty alleviation."

He said their work highlighted the important role aqua farming plays in promoting healthy living and in fighting poverty and hunger.

Syed said aqua farming was a big industry across the world and it involved the farming of fish, shrimps, prawns, squid and octopus and is considered by the UN as an important sector that can provide a livelihood for more than 60 million people in Africa and Asia.

"Consumption of these animals remains a vital source of protein and essential nutrients, especially for developing countries, where they constitute almost half of the total value of their traded communities," Syed said.

"The results of this study have been accepted for publication in the Nature Publication Group journal Scientific Reports, a prestigious multidisciplinary scientific international journal with an impact factor of 5.1."

dag 16 Junie 2015 **Volksblad**

Navorsers by SUT maak deurbraak

Kaydene Davids

'n SPAN navorsers aan die Sentrale Universiteit vir Tegnologie (SUT) het 'n wegspringpunt vir 'n middel ontdek wat groot potensiaal toon om siektes, wat deur parasiete veroorsaak word, in seediere te bestry.

Navorsers van oor die wêreld heen het jare lank probeer om die swamagtige mikro-organismes te verstaan ten einde die siekte, wat onder meer in visse, garnale, inkvisse en seekatte voorkom, te beheer en 'n middel daarteen te ontwikkel.

Maar meer as 50 jaar lank was dit tevergeefs – totdat die navorsers aan die SUT en wêreldbekende wetenskaplikes koppe bymekaargesit het.

Dan Maritz, woordvoerder, sê hul span jong navorsers onder leiding van prof. Samson Sitheni Mashele en dr. Khajemohiddin Syed loop nou voor om oplossings te vind wat die maatskaplik-ekonomiese uitdagings wat die siektes vir veral die visserybedryf bied, te beëindig.

Syed sê miljoene seediere vrek jaarliks weens siektes wat deur parasiete veroorsaak word en dit het 'n regstreekse invloed op die visserybedryf.

Volgens hom is dit alombekend in die wetenskaplike wêreld dat die parasiete (*oomycete*) chaos in die visserybedryf wêreldwyd saai en die lewensduur van die diere aansienlik verkort.

Die navorsers het die *P450 monooxygenase*-proteïen in 13 patogeniese genome bestudeer en bevind dit kan as wegspringpunt gebruik word vir 'n middel om die swamagtige mikro-organismes te beveg.

Die navorsers ondersoek dit nou om oplossings te vind om seehulpbronne te beskerm.

Maritz sê hul (die navorsers se) werk beklemtoon die belangrikheid van die visserybedryf in die bevordering van gesonde lewe en die stryd teen armoede en honger.

Die uitkoms van die navorsing is reeds vir plasing in die *Nature Publication Group Journal* aanvaar.

■ Die wêreldbekende wetenskaplikes wat ook deel van die navorsingspan is, sluit in prof. David R. Nelson van die Universiteit van Tennessee, prof. Jae Hyuk Yu van die Universiteit van Wisconsin-Madison, en dr. Wanping Chen Huazhong van die Landbou-universiteit in China.

Die span navorsers aan die Sentrale Universiteit vir Tegnologie (SUT) wat die wegspringpunt vir 'n middel ontdek het om siektes wat deur parasiete veroorsaak word, in seediere te bestry. Hier is van links, voor: dr. Khajamohiddin Syed; middel: Lorato Mongale, dr. Ntsoaki Malebo, Poojah Jawallapersanel en Mohammad Parvez; agter: prof. Sitheni Mashele, Mopeli Sello, Seiso Raselemana, Lehlohonolo Qwanya, Mthakathi Ntsane, Thapelo Makhele, Euginiah Rampana en Richie Monyaki.

Foto: MLUNGISI LOUW

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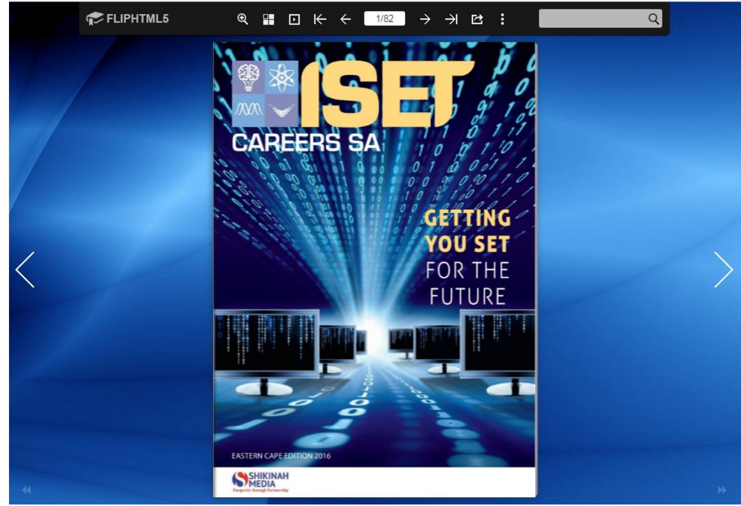

Q&A

What factors influenced you to choose this career path?

What kind of education, training, or background does your job require?

What do you do? What are the duties/functions/responsibilities of your job?

What is a typical day like for you?

What part of this job do you personally find most satisfying?

What has been the greatest challenge you have faced in your career to date?

What kinds of accomplishments tend to be valued and rewarded in this field?

Publications, contributions and collaborations in highly rated journals.

What are the major qualifications for success in this occupation?

A Ph.D. in Microbiology & Biochemistry.

What particular skills or talents are essential to be effective in your job?

What advice would you offer learners who are interested in pursuing this career path?

What is the future of the field in terms of new and expanding opportunities?

What educational preparation would you recommend for someone who wants to advance in this field?

What sorts of changes are occurring in your occupation?

Who would you say has been the most help in your career? How did they help you?

"THE MOST SATISFYING PART OF WHAT I DO SHARING MY RESEARCH NATIONALLY OR INTERNATIONALLY, TO SHARE AND LEARN FROM THE EXPERTS IN MY FIELDS OF STUDY."

How can I evaluate whether or not I have the necessary skills for a position such as yours?

Can you suggest some ways a learner could obtain this necessary experience?

What exactly does the word 'success' mean to you?

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