ISOLATION AND CHARACTERISATION OF MICROBES INVOLVED IN PHOSPHATE SOLUBILIZATION AND PESTICIDE DEGRADATION FROM SELECTED AGRO ECOLOGICAL ZONES OF MALAWI

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156/82140/2015

COLLEGE OF BIOLOGICAL AND PHYSICAL SCIENCES

UNIVERSITY OF NAIROBI

2018

A thesis submitted to Graduate School of the University of Nairobi in partial fulfillment of the requirement for the degree of Master of Science in Biotechnology at The Centre of Biotechnology and Bioinformatics

DECLARATION

I declare that this research is my own work and has not been submitted for examination in any other university

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ACKNOWLEDGEMENT

I would like to give thanks to God for effective grace to carry out this research work. First and foremost, I would like to express my sincere appreciation to my supervisors Dr Aboge, Dr Obiero and Dr Mpeketula, for synergistic relentless support throughout the course of this research. Dr. Obiero valuable and constructive suggestions have guided me through many difficulties and challenges faced during my masters. His willingness to give precious time and effort in this project highlighted his generosity and benevolent personality, which has been very much appreciated.

Their guidance and endless encouragement helped me all the way through. The success and final outcome of this research project would simply not have been possible without them. Advice and laboratory supervision given by Dr. Mpeketula and Dr. Obiero

I wish to acknowledge the help provided by the following colleagues from Department of Biology Chancellor College; Kwenda, Kazembe and Kondwani for technical support and assistance in the lab

This material is based upon work supported by CHASATHA BIOTECH and the United States Agency for International Development, as part of the Feed the Future initiative, under the CGIAR Fund, award number BFS-G-11-00002, and the predecessor fund the Food Security and Crisis Mitigation II grant, award number EEM-G-00-04-00013.

Last but not least, I would like to express my deepest gratitude and appreciation to the following people my mother B Chilongo, H Gondwe, Jennet, Mr. and Mrs. Kanyada, James, Chindikani, Queen, Thandi, Miracle, Robert, Mbeere for their support both morally and financially. Without all of your constant encouragement and support I would have never made it this far.

ABSTRACT

Microbes involved in phosphate solubilising and pesticide degradation were isolated from different agro ecological zones of Malawi to solve problems of available phosphorous deficiency and xenobiotics of green revolution technologies. Phosphorous is deficient only in soluble state in tropical countries and the use of green revolution technologies like pesticides application interfere with rhizospheric microbes that help in phosphate solubilisation due to xenobiotics production. These compounds also have an impact on bio-magnification, and environment. It is therefore important to investigate synchronised strategies that will improve the utilisation of fixed phosphate in soil-plant systems and also degradation of xenobiotics using microorganisms. Extensive research done show prospect microbes for biofertilisation and bioremediation but little was known about presence of Phosphate Solubilising Microbes in Malawi. In this study microbes were isolated for solubilisation of phosphates using pikovskaya's medium and pesticide degradation for their capacity to utilise pesticide as sole carbon source complimented by presence laccase gene. Isolates were selected based on solubilisation of inorganic tricalcium phosphate, soil and rock phosphates and also characterised for the presence of Plant Growth Promoting Traits. Biochemical test and molecular characterisation using 16S rRNA and 18S rRNA genes for bacteria and fungus respectively were used in identification. Six strains that had higher solubilisation index of 1.5 and 30 microorganisms that utilised pesticides as sole carbon source were isolated. The strains for P solubilisation were identified as *Aspergillus niger*, *Enterococcus casseliflavus, Klebsiella pneumoniae, E. cloacae, Pseudomonas putida* and *Penicillium janthinellum* while for degradation of pesticides *Mucor irregularis, Fusarium oxysporum, Meyerozyma caribbica, Aspergillus parasiticus* for fungus and genus *Klebsiella, Pseudomonas, Pantoea, Bordetella and Enterobacter* for bacteria. One strain of *Klebsiella pneumoniae* was found to degrade xenobiotics and solubilise P while other strains had PGP traits besides potential in bioremediation and biofertilisation. The study reveals new strains and shows diversity at strain level for Malawian isolates. Isolated microbes showed strong statistically significant difference in solubilising of P and the values were greater than commercial strains which indicate that indigenous microbes have high potential in solubilisation of P and degradation of xenobiotics. The study also reveals that evolutional relationships of isolated microbes are based on agro ecological zones and xenobiotics present. The study concludes that indigenous microbes have higher potential in P solubilisation and bioremediation.

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LIST OF ABBREVIATIONS

ABTS	2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
ACC deaminase	1-aminocyclopropane-1-carboxylate deaminase
Al_3PO4	Aluminum Phosphate
ANOVA	Analysis of Variance
BNF	Biological Nitrogen Fixation
CAS	
CEBIB	Centre for Biotechnology and Bioinformatics
CFU	Colony Forming Units
Fe3PO4	Iron Phosphate
HCN	hydrogen cyanide
IAA	Indol Acetic Acid
ML	Maximum Likelihood
MSM	Mineral Salt Medium
NA	Nutrient Agar
NFB	Nitrogen-Fixing Bacteria
NJ	Neighbour Joining
P	Phosphorous
PDA	Potato Dextrose Agar
PCR	Polymerase Chain Reaction
PGPM	Plant Growth Promoting Microbes
PGRT	Plant Growth Regulatory Traits
PSB	Phosphate Solubilising Bacteria
PSF	Phosphate Solubilising Fungi
PSM	Phosphate Solubilising Microbes
PVK	Pikovskaya's
SDA	Sabouraud Dextrose Agar
SI	Solubilisation Index
RP	Rock Phosphate
RUP	Restricted Use Pesticide

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Soil fertility is complex and dynamic due to interaction of several factors involved in production. Attempt to increase agricultural productivity from a degrading land and ecological footprint has huge negative impact on agro-ecosystems (Wang *et al.*, 2016)**.** The current strategy for improving and maintaining productivity, involves the use of green revolution techniques, that are based on inorganic chemicals such as pesticides and fertilisers (Sumatera, 2016).

Green revolution technologies promote usage of inorganic chemicals in which inorganic fertilisers provide macronutrients such as nitrogen (N), phosphorous, and potassium (K) (Tortella *et al.*, 2010). Phosphate (P) is an essential element and is second to N for limiting plant growth and its cycle in soil involves both organic and inorganic P (Chotchutima *et al.*, 2016). Most important metabolic processes involve P in their activities including energy transport, respiration of plants, signal transduction, growth and cell division, photosynthesis and macromolecular biosynthesis. The use of inorganic fertilisers containing phosphorous results into long-term accumulation of P, together with heavy metals, such as cadmium and fluoride. These contaminants can be passed in the food chain and are potentially toxic to animals and humans. Excessive inorganic fertilisers also accelerate eutrophication via leaching and run-off to waterways (Hundey *et al.*, 2016; Savci, 2012).

In Malawi, agriculture soils contain high reserves of insoluble P, which has accumulated as a result of persistent application of phosphate-based inorganic fertilisers and from rock phosphate

(Mikkelsen, 2004). The current levels of total P is in the range of 400–1200 mg/kg in the soil but soluble P is extremely deficient with the concentration which can't be available for crops (Aferi, 2014). Large portion of soluble P from other sources is rapidly immobilised and fixed because of high sorption to form compounds like tricalcium phosphate, aluminum phosphate (Al_3PO_4) and iron phosphate (Fe_3PO_4) (Yadav *et al.*, 2015; Song *et al.*, 2008). Strong bonds between P and iron (Fe) or aluminum (Al) in acidic soils and with Ca in alkaline soils leads to high sorption (Gupta *et al.*, 2015).

Naturally soil microorganisms called phosphate solubilising microorganisms (PSM) transform organic and fixed (inorganic) P by mineralising and solubilising process mainly in the rhizosphere (Tortella *et al.*, 2010)**.** Solubilisation of insoluble P forms is mediated by three processes; ion exchange reactions, chelation and organic acid production (Sharma *et al.*, 2013a). PSM which is a combination of Phosphate Solubilising Fungi (PSF) and Phosphate Solubilising Bacteria (PSB) are environmental friendly strategy for the provision of less costly P to crops (Souza *et al.*, 2015; Sharma *et al.*, 2013b)**.**

Green revolution technologies have raised environmental concern due to xenobiotics contamination to aquatic life (Hundey *et al.* 2016; Maisnam and Abhik 2014; Savci, 2012). Also, rhizosphere has been contaminated by components of pesticides and fertilisers which renders usage of PSM and other biofertilisation useless (Savci, 2012)**.** Pesticides are of paramount importance for controlling weeds and insect pests by farmers (Carvalho, 2017). Inorganic chemicals contain xenobiotics which have impact on beneficial microbes, bio-magnification (Kucharski *et al.*, 2014), acidification of the soil (Savci, 2012; Hundey *et al.*, 2016) and ecosystems (Thatheyus and Selvam, 2013). Pesticide have direct impact to yield and yield components because they make the environment not conducive for soil beneficial microorganism as well as interfere with plant growth and nutrition (Ahmad and Khan 2008).

Bioremediation is gaining momentum for its low cost and environmentally friendly impact compared to other methods (Annibale *et al.*, 2006). Fungi and bacteria display many of these features and could be important components of biotechnologies designed to remediate polluted soil and water. Bioaugmentation, in which biostimulation of indigenous microflora, is used effectively works when dealing with heavily or historically contaminated sites. Some bioremediation fungi may also act as biofertilisers by using natural processes of nitrogen fixation, solubilising phosphorus, and production of phytohormones (Barroso and Nahas, 2013; Sharma *et al.*, 2013a)

The purpose of the study was to isolate potential microbial strains for use as biofertilisers to solubilise phosphorous and also as bioremediation tool to degrade commonly used pesticides in contaminated soils of Malawi as illustrated in figure 1. In this study, isolation and characterization of PSM and pesticide degrading microbes were examined with the hope of increasing available P and decreasing pesticide contamination in the environment. Isolates were evaluated for their capability to solubilise insoluble P from Rock Phosphate (RP) and field soil and as well as assessed for synergistic effect of co-inoculation with rhizobium. Quantitative and qualitative values of P solubilisation of isolates were carried out using different media. The outcome of the study suggested that isolated microbes have the potential to be used in biofertilisation and bioremediation as compared to commercial strains in Malawi environment.

1.2 Literature review

1.2.1 Phosphorous levels and plant growth promoting microbes in tropical countries

Plant Growth Promoting Microbes (PGPM) is rhizosphere and soil microbes that help in plant growth by several mechanisms. Phosphorous is the second important plant macronutrient after N which markedly affects the overall plant development and growth. It is present in tropical soils at concentration of between 400 and 1200 mg/kg of soil (Mikkelsen, 2004). The level of available P in the soil is 1 ppm or less, which is low. Inorganic compounds of P usually contain manganese (Mn), Fe and Al, in acidic soils while Ca and magnesium are found in alkaline soil. Between 70–95% of applied inorganic soluble P is easily fixed in the soil by Ca^{2+}, Mn, Fe and Al cations to form compounds like $Ca_3(PO_4)_2$ and Al $(PO_4)_3$ (Gupta *et al.*, 2012; Song *et al.*, 2008). Total phosphorous built up in these tropical soils can sustain P demand by crops for about 100 years (Adhya *et al.*, 2015). Excess P application is contributes to high P potential loss through subsurface flow or on land-surface causing eutrophication to freshwater (Sharma *et al.*, 2013b; Savci, 2012).

Some rhizospheric soil microorganism change fixed P to soluble P hence enabling crops access some soluble (available) P symbiotically. These microorganisms are called PSM and besides being ecofriendly option for provision of cheap P to crops they also facilitate the growth and development of crops by several other mechanisms. These mechanisms include uptake of trace elements and nutrients, disease suppression (Jahangir *et al.*, 2016) and plant growth promoting traits (Thimmappa *et al.*, 2016; Hameed, 2015; Sharma *et al.*, 2013b). The PSM perform best in areas deficient in soluble P but having high insoluble P to increase crop yield (Dinesh *et al.*, 2015; Bhardwaj *et al.*, 2014; Pingale and Virkar, 2013). Plants can only take up orthophosphate ions as nutrients, which are soluble form of P.

1.2.2 Phosphate Solubilizing Microbes

The insoluble phosphate forms are converted to soluble P by PSM (Aferi, 2014) and making it available to plants by mineralising and solubilising processes (Khan *et al,*. 2009; Khan *et al.*, 2007). The main proposed theories of P solubilisation are proton-enzyme and acid production theory. Acid production theory which is widely recognized, propose that organic acids (OA) secreted by microorganisms are responsible for P solubilisation (Liu *et al.*, 2016). Various OA have been identified and quantified in relationship to solubilisation process. These acids include malic, gluconic, acetic , ketogluconic, lactic, citric, and succinic (Stella and Halimi, 2015). Acidification of soil by microbes is responsible for the release of Phosphate ions through H^+ substitution for Al and Ca^{2+} from insoluble particles (Liu *et al.,* 2016).

The use of less expensive sources of P accompanied by environmental friendly methods is advocated for sustainable agriculture (Bhattacharya *et al.*, 2015; Dinesh *et al.*, 2015) . RP has been used as source of P for plants but promotion has been affected due to poor solubility. Many studies have found Fungi of Aspergillus and Penicillium genera solubilize rock phosphate in-vitro (Sane and Mehta, 2015). Some researchers have shown that combinatorial application of PSM and RP or co-inoculation with nitrogen fixing rhizobia is important to reduce depletion of high-grade RP reserves (Investigación *et al.*, 2015; Sane and Mehta, 2015).

Microbial mediated RP solubilisation technology has several advantages over conventional chemical fertilisers for sustainable agriculture. These advantages are as follows: (1) microbial products are considered safer than inorganic fertilisers, (2) no toxic substances or microbes can accumulate in food chain; and (3) fast and self-replication through biostimulation (Investigación *et al.*, 2015; Goudjal *et al.*, 2014; Mehrvarz and Chaichi, 2008).

1.2.3 Microbial biodegradation of pesticides

1.2.3.1 Pesticides

Pest infestation is one of the serious problems for decrease in yield and yield components for all crops. To minimize losses several methods of pest control are used such as mechanical, cultural, biological and chemical control (Liang *et al.*, 2014). Chemical control method is also advantageous because it's quick, more effective, time and labor saving method than other methods (Khan *et al.*, 2016; Jhala *et al.*, 2015). Currently the use of different pesticides including herbicides and insecticides in one field is essential to control diverse weeds and insects due to compatibility effectiveness of pesticides (Jhala *et al.*, 2015).

However, regardless of benefits these synthetic organic chemicals contain xenobiotics which have a negative impact on beneficial microbes, bio-magnification (Kucharski *et al.*, 2014) and ecosystems (Thatheyus and Selvam, 2013). They also have direct impact on yield because they make the environment not conducive for beneficial soil microorganisms including PSM, and interfere with plant growth and nutrition (Ahmad and Khan 2008)**.**

1.2.3.2 Biodegradation

There are several clean-up mechanisms for pesticides degradation including chemical treatment, volatilization and incineration (Kikuchi and Tanaka, 2016; Mohamed *et al.*, 2013). These methods have met public opposition due to association with large volumes of acids and alkalis, which create a problem of disposal as well as emissions of toxic chemicals (Eman *et al.*, 2013). These methods are also expensive, inefficient, non-convenient and economically not feasible because the contaminated soil has to be excavated and transported to a storage area where it can be processed (Guermouche *et al.*, 2015).

Therefore, biological technique involving biodegradation of organic compounds by microorganisms have been developed. The technique involves the use of microorganisms, either naturally occurring or introduced, to degrade xenobiotics by a process called bioremediation (Bhawana and Fulekar 2012; Harms *et al.*, 2011a). Some microbes involved in bioremediation may also act as biofertilisers by using natural processes of N fixation, P-solubilisation, and production of phytohormones (Ahuja *et al.*, 2016). Bioaugmentation through biostimulation of indigenous microflora is also another promising technique when dealing with heavily contaminated fields. The use of filamentous fungi is more effective in bioremediation however, most studies prefer combination of both (Bhawana and Fulekar, 2012)**.**

In Malawi, the most commonly used pesticides are glyphosate, acetochlor (harness), dimethoate and cypermethrin. Glyphosate product of Monsanto is a broad-spectrum systemic herbicide while acetochlor is pre-emergence herbicide (Tahir *et al.*, 2017). Acetochlor is one of Restricted-Use-Pesticide (RUP) and is classified in level I (Highly toxic) because it has an effect on chromosomal aberrations and induction of micronuclei and also pose a risk to aquatic organisms mainly amphibians and fish (Hayes *et al.*, 2006). Product claim of selective toxic to insect’s pest only, synthetic pyrethroid like cypermethrin at levels same to those used for controlling mosquito and black fly, are also extremely toxic to aquatic organisms. Cypermethrin has also been linked to the disruption of endocrine system, reproduction and sexual development, and induction of breast cancer (Carvalho, 2017; Piotrowska-seget, 2016; Thatheyus and Selvam, 2013). Dimethoate are organophosphorus insecticides that are neurotoxic through effect on nervous system and inhibition of the Acetyl cholinesterase (AChE) (Shubhamsingh and Tejashree, 2014).

Studies in morphological, behavioral and physiological changes are dominating in toxicity assessments in unicellular organisms, along with fish rotifers, rodents and insects (Shubhamsingh and Tejashree, 2014). Studies show that indigenous microorganisms are responsible for detoxifying and degrading of xenobiotics residues in contaminated environment. Many studies have isolated potential microbes in bioremediation of glyphosate, cypermethrin, dimethoate and Acetochlor (Harms *et al.*, 2011b), but there is no research describing biodegradation of pesticides by indigenous microbes in Malawi or Southern African Development Community (SADC).

Microbes degrade organic compounds using a range of extracellular oxidoreductases which are relatively nonspecific in its activity (Castilho *et al.*, 2009). Evolution has supported these microbes growth on recalcitrant substrates of random structure that are not accessible by other microbes (Harms *et al.*, 2011b). Extracellular oxidoreductases are a source of a large number of secondary metabolites, enzymes, ergotrate, statins, penicillin and laccase enzymes (Ahuja *et al.*, 2016)**.** Among enzymes, laccase are the most commonly produced and are of significant in bioremediation and other applications (Castilho *et al.*, 2009).

1.2.3.3 Enzymes involved in biodegradation

Laccase is copper containing oxidase enzymes that's found in many plants, fungi, and microorganisms (Castilho *et al.*, 2009). Potential applications of laccases are related to bioremediation and waste treatment like degradation and detoxification of recalcitrant wastewater pollutants containing EDCs, chlorophenols, PAhs, pesticides and others (Nasir *et al.*, 2015). Laccase is also involved in detoxification of hazardous compounds arising from coal processing such as Sulphur-containing compounds, phenols, lignolytic degradation, detoxification studies, plant pathogenesis, odour control in decomposition of wastes, and pigment production (Viswanath *et al.*, 2016; Rohilla and Salar, 2012; Kunamneni *et al.*, 2007). The expression of laccase is influenced by several factors including nature and concentration of carbon source, nitrogen source, temperature, pH etc. (Piscitelli *et al.*, 2016).

Laccases has several inhibitors of its enzymatic activity such as cyanide, thiocyanide, halides, fluoride, hydroxide and azide (Kunamneni *et al.*, 2007)**.** Heavy metals and xenobiotics induce laccase production due to the presence of receptors (putative *cis*-acting responsive elements) in the promoter regions of the laccase encoding genes (Castilho *et al.*, 2009).

In addition to P solubilisation, some PSM and degrading pesticides have also been known to produce phytohormones (Ahmad and Khan 2008) for plant growth and for biocontrol of plant pathogens (Almaghrabi *et al.*, 2013; Beneduzi *et al.*, 2012).

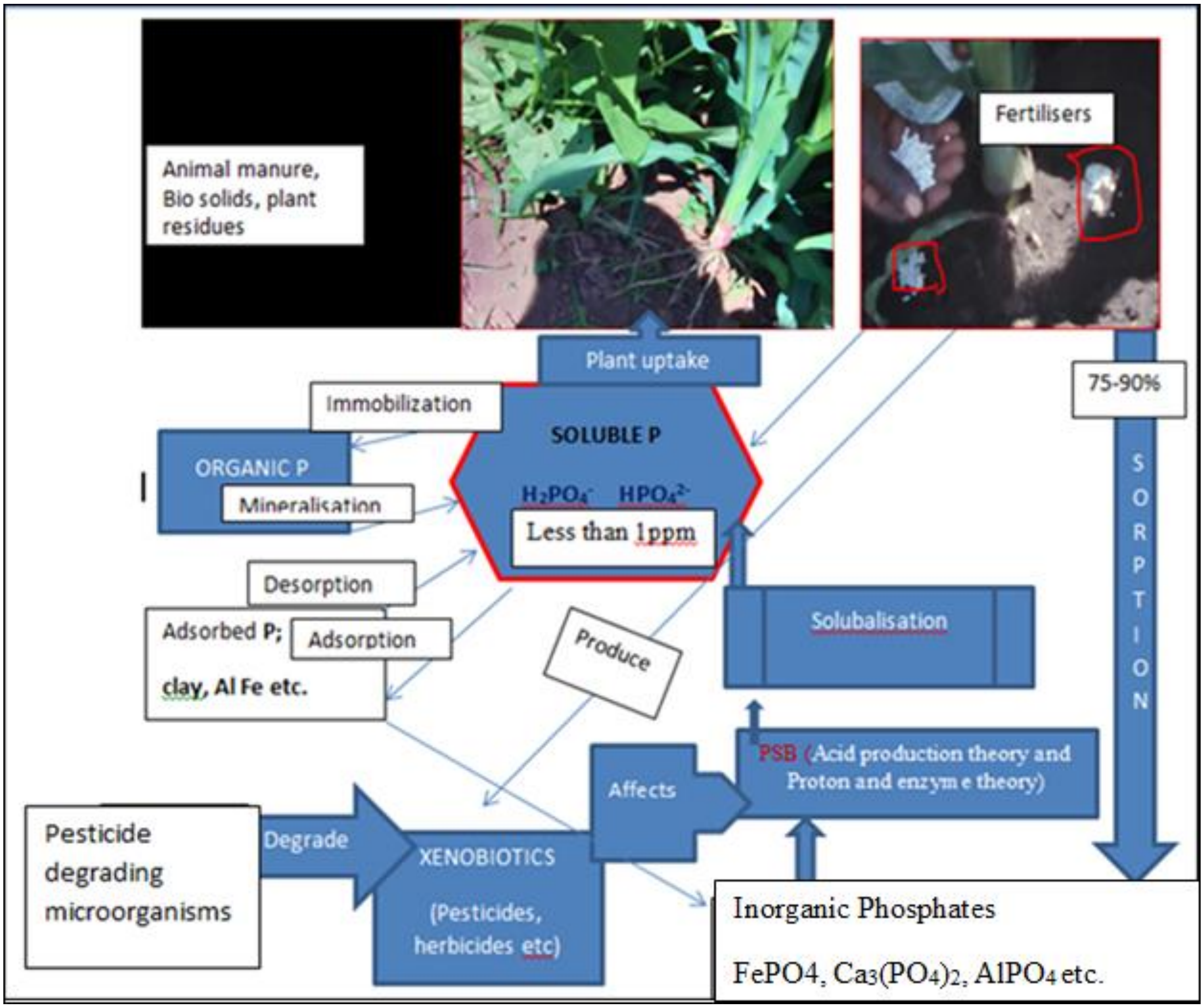

Figure 1: A proposed overview of xenobiotics effects, chemical, physical and microbiological processes influencing the direction of phosphorous in the soil according to this study.

1.2.4 Other traits of growth-promoting rhizosphere microorganisms

Rhizosphere microorganisms promote plant growth and are categorized into three major groups; nitrogen-fixing bacteria, mycorrhizal fungi, and PGPR (Parray *et al.*, 2013). Mutualism and symbiosis exist within the rhizosphere where plants provide carbon sources for microorganisms via root exudates (Castro-Sowinski *et al.*, 2007) and PGPR provide nutrients, hormones and antibiotics that promote plant growth (Abbas *et al.*, 2013; Beneduzi *et al.*, 2012).

1.2.4.1 Production of Indole Acetic Acid

Indole Acetic Acid (IAA) is well known as an important plant growth-promoting factor due to its role in the initiation of cell division, differentiation, root elongation and proliferation (Kavamura *et al.*, 2013). PGPR can synthesise IAA via three major tryptophan-dependent pathways (Souza *et al.*, 2015; Ahemad and Kibret 2014). Auxin has been detected in liquid culture supernatants of some rhizobacteria and has been suggested as a signaling molecule that activates several plant colonisation and adaptation genes (Ahemad and Kibret, 2014).

1.2.4.2 Production of siderophore

Fe is essential for metabolic function but is not readily bioavailable because of its low solubility of the iron-oxide forms in the soil (Barroso and Nahas, 2013). Soil microorganisms secrete siderophores to facilitate cellular absorption iron from their environment. Five hundred different siderophores have been documented, which are high affinity Fe^{3+} chelating compounds, low molecular weight and form ferri-siderophore complexes (Shobha and Kumudini, 2012). These compounds when produced by PGPR are important because they inhibit pathogenic fungal growth in the rhizosphere due to lower affinity for Fe^{3+} fungal siderophores (Saraf *et al.*, 2014). *Pseudomonas fluorescens* strains MPF47 suppress the proliferation of the fungal pathogen *Rhizoctonia solani* and facilitated iron uptake by plants. Siderophore producing rhizobacteria as bioinoculants have potential to replace conventional chemical fungicides for suppressing root diseases and promoting plant growth (Pérez-Montaño *et al.*, 2014).

1.3 Problem statement and justification

Most tropical and subtropical agriculture soils have large reserves of insoluble P which is due to regular applications of phosphate based inorganic fertilisers and rock minerals (Aferi, 2014;

Mikkelsen, 2004). Phosphate based fertilisers have an impact on bacterial and fungal activity because they contain heavy metals (Hundey *et al.*, 2016). Efficiency of applied P based fertilisers is usually less than 20%, suggesting that the accumulated P in tropical soils would be enough to sustain potential yields and yield components for about 100 years (Khan *et al.*, 2007).

Large quantity of P is available in insoluble form due to high sorption of P to form compounds like $Ca_3(PO_4)_2$, and $Al(PO_4)_3$. Formation of these compounds have led to deficiency of soluble P in such soils with the levels of 1 ppm or less documented (Aferi, 2014) and has a big impact on agricultural productivity and food security in Malawi. On the other hand, rhizosphere xenobiotics due to pesticides makes environment not favourable for PSM. The use of commercial PSM and other biofertilisers efficiency is low due to edaphic biotic and abiotic stress.

A number of studies exploiting indigenous microbes have been done by characterising microbes involved in P solubilisation and pesticide degradation. The indigenous microbes have been commercialized for P solubilization (Nadu *et al.,* 2013). However, in Malawi, the actual characteristics of soil microbes involved in P solubilisation and pesticide degradation remains unknown yet this information is needed for getting microbes that may help in solubilising rock P and biodegrade harmful pesticides. The current study is important because it has provided valuable preliminary data on the microbes involved in phosphate solubilisation and pesticide degradation in Malawi. This may go a long way in developing biofertilisers and bioremediation strategies. The data generated here may also provide a basis for further studies on co-inoculation, bioargumentation and biostimulation.

1.4 Study hypotheses

The soil profiles of different ecological zones in Malawi have significant impact on microbes involved in phosphate solubilisation and bioremediation.

1.5 Objectives

1.5.1 Main objective

To isolate and characterise microbes involved in P solubilisation and bioremediation from selected agro ecological zones of Malawi.

1.5.2 Specific objectives

1. To isolate, characterise and evaluate indigenous Phosphate Solubilising Microorganisms as potential biofertiliser.
2. To isolate, characterize and evaluate indigenous microorganisms involved in degradation of pesticides.
3. To characterize genetic diversity of the isolated microorganisms involved in Phosphate solubilisation and Pesticide degradation from different ecological regions.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Study design

The study used purposive sampling which was done in a Completely Randomised Design (CRD) manner using two replicates. Asia green products which are commercial strains were used as positive control for comparison for PSM.

2.2 Study site

Samples were collected in some of the 4 main agro-ecological zones based on the crops for PSM, and previous history of pesticides application for xenobiotic degrading microbes. The classifications of the zones were based on the altitude and climatic characteristics of the country (DARS, 2016**)**. Soil map of available P was taken from the Department of Agricultural Research and Services while data on pesticides was taken from Pesticide Control Board. The laboratory work was conducted at Department of Biology, Chancellor College (constituent college of University of Malawi) and Chitedze Agricultural Research Station in Malawi.

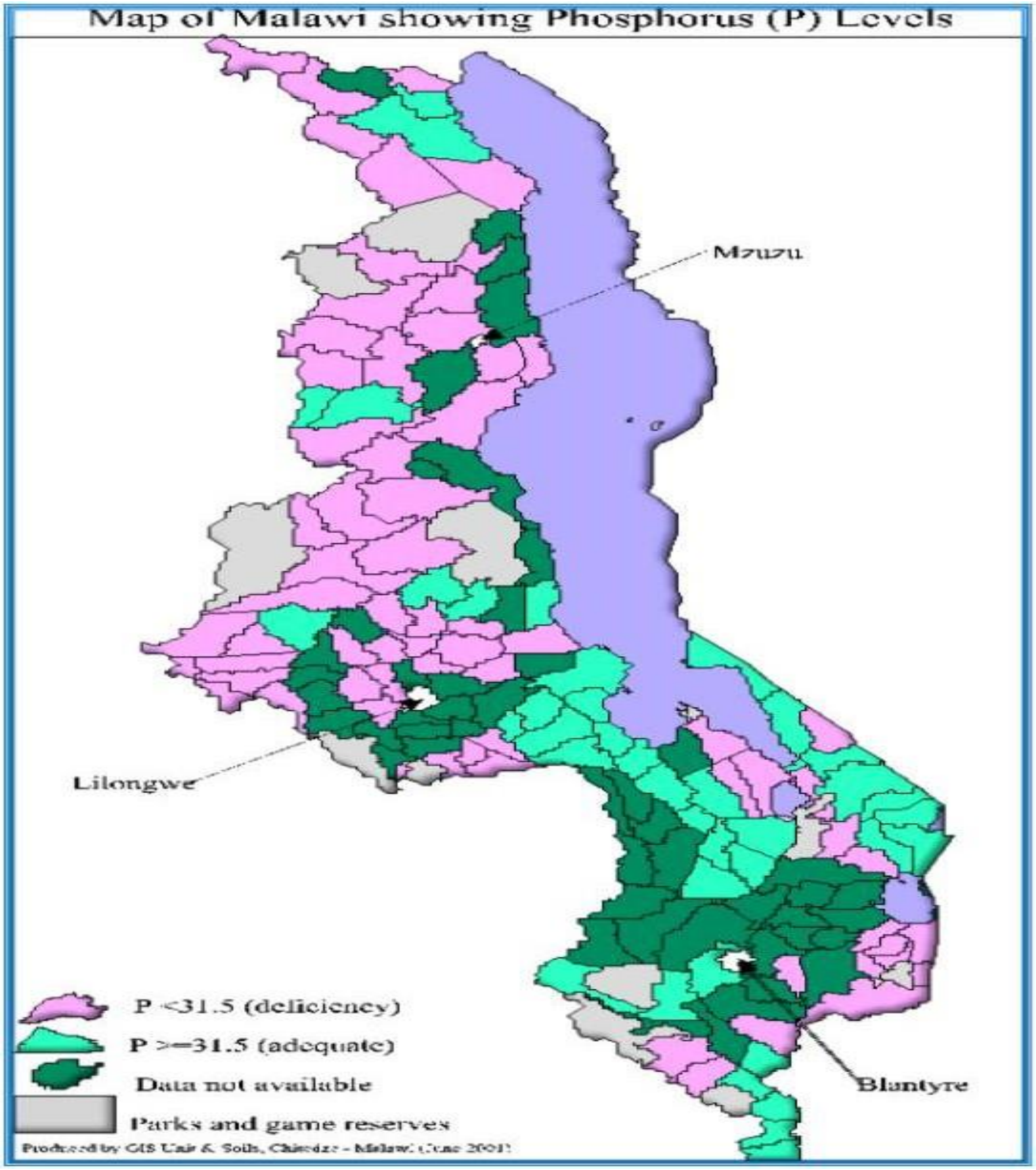

Figure 2: Map of Malawi showing available phosphorous for 2015-2016 soil survey by Department of Agricultural Research under Ministry of Agriculture and Food Security (Malawi) (DARS, 2016)

2.3 Isolation of microorganisms

2.3.1 Isolation of phosphate solubilising microbes

The soil samples were collected from maize, rice, okra, and amarathus rhizosphere grown in areas without application of inorganic fertilisers. Rhizosphere from black soil was used because colony forming units (CFU) of PSM are higher in rhizosphere than in non-rhizosphere (Krishnaveni, 2010). Soil map from Department of Agriculture Research was used as a guide in identification of these sites as shown in figure 2. Plants were uprooted and the rhizosphere soil was obtained using a method described by Nadu *et al.* (2013) with slight modifications. Briefly root system was separated from the bulk soil by shaking while the remnant soil (rhizospheric soil) was removed by using a brush. Thereafter, the rhizospheric soil was collected in polythene bags and were placed in iced cooler boxes during transportation to the laboratory and stored at 4 ^{0}C.

After homogenisation, 1 g of each soil sample was 10 fold serially diluted from neat homogenate to 10^{-4}. Thereafter, 1 ml of 10^{-3} and 10^{-4} dilutions were plated on selective Pikovskaya's (PVK) Agar Medium using the streaking method and then incubated at 28 °C for 2 to 4 days. Single colony showing clear zones on agar plates was streaked onto new PVK agar plate for further quantitative tests. The PVK medium contained $(NH_4)_2SO_4$ 0.5 g; $Ca_3(PO_4)_2$ 5 g; glucose, 10 g; NaCl 0.2 g; $MgSO_4 \cdot 7H_2O$ 0.1 g; KCl 0.2 g; $FeSO_4 \cdot 7H_2O$ 0.002 g; yeast extract 0.5 g; $MnSO_4 \cdot H_2O$ 0.002 g and agar 18 g, all dissolved in 1 liter of pure distilled water followed by autoclaving at 105 kPa and at 121 °C for 15 min.

2.3.1.1 Determination of solubilisation index

All isolates that showed clear halo zone were screened for phosphate solubilisation on Pikovskaya's medium. The isolates were inoculated at the center of pikovskaya medium plate and incubated at 28 ^{o}C. This was followed by consecutively measuring diameter of clearance zone and colonies at 2 days interval for 7 days. The PSI (Phosphate Solubilisation Index) was measured by addition of colony and halo zone diameters and then was dividing by colony diameter. Those with PSI of greater than 1.5 were selected for further testing. The commercial strains were used as positive control for comparison.

2.3.1.2 Determination of solubilisation efficiency by microbes

The isolates with PSI more than 1.5 and commercial strain (with approximately $1*10^{10}$ CFU ml^{-1} for bacteria and five disks measuring 8 mm impregnated with mycelium of fungi) were grown on PVK broth as described by Karpagam & Nagalakshmi, (2014). The pH was adjusted to 6.6 in order to test their ability to solubilise $Ca_3(PO_4)_2$. $Ca_3(PO_4)_2$ acted as sole source of P in the medium incubated in an orbital shaker at 28 ^oC for 14 days and turbidity acting as a growth indicator.

2.3.1.3 Determination of phosphorous in-vitro

Isolates and commercial strain were tested for solubilisation of autoclaved grounded rock phosphate and pure field soils. Two millilitres of every bacteria isolates with approximately $1*10^{10}$ CFU ml^{-1} and five disks of 8 mm of the mycelium of fungi was inoculated on 5 g soil and incubated at 28 ^oC. The rock phosphate was from Phalombe district.

2.3.1.4 Evaluating synergistic effect of co-inoculation of rhizobia and PSM

Co-inoculated *Bradyrhizobium japonicum*/*Bradyrhizobium archis* and PSM were tested for their ability to solubilise rock phosphate and pure field soils. Broth for PSB, rhizobium and PSF was Nutrient Agar, Yeast Extract Mannitol and Sabouraud Dextrose respectively. Two millilitres of each isolate with CFU of $1 * 10^9$ was inoculated a sole inoculant or in combination with rhizobia on 5 g soil and incubated at 32 ^oC.

2.3.1.5 Determining phosphorous levels by Mehlich III method

Mehlich III method was used to measure available P because it is the only available method in our laboratory and that the pH of Malawi soils favours the method. Twenty five millilitres of Mehlich III extractant was added to 2.5 g soil followed by vigorous shaking for 5 minutes. The culture broth of PVK was filtered by Whatman filter paper No. 42. Combined reagent containing H_2SO_4, antimony potassium tartrate, ammonium molybdate, and 0.1 M ascorbic acid was added to the clear filtrate and development measure at OD_{880nm}. The soluble P was calculated by interpolation using a standard curve

2.3.2 Isolation of pesticide degrading microbes

2.3.2.1 Sites for soil sample collection

Soil samples were collected from sites having more than one year history of pesticides application except Chasatha Farm, which had 1 year history of pesticide application. Soil samples were collected in late December 2016 from the 3-15 cm top layer of cultivated soil from several fields of the farms. The sites for soil samples collection were Chasatha farm in Karonga district, Nkhozo farm in Rumphi district and Khongoloni Tea Estate in Mulanje district. Samples were collected in polythene bags, placed in iced-box, transported to the laboratory and then stored at 4 ^{o}C pending analysis. Each sampling site had three sampling points. These 3 points of sampling site were 500m outside area of the farm where there was no history of application of pesticide (upstream of drainage and wind), inside the farm with long history of pesticide, and downstream in the drainage system of the farm.

2.3.2.2 Types of pesticides used

The commonly used pesticides namely cypermethrin, glyphosate, dimethoate and acetochlor were used and were purchased from the Farmers Organisation Limited shop.

2.2.2.3 Isolation of microorganisms

The procedure was as described by Eman *et al.* (2013) with some modification. Microorganisms were isolated from soil samples using enrichment culture technique. Firstly, 5 gm of soil sample was put into a 250 ml flask containing 50 ml of sterile liquid Mineral Salt Medium (MSM) having 100 ppm of pesticide. MSM contained (g/l) KH_2PO_4 (1.5), Na2HPO$_4$ (0.6), NaCl (0.5), NH_4SO_4 (2), $MgSO_4.7H_2O$ (0.2), $CaCl_2$ (0.01) and $FeSO_4.7H_2O$ (0.001). Microbe isolation was carried out at different concentrations of pesticides (100, 500, 1000 and 10000 ppm) on Czapek Dox agar (Akbar *et al.*, 2015; Shamsuddeen and Inuwa, 2013). Microbes that tolerated pesticide up to 1000 ppm were selected for further studies.

2.3.2.4 Determination of pesticide utilisation patterns

Pesticide utilisation was determined using a method described by Shamsuddeen and Inuwa, (2013) and Akbar *et al.* (2015) with slight modifications. The individual pesticide tolerant microorganisms were inoculated into three 250-ml flask containing 50 ml MSM 1, MSM 2 and MSM 3 each containing 20 ml of pesticides as sole carbon and phosphorous sources. Negative

Controls were not inoculated. The composition of MSM-1 was KH_2PO_4 (1.5g), MgSO4.7H2O (0.2g), Na2HPO$_4$ (0.6g), NaCl (0.5g), NH_4SO_4 (2g), $CaCl_2$ (0.01g) and $FeSO_4.7H_2O$ (0.001g) dissolved in 1 L of water, pH (7.0). MSM-2 had no phosphate source and targeted pesticides to be sole P source, and contained glucose (10g), Tris buffer (12g), CaCl2 (0.01g), NaCl (0.5g), NH4SO4 $CaCl_2$ (0.01g) and $FeSO_4.7H_2O$ (0.001g) dissolved in 1 liter of distilled water, pH (7.0). MSM-3 was used for isolating microbes using pesticides as sole source of P and carbon and contained NaCl (0.5g), MgSO4.7H2O (0.2g), KCl (0.5g) NH_4SO_4 (2g). Degradation was observed by growth of microorganism in the media. Thereafter, medium turbidity measurement was done periodically at 625 nm using a spectrophotometer and also by streaking on Czapek Dox agar plates.

2.3.2.5 Determination of laccase enzyme presence

The presence of laccase enzyme production was determined by streaking isolates on Sabouraud Dextrose Agar (SDA), supplemented with 1% ABTS (2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)). Production of laccase enzyme was confirmed when dark green to purple colour was seen around the colonies (Singh and Abraham, 2013).

2.4 Determination of Plant Growth Regulatory Traits

PGRT were determined by conducting test for production of ammonia, IAA, catalase, hydrogen cyanide, siderophores and nitrogen-fixing ability. For ammonia production test, broth cultures of isolated microbes ($1*10^9$ CFU or 4 mm diameter of fungus) were inoculated in 10 ml tube of peptone water and incubated at 36 ± 2°C for 48-72 hrs. Thereafter, 0.5 ml of Nessler's reagent was added. Positive test for production of ammonia was confirmed by development of either yellow or brown colour (Ahmad and Khan, 2008). The production of IAA by isolated microbes was determined as described by Ahmad and Khan, (2008) with slight modification. The isolates were grown in nutrient broth supplemented with tryptophan (100µg/ml) maintained at 30 °C for 48 hours in an orbital incubator while shaking at 120 rpm. The broth media with the isolates was centrifuged at 3000 rpm for 30 minutes. Thereafter, 2 ml of supernatant was recovered, this was followed by addition of 2 drops of o-phosphoric acid, and 4 mls of Salkowski reagent. Positive test for production of IAA was confirmed by development of pink colour. Catalase production was determined by addition 2 drops of 3% hydrogen peroxide to grown the culture of isolated microbes on a slide using wire loop in a biosafety cabinet. Positive test for production of catalase was confirmed by effervescence. Hydrogen Cyanide production (HCN) was determined in-vitro by a method described by Ahmad and Khan, (2008) with some modifications. Isolates grown in Nutrient Broth and Sabouraud Dextrose Broth supplemented with glycine (4.4 g /L) were streaked on modified NA and SDA plates for bacteria and fungi respectively. Sterile Whatman filter paper No. 1 was dipped in 2.5% sodium carbonate in 0.5% picric acid solution and later placed on top of the grown cultures on agar plate. Agar Plates were tightly sealed and incubated at 36 °C for 4 days. Positive test for production of HCN was confirmed by colour change from yellow to orange-red on Whatman filter paper. Qualitative production of siderophore by the isolates was done using universal Chrome azurol S (CAS) agar plate assay as documented by Liu *et al.* (2016). This was done using CAS agar plates, because siderophore producing microbes forms orange halo around the colonies after 7 days incubation at 28 °C (Ahmad and Khan, 2008). Nitrogen fixing ability of microbes was assessed using a method described by Liu *et al.* (2016) with some modifications. The isolates were streaked on modified nitrogen deficient Ashby's agar medium [0.2 g NaCl, 0.1 g $CaSO_4 \cdot 2H_2O$, 10 g sucrose (dextrose for fungus), 5 g $CaCO_3$, 0.2 g KH_2PO_4, 0.2 g MgSO4·7H2O and 15 g agar in 1 L distilled water; pH 7.0 incubated at 28 °C for 7 days. The growth of the isolates on the media was considered as an ability to fix atmospheric nitrogen.

2.5 Identification of the microbes

Preliminary identification of the microbes was done based on colony morphology as outlined below. The isolates were preliminary observed for colony morphology using magnifying glass. The morphological characters such as colony surface, texture, margins, elevation, pigmentation and shape, were observed using microscope. Gram staining was done to determine cell structure, shape and size. Smears were made from the colonies on a microscopic slide heat fixed and then stained. The stained smear was observed under oil immersion lense-100x of microscope. Fungal characterisation was based on colony characteristics on PDA plates and microscopic examination was done on slide using lacto phenol blue stain. The microbes were confirmed by using molecular techniques employing 16S rRNA gene and ITS (internal transcribed sequences) of 18S rRNA of bacteria and fungus respectively. These genetic markers were used because they are conserved, and ready available in the database (Yang *et al.*, 2016; Hejazi *et al.*, 2010; Anderson *et al.*, 2003).

2.5.1 Extraction of genomic DNA from the microbes

Genomic DNA of bacteria and fungi was extracted and purified using the ZR-kit according to manufacturer's manual. Approximately 70 - 100 mg of bacteria (10^9 CFU) and mycelia for fungi were put in ZR bashing bead lysis tube and processed in a cell disruptor at maximum speed for 5 min. This was followed by centrifugation at 10,000 x g for 1 min. Four hundred microliters of supernatant was transferred to a Zymo-Spin™ IV Spin Filter in a collection tube and centrifuged at 7,000 rpm for 1 min. Approximately 1.2 ml of fungal/bacterial DNA binding buffer was added to the filtrate in the collection tube. Approximately 800 µl of the mixture was transferred to a Zymo-Spin™ IIC column in a collection tube and centrifuge at 10,000 x g for 1 min. Two hundred microlitres of DNA pre-wash buffer was added to the Zymo-Spin™ IIC column and centrifuged at 10,000 x g for 1 min. The column was transferred to a clean 1.5 ml micro centrifuge tube and followed by addition of 100 µl DNA elution buffer directly to the column matrix. The genomic DNA was eluted by centrifuging at 10,000 x g for 30 min.

2.5.2 Polymerase Chain Reaction

The amplification was done following a modification of Srinivasan *et al*, (2012) method where by a conventional PCR amplifying 1500, and 700 bp fragments for 16S rRNA gene and ITS (internal transcribed sequences) of 18S rRNA gene for bacteria and fungus respectively were used. The primers used were 907R (5'- CCGTCAATTCMTTTRAGTTT-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') for bacteria and ITS1 (5'-TCCGTAGGTGAACCTGCGG -3') and ITS4 (5'- TCCTCCGCTTATTGATATGC -3') for fungus. The final 20 µl PCR reaction volume consisted of 10 ng of purified genomic DNA, 1.5 mM $MgCl_2$, 250 of µM dNTPs, 10 pmol of each 2 primers and 2.5 of Taq DNA polymerase. The thermocycling conditions for the full-length amplicons were as follows: pre-denaturation at 94 ^oC for 5 minutes followed by 35 cycles of denaturation at 94 ^oC for 5 minutes, annealing at 54 ^oC for 1 minute and extension at 72 ^oC for 1 minute. Thereafter one cycle of final extension at 72 ^oC for 5 minutes was done.

2.5.3 Sequencing and bioinformatics analysis

Sequencing of the isolates 16S rRNA and 18S rRNA genes was done by Inqaba Biotech Ltd in South Africa using Sanger sequencing. Consensus sequences of two PCR products of 16S rRNA and 18S rRNA sequence was obtained using BioEdit software. The consensus sequence obtained in BioEdit was analysed by BLAST algorithm for comparison of a nucleotide query sequence against public nucleotide sequence database to find the homologous strains**.** The nucleotide sequences of the 16S rRNA were subjected to BLAST analysis based on the National Center for Biotechnology Information (NCBI) database (http://blast.ncbi.nlm.nih.gov/Blast.cgi. Sequences with high similarity scores were downloaded from the NCBI database. This was based on maximum identity score, whereby the first sequences were selected and aligned with isolate sequences using MUSCLE to show microorganism diversity and richness. The Neighbour Joining (NJ) phylogenetic tree was used for defining dataset as it establishes relationships between sequences according to their genetic distance (a phenetic criterion), without taking into account an evolutionary model (Kuan *et al.*, 2016). Maximum Likelihood (ML) phylogenetic tree was preferred because it investigates the spaces of all possible phylogenetic trees. The phylogenetic trees were constructed with Seaview software version 4.5.0 http://doua.prabi.fr/software/seaview calculated by the method of Kimura two-parameter model with a discrete Gamma distribution. Gaps were treated by partial deletion and bootstrap analysis was done by using 100 replicates.

2.6 Data analysis

Phosphate solubilisation data analysis was done using ANOVA followed by pairwise multiple comparisons (post hoc testing), using the Tukey method and Microsoft excel.

CHAPTER THREE

RESULTS

3.1 Phosphate solubilising microorganisms

A total of 13 different PSM were isolated out of which 6 were selected based on solubilisation index and used for further analysis. As shown in figure 3, the non PSM initially grew on the medium revealing yellow colonies without halozone. After 7 days the isolated *Aspergillus niger* revealed yellow colonies surrounded by translucent halozones indicating solubilisation of inorganic phosphorous. In particular 73A, 72A, 75A, 77A, 74B and 3100A had PSI more than 1.5 as presented in table 1. *A. niger* had the high PSI, followed by *P. putida*. *E.casseliflavus* had the lowest PSI, though the value was more than 1.5. The growth of *Aspergillus niger* was characterised by yellow black colonies while those of *P. putida* consisted of regular white colonies. All microbes were able to fix nitrogen, produce IAA, ammonia as well as catalase. None of the isolated microbes produced HCN. *Aspergillus niger* and *Klebsiella pneumoniae* (from okra) were isolated from Lilongwe while *E.casseliflavus*, *P. putida* and *Penicillium janthinellum* were isolated from Karonga. *Klebsiella pneumoniae* obtained from amarathus was isolated from soil collected from Rumphi. Futher genetic analysis revealed that isolates 72A, 75, 77A, 73A, 3100A, and 74B were *Enterococcus gallinarum*, *Pseudomonas sp.*, *Klebsiella pneumoniae*, *Aspergillus niger, E. cloacae* and *Penicillium janthinellum.*

Phosphate solubilisation kinetics of the isolates obtained from the fields and commercial strains available in Malawi were compared. Results showed that stain 73A and 77A had higher phosphate solubilisation efficiency of 225 and 140 mg/kg respectively in rock phosphate as compared to values 40 mg/kg for commercial strain (figure 4 A). The results of co-inoculation of isolated PSM and *B. japonicum* or *B. archis* showed that isolates, 73A, 75A, 77A, and 72A increased P solubilisation through synergistic effect mainly on soil medium except *K. pneumoniae* strain 3100B had the least efficiency as shown figure 4 B. Isolates that had high P solubilisation on RP had negative synergistic effect on co-inoculation in relation to P solubilisation as shown in figure 4 C. Isolated microbes showed strong statistically significant difference ($P \leq 0.03$) in solubilising P and the values were greater than commercial strain as shown in table 2. Further analysis was done to determine solubilisation rate of PSM grown under different conditions as shown in figure 5. *E.casseliflavus*,

Was able to solubalise P for up to 18 days. The other isolates including, *Pseudomonas putida*, *Klebsiella pneumoniae*, *Aspergillus niger, E. cloacae* and *Penicillium janthinellum* solubilised P similary but the efficiency decreased as analysis proceded from day 6 to day 18.

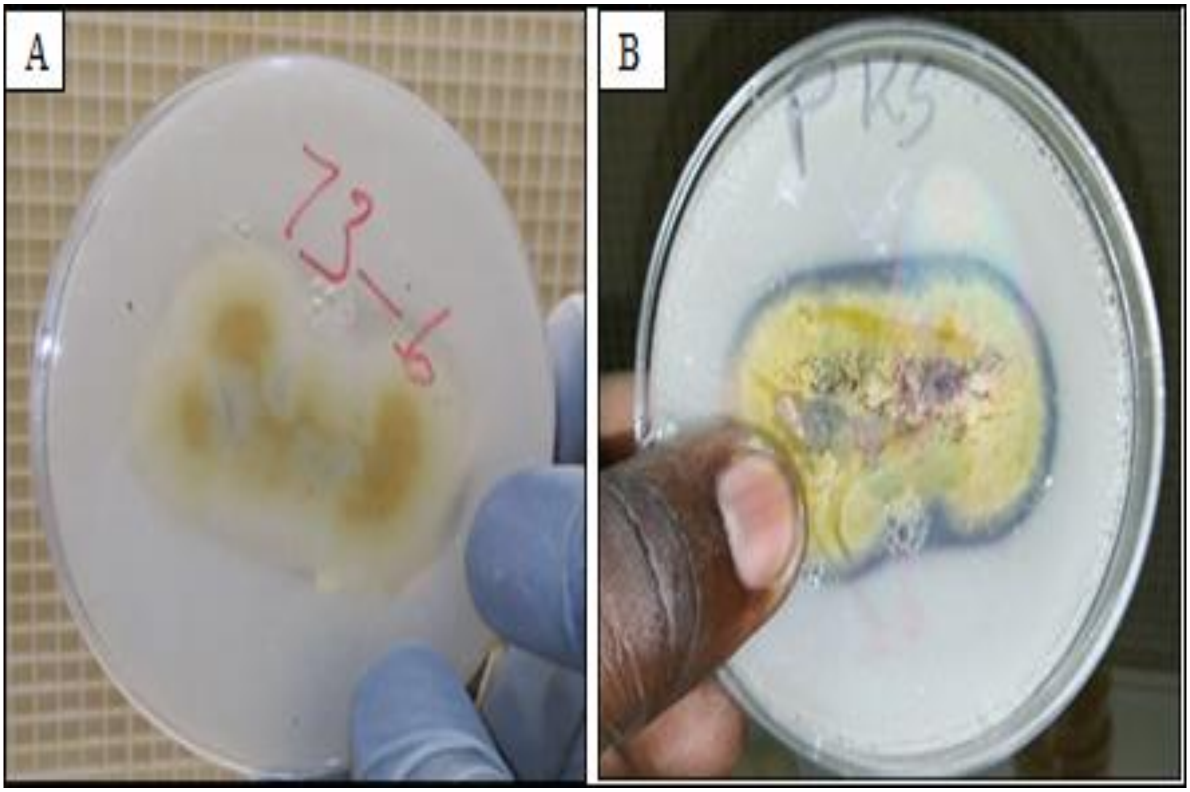

Figure 3: *Aspergillus niger* showing halo zone on Pikovskaya's agar plate. A: *Aspergillus niger* with no halo zone around the yellow colony. B: The presence of halo zone around the yellow colony of *Aspergillus niger* on Pikovskaya's agar plate.

Table 1: Phosphate Solubilising Microorganisms from selected agro ecological zones of Malawi.

Characteristic feature	**73A**	**72A**	**77A**	**3100A/74B**	**75A**	**74B**
Genus/Spp	*A. niger*	*E.casseliflavus*	*K.pneumoniae*	*K.pneumoniae*	*P.putida*	*P. janthinellum*
Plant	Amarathus	Maize	Okra	Amarathus	Maize	Rice
Location	Lilongwe	Karonga	Lilongwe	Rumphi	Karonga	Karonga
Nitrogen fixing	+	+	+	+	+	+
IAA	+	+	+	+	+	+
Ammonia	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
Gram staining		+	-	-	-	
Siderophore	-	+	+	+	+	+
HCN	-	-	-	-	-	-
Shape		Coccoid	Rod	Rod	Rod	
Colony Characteristics	Yellow/ black	yellow Circular &smooth	shiny and mucoid	shiny and mucoid	White regular	dark green
Solubilisation index	3.5	2.11	3.14	2.86	3.33	2.75

HCN= Hydrogen Cyanide

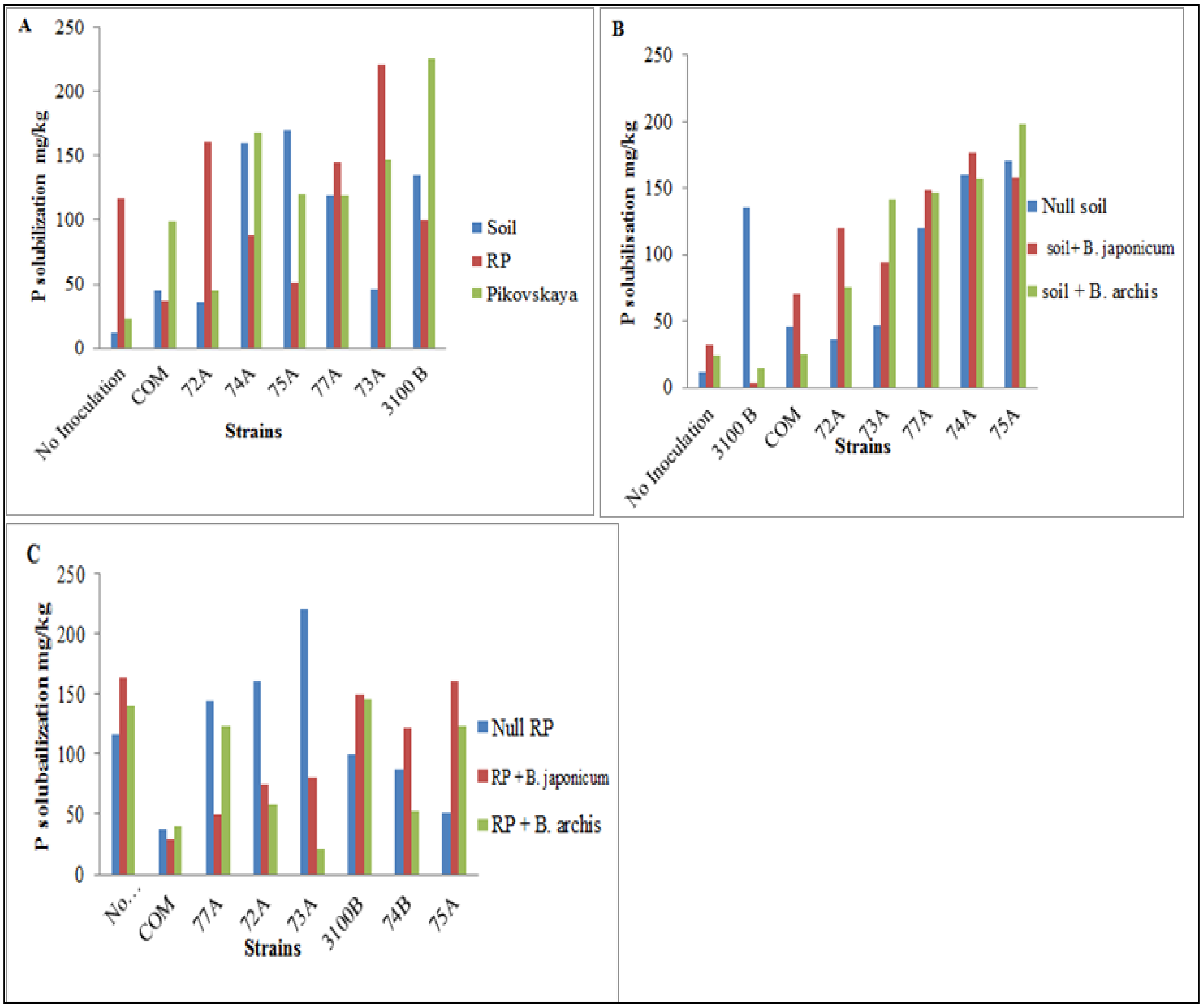

Figure 4: Solubilisation efficiency of Phosphorous by different strains and their co-inoculation with *Bradyrhizobium japonicum* or *Bradyrhizobium archis* on different media. **A**: Solubilisation efficiency by different strains on different media **B**: Effect of co-inoculation of Phosphate Solubilising Microorganisms with rhizobium on solubilisation of Phosphorous on soil **C**: Effect of co-inoculation of Phosphate Solubilising Microorganisms with rhizobium on solubilisation of Phosphorous on Rock phosphate. 72A (*Enterococcus gallinarum*); 75A (*Pseudomonas sp.);* 77A (*Klebsiella pneumoniae)*; 73A (*Aspergillus niger);* 3100A (*E. cloacae)*; 74B (*Penicillium janthinellum*)

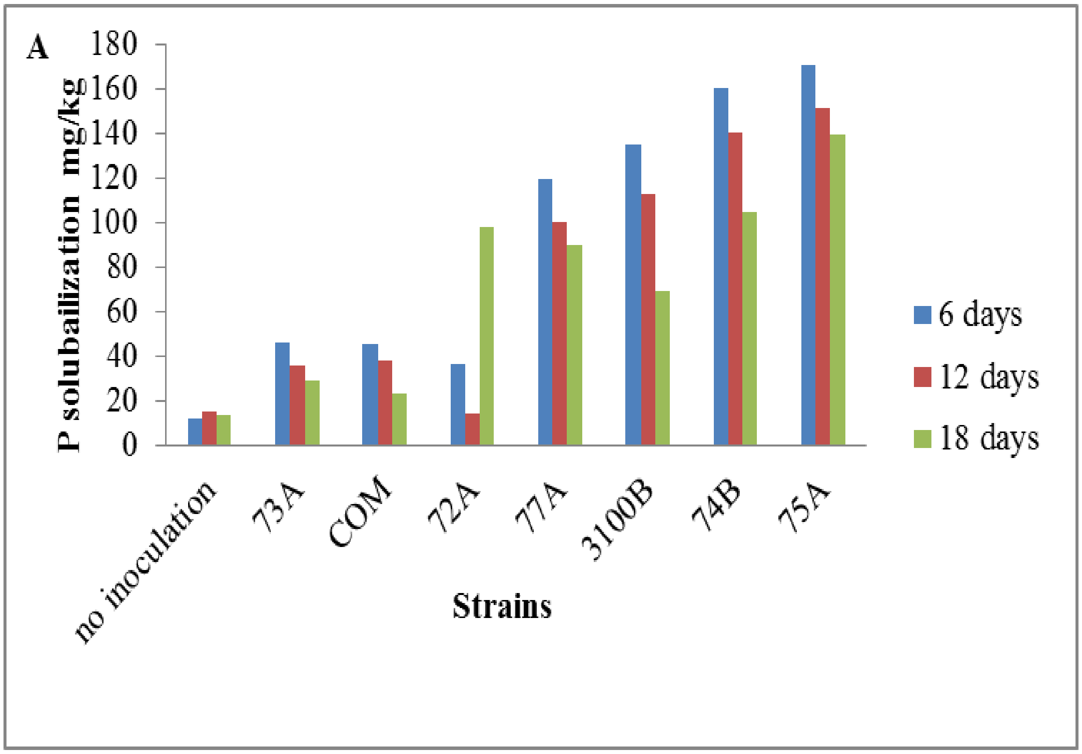

Figure 5: Solubilisation rate of isolated Phosphate Solubilising Microorganisms grown under different soil conditions. 75A (*Pseudomonas sp.);* 77A (*Klebsiella pneumoniae)*; 73A (*Aspergillus niger);* 3100A (*E. cloacae)*; 74B (*Penicillium janthinellum*)

Table 2: Phosphate solubilisation of the isolated and the commercial strains

(I) Bacterial strain	(J) Bacterial strain	Mean Difference (I-J)	P-values	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
	73A	-34.2024*	.001	-52.8803	-15.5244
	73B	-37.6131*	.001	-56.2911	-18.9351
	74A	-58.9295*	.000	-77.6075	-40.2516
No inoculation	75A	-67.2138*	.000	-85.8918	-48.5359
	77A	-48.5393*	.000	-67.2173	-29.8613
	72A	-8.3879	.649	-27.0659	10.2901
	COM	26.3982*	.007	7.7203	45.0762
	No inoculation	34.2024*	.001	15.5244	52.8803
	73B	-3.4107	.993	-22.0887	15.2672
	74A	-24.7272*	.011	-43.4051	-6.0492
73A	75A	-33.0115*	.002	-51.6894	-14.3335
	77A	-14.3369	.162	-33.0149	4.3410
	72A	25.8145*	.008	7.1365	44.4924
	COM	60.6006*	.000	41.9226	79.2785
	No inoculation	37.6131*	.001	18.9351	56.2911
	73A	3.4107	.993	-15.2672	22.0887
	74A	-21.3164*	.025	-39.9944	-2.6385
73B	75A	-29.6007*	.003	-48.2787	-10.9228
	77A	-10.9262	.385	-29.6042	7.7518
	72A	29.2252*	.004	10.5472	47.9032
	COM	64.0113*	.000	45.3334	82.6893
	No inoculation	58.9295*	.000	40.2516	77.6075
	73A	24.7272*	.011	6.0492	43.4051
	73B	21.3164*	.025	2.6385	39.9944
74A	75A	-8.2843	.660	-26.9623	10.3937
	77A	10.3902	.435	-8.2877	29.0682
	72A	50.5416*	.000	31.8637	69.2196
	COM	85.3278*	.000	66.6498	104.0057
	No inoculation	67.2138*	.000	48.5359	85.8918
	73A	33.0115*	.002	14.3335	51.6894
	73B	29.6007*	.003	10.9228	48.2787
75A	74A	8.2843	.660	-10.3937	26.9623
	77A	18.6745	.050	-.0034	37.3525
	72A	58.8259*	.000	40.1480	77.5039
	COM	93.6120*	.000	74.9341	112.2900
	No inoculation	48.5393*	.000	29.8613	67.2173
	73A	14.3369	.162	-4.3410	33.0149
	73B	10.9262	.385	-7.7518	29.6042
77A	74A	-10.3902	.435	-29.0682	8.2877
	75A	-18.6745	.050	-37.3525	.0034
	72A	40.1514*	.000	21.4734	58.8294
	COM	74.9375*	.000	56.2596	93.6155
	No inoculation	8.3879	.649	-10.2901	27.0659
	73A	-25.8145*	.008	-44.4924	-7.1365
	73B	-29.2252*	.004	-47.9032	-10.5472
72A	74A	-50.5416*	.000	-69.2196	-31.8637
	75A	-58.8259*	.000	-77.5039	-40.1480
	77A	-40.1514*	.000	-58.8294	-21.4734
	COM	34.7861*	.001	16.1082	53.4641
	No inoculation	-26.3982*	.007	-45.0762	-7.7203
	73A	-60.6006*	.000	-79.2785	-41.9226
	73B	-64.0113*	.000	-82.6893	-45.3334
	74A	-85.3278*	.000	-104.0057	-66.6498
COM	75A	-93.6120*	.000	-112.2900	-74.9341
	77A	-74.9375*	.000	-93.6155	-56.2596
	72A	-34.7861*	.001	-53.4641	-16.1082

This is Analysis of Variance based on pairwise multiple comparisons (post hoc testing), using the Tukey method for phosphate solubilising microorganisms using different media. 75A (*Pseudomonas sp.);* 77A (*Klebsiella pneumoniae)*; 73A (*Aspergillus niger);* 3100A (*E. cloacae)*; 74B (*Penicillium janthinellum*)

3.2 Degradation of pesticides by microbes

The ability of microbes to degrade cypermethrin, acetochlor and glyphosate was evaluated in-vitro. A total of 25 bacteria and 6 fungi with ability to degrade cypermethrin, acetochlor and glyphosate were isolated as shown in table 3 and figure 7. These microbes were isolated from different agro ecological zones and showed production of laccase enzyme and plant growth regulatory traits. *E. cloacae* and *Achromobacter sp* degraded glyphosate and used the compound as sole phosphorous source. All isolates that degraded cypermethrin used the pesticide as sole carbon source. The isolates that degraded cypermethrin didn't use the insecticide as P source. The microbes that degraded acetochlor utilised the herbicide as a source of both carbon and P. two F. oxysporum isolates separately utilised C and P from glyphosate. However one isolate was able to utilise P and C and also P and C at the same time. Microbes were found to be inside the farms where pesticide were applied as shown in table 4. In this study no microbe was found to degrade Dimethoate. The study found diversity in Nkhozo and Mulanje which has longer history of pesticide application compared to Chasatha farm in Karonga which had one year of application (table 4). The results also show that aerial application has an impact to non-target sites shown by diversity of microbes responsible for degrading xenobiotics outside the farm as is a case in Mulanje. Diversity was also shown by pesticide utilisation pattern as indicated in table 3. As shown in figure 6, some microbes grew in medium with cypermethrin indicating their ability to use the pesticide as the sole carbon source. The colour from milky white to three layers of different colours.

Evaluation of growth kinetics show that the various microorganisms had different growth rates under different conditions. Growth was lower when microorganisms utilised glyphosate as phosphorous and carbon sources with OD values of less than 0.09 at 144 hours of growth (Figure 7 A) compared to when they utilised it as either carbon or phosphorus source revealing OD values of 0.15 at 144 hours of growth (figure 7 B and C).

Analysis of growth kinetics for acetochlor and cypermethrin showed that the various microorganisms isolated had different growth rates under different conditions. Isolate 3106r utilised acetochlor and cypermethrin as carbon sources but at different growth rate. Bacteria utilised acetochor as carbon and phosphorous sources but only utilised cypermethrin as carbon source. Microbes showed higher growth rate by utilisation of acetochlor as carbon source than P source as shown in figure 8 B and 8 D. The microbes revealed slower growth rate in utilisation of cypemethrin than acetochlor as carbon sources as shown in figure 8 A and 8 D. By utilising carbon from pesticide they are degrading the pesticide using laccase enzyme. All microbes that were able to utilise glyphosate, cypermethrin and acetochlor produced laccase enzyme as also indicated on table 3.

Table 3: Pesticide degrading microbes from selected agro ecological zones of Malawi

LAB NO	Microbe	Site	Pesticide	Microorganism	SI	IAA	Gram stain	Ammonia	Catalase	Siderophore	Shape	sole source			Laccase
												C	P	C&P	
3106r	*Enterobacter*	M	C	B	1.16	+	-	+	+	+	R	+	-	-	+
3103	*Enterobacter*	N	C	B	1	+	-	+	+	+	R	+	-	-	+
3100 a	*Enterobacter cloacae*	N	C	B	1.1	+	-	+	+	+	R	+	-	-	+
3100 b	*Klebsiella pneumonia*	N	C	B	2.86	+	-	+	+	+	R	+	-	-	+
3106br	*Enterobacter asburiae*	M	C	B	1.5	+	-	+	+	+	R	+	-	-	+
3104 b	*Klebsiella pneumonia*	K	C	B	1.44	+	-	+	+	+	R	+	-	-	+
3106b	*Leclercia sp.*	M	C	B	1.4	+	-	+	+	+	R	+	-	-	+
3102	*Klebsiella oxytoca*	N	C	B	1.48	+	-	+	+	+	R	+	-	-	+
3104 a	*Pseudomonas aeruginosa*	K	C	B	1.3	+	-	+	+	-	R	+	-	-	+
2101	*Enterobacter cloacae*	N	A	B	1.4	+	-	+	+	+	R	+	+	+	+
2106 r	*Enterobacter asburiae*	M	A	B	1.4	+	-	+	+	+	R	+	+	+	+
2100a	*Enterobacter asburiae*	N	A	B	1.4	+	-	+	+	+	R	+	+	+	+
2100B	*Enterobacter cancerogenus*	N	A	B	1.1	+	-	+	+	+	R	+	+	+	+
2103-2	*Enterobacter tabaci,*	N	A	B	1.12	+	-	+	+	+	R	+	+	+	+
2103	*Enterobacter asburiae*	N	A	B	1.39	+	-	+	+	+	R	+	+	+	+
2106a	*Enterobacter xiangfangensis*	M	A	B	1.22	+	-	+	+	+	R	+	+	+	+
2104-	*Enterobacter cloacae*	K	A	B	1.13	+	-	+	+	+	R	+	+	+	+
2106b	*Enterobacter xiangfangensis*	M	A	B	1.12	+	-	+	+	+	R	+	+	+	+
2107b	*Pantoea agglomerans,*	M	A	B	1.3	+	-	+	+	+	R	+	+	+	+
2104	*Enterobacter cloacae*	K	A	B	1.3	+	-	+	+	+	R	+	+	+	+
2105	*Enterobacter tabaci,*	M	A	B	1.6	+	-	+	+	+	R	+	+	+	+
1104	*Enterobacter cloacae*	K	G	B	1.23	+	-	+	+	+	R	-	+	-	+
1107	*Achromobacter sp*	M	G	B	1.3	+	-	+	+	+	R	-	+	-	+
1103	*Enterobacter aerogenes*	N	G	B	1.2	+	-	+	+	+	R	+	+	+	+
1105	*Enterobacter tabaci*	M	G	B	1.13	+	-	+	+	+	R	+	+	+	+
6106b	*Mucor irregularis*	M	G	F	1.13	+		+	+	+		+	+	+	+
6106	*Fusarium oxysporum*	N	G	F	1.13	+		+	+	+		+	+	+	+
6102b	*Fusarium oxysporum*	N	G	F	1.13	+		+	+	+		+	+	-	+
6102	*Fusarium oxysporum*	N	G	F	1.13	+		+	+	+		+	+	-	+
6101b	*Meyerozyma caribbica*	N	G	F	1.13	+		+	+	+		+	+	+	+
6100	*Aspergillus parasiticus*	N	G	F	1.13	+		+	+	+		+	+	+	+

C= Cypermethrin, A= Acetochlor, G= Glyphosate, B= bacteria, F=Fungus, M=Mulanje, K=Karonga, N=Nkhozo, SI=solubilization index, R=Rod shaped,

Pesticide utilisation pattern showed diversity of microbes at species level which was also observed at molecular level figure 13.

Table 4: Diversity of microorganism in the selected sites and their sampling points

Pesticide	Sites								
	Karonga			**Rumphi (Nkhozo)**			**Mulanje**		
	Outside the farm	Inside the farm	Downstream (drainage system)	Outside the farm	Inside the farm	Downstream (drainage system)	Outside the farm	Inside the farm	Downstream (drainage system)
C	Nil	3104b 3104a	3104 b,	Nil	3103 3100a 3100b 3102	3100b 3102	3106 3106r 3106b	3106 3106r 3106b	3106 3106r 3106b
A	Nil	2104	2104	Nil	2101 2100a 2100B 2103-2 2103	2103-2 2103	2106r 2106a 2106b 2107b 2105	2106r 2106a 2106b 2107b 2105	2106r 2106a 2106b 2107b 2105
G	Nil	1104	Nil	6100	6103 6102 6101b 6100	6102 6101b	1105 6106	1107 1105 6106	1107 1105 6106

C= cypermethrin, A= Acetochlor, G= Glyphosate, Nil= no microbe isolated, Numbers= Lab no of isolate. 3106r= *Enterobacter sp;* 3103= *Enterobacter sp;* 3100a= *Enterobacter cloacae;* 3100b= *Klebsiella pneumoniae;* 3106br= *Enterobacter asburiae;* 3104b= *Klebsiella pneumoniae;* 3106b= *Leclercia sp.;* 3102= *Klebsiella oxytoca;* 3104a= *Pseudomonas aeruginosa;* 2101= *Enterobacter cloacae;* 2106 r= *Enterobacter asburiae;* 2100a= *Enterobacter asburiae;* 2100B= *Enterobacter cancerogenus;* 2103-2= *Enterobacter tabaci;* 2103= *Enterobacter asburiae;* 2106a= *Enterobacter xiangfangensis;* 2104a= *Enterobacter cloacae;* 2106b= *Enterobacter xiangfangensis;* 2107b= *Pantoea agglomerans;* 2104b= *Enterobacter cloacae;* 2105= *Enterobacter tabaci*, 1104= *Enterobacter cloacae*, 1107= *Achromobacter sp*, 1103= *Enterobacter aerogenes*, 1105= *Enterobacter tabaci*, 6106b= *Mucor irregularis*, 6106= *Fusarium oxysporum*, 6102b= *Fusarium oxysporum*, 6102= *Fusarium oxysporum*

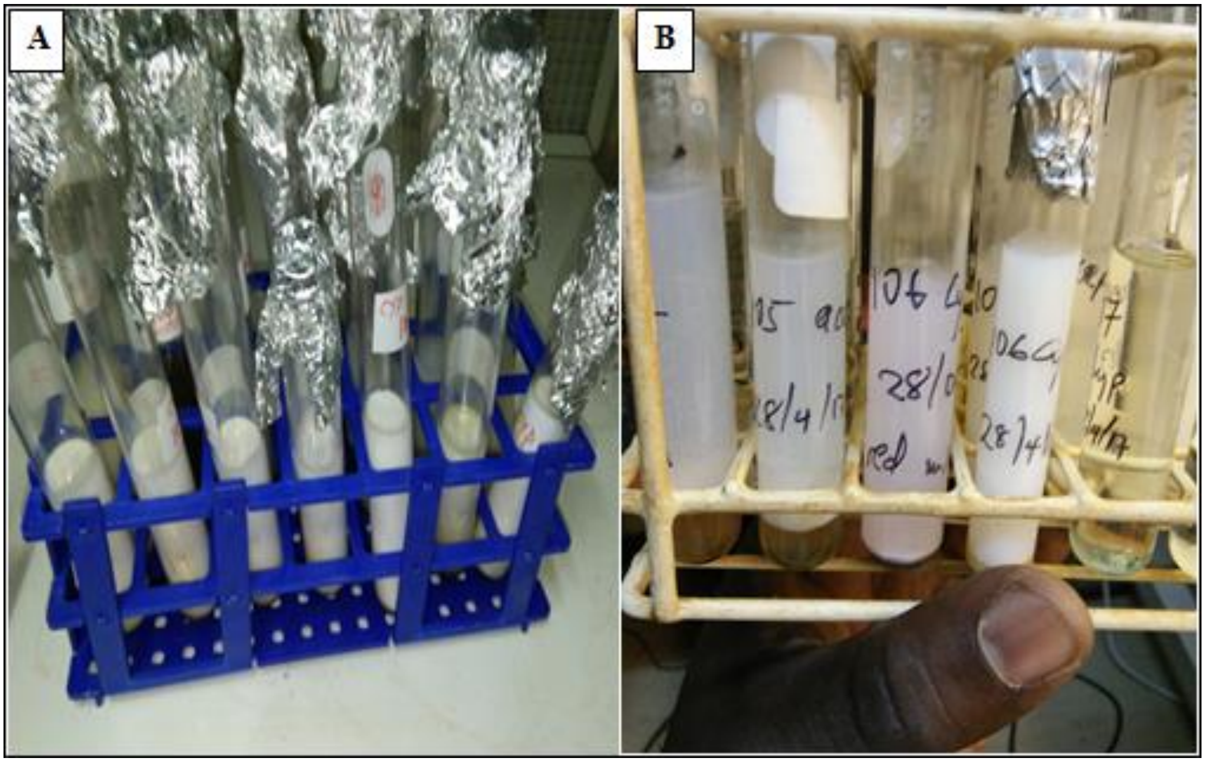

Figure 6: Illustration of growth of some microbes in Mineral Salt Medium (where cypermethrin is the only carbon source) after 40 days. A: Before inoculation (No growth) and B: Growth after 40 days of incubation in the presence of cypermethrin.

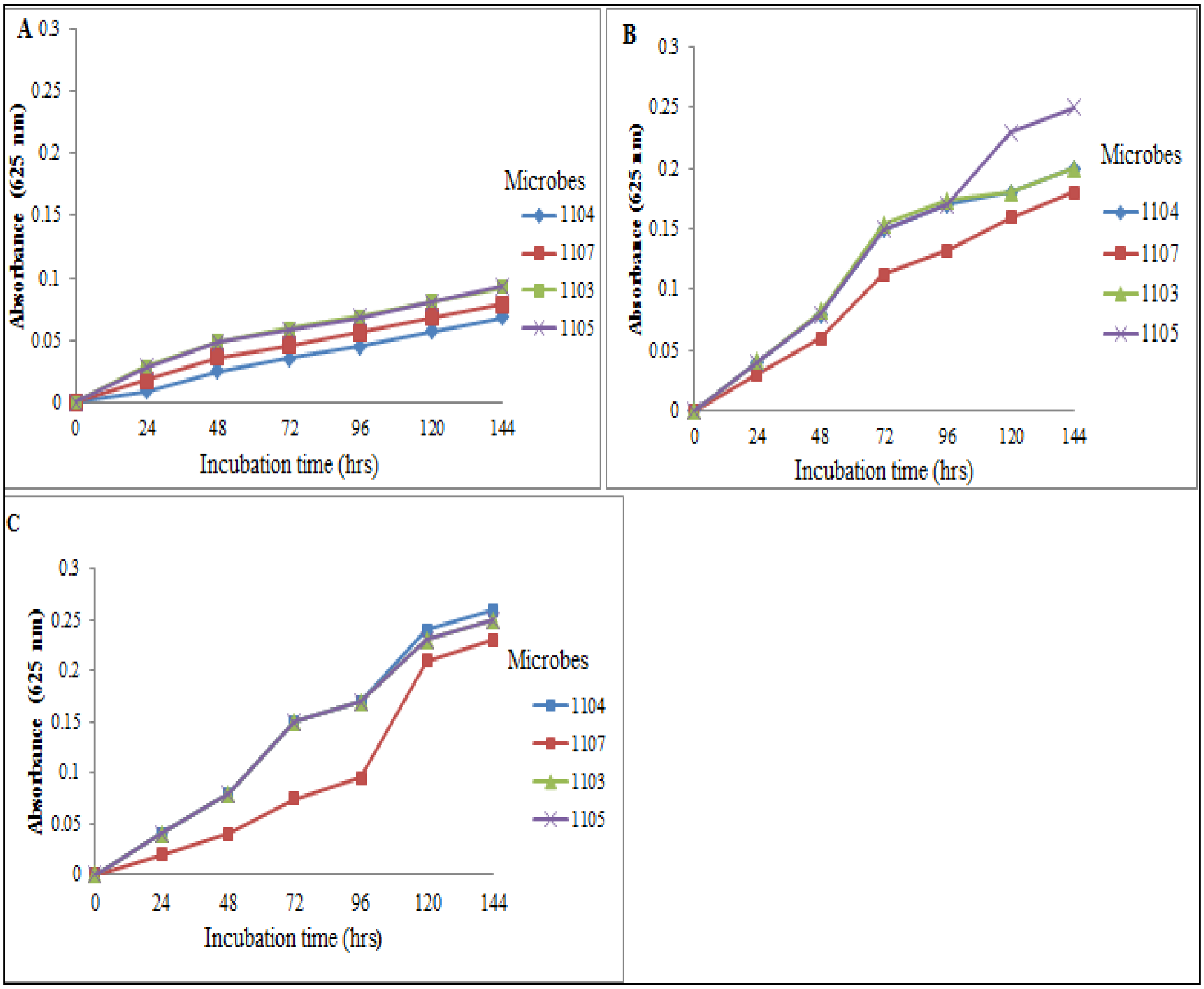

Figure 7: Growth kinetics of glyphosate degrading bacteria through utilisation of pesticide as sole carbon and phosphorous source for 144 hours. **A**: Growth kinetics of isolates in glyphosate as sole phosphorus and carbon sources (MSM 3), B: Growth kinetics of isolates using glyphosate as sole phosphorus source (MSM 2) and C: Growth kinetics of isolates using glyphosate as sole carbon source (MSM 1). 1104= *Enterobacter cloacae*, 1107= *Achromobacter sp*, 1103= *Enterobacter aerogenes*, 1105= *Enterobacter tabaci*, 6106b= *Mucor irregularis*, 6106= *Fusarium oxysporum*, 6102b= *Fusarium oxysporum*, 6102= *Fusarium oxysporum.*

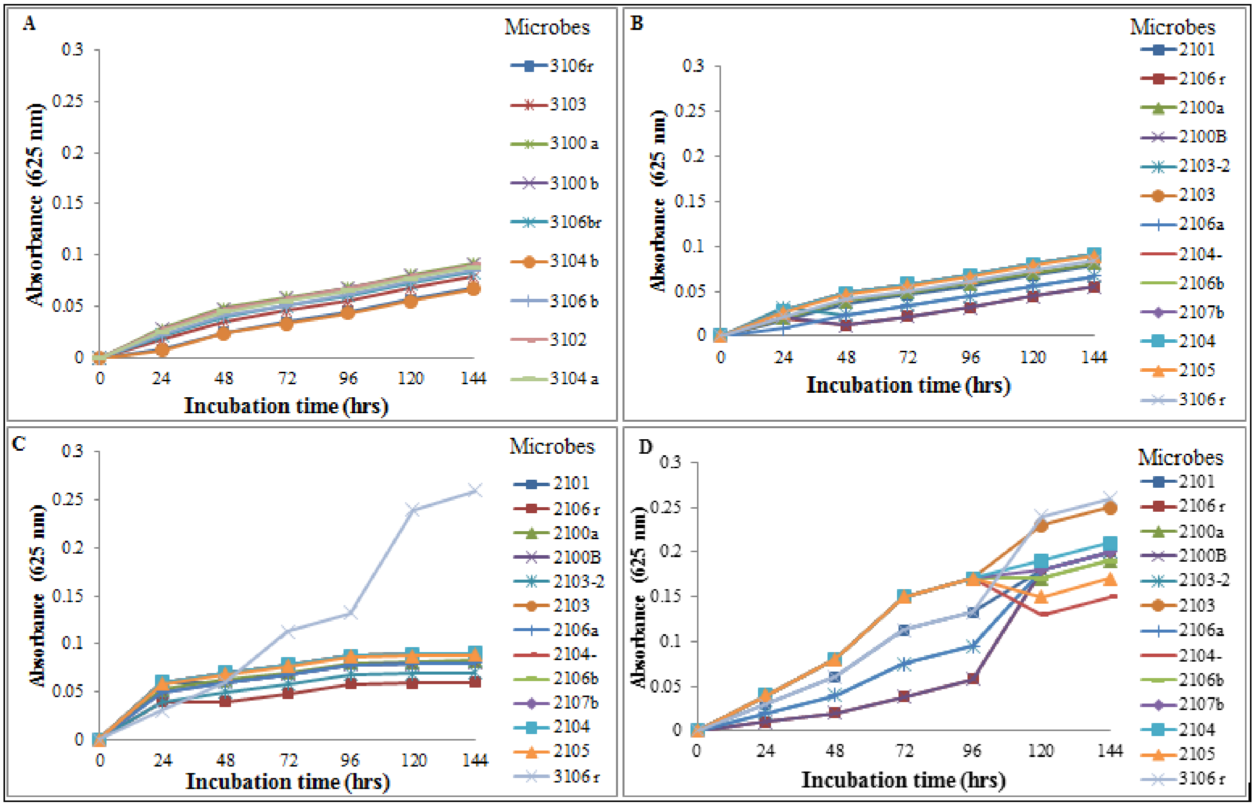

Figure 8: Growth kinetics of cypermethrin and acetochlor degrading bacteria through utilisation of pesticides as sole carbon and phosphorous source for 144 hours. **A**: Growth kinetics of isolates using cypermethrin as sole carbon source, **B**: Growth kinetics of isolates using acetochlor as sole carbon and P source, **C**: Growth kinetics of isolates in acetochlor as sole phosphorus source, **D**: Growth kinetics of isolates using Acetochlor as sole carbon source. 3106r= *Enterobacter sp;* 3103= *Enterobacter sp;* 3100a= *Enterobacter cloacae;* 3100b= *Klebsiella pneumoniae;* 3106br= *Enterobacter asburiae;* 3104b= *Klebsiella pneumoniae;* 3106b= *Leclercia sp.;* 3102= *Klebsiella oxytoca;* 3104a= *Pseudomonas aeruginosa;* 2101= *Enterobacter cloacae;* 2106 r= *Enterobacter asburiae;* 2100a= *Enterobacter asburiae;* 2100B= *Enterobacter cancerogenus;* 2103-2= *Enterobacter tabaci;* 2103= *Enterobacter asburiae;* 2106a= *Enterobacter xiangfangensis;* 2104a= *Enterobacter cloacae;* 2106b= *Enterobacter xiangfangensis;* 2107b= *Pantoea agglomerans;* 2104b= *Enterobacter cloacae;* 2105= *Enterobacter tabaci*.

3.3 Genetic diversity of the isolated microbes

In this study, isolates were identified with the best matching 16S rRNA and 18S rRNA genes with those of NCBI database with result list in Table 5. All bacteria were above 97% of 16S rRNA similarity level. All the gene sequences of PSM and pesticide degrading microbes were submitted to the GenBank nucleotide database and accession numbers are on table 5.

The genetic diversity of phosphate solubilising and pesticide degrading microbes isolated from different ecological zones were characterised using a range of molecular techniques. Blast analysis of sequenced genes released homologous bacteria and fungi (table 5).

The homologous had higher nucleotide identity to the isolates obtained from this study. The identity ranged from 84% to 100%. All fungal organisms revealed the highest sequence identity of 100%. Most of the isolates analysed in this study were homologous to the various species of *Enterobacter*. The other notable genera were *Klebsiella* and *Pseudomonas*. The accession numbers of the nucleotide sequences of the microbes isolated in this study are given in table 5.

Phylogenetic analysis of fungal microbes solubilising P and degrading pesticides revealed *Fusarium oxysporum* (MF977405) as an outgroup. The other two isolated belonged to the same clade with those previous isolated.

Table 5: Isolates and their BLAST related species and GenBank deposit accession numbers

LAB NO	RELATED SPECIES(homologs)	NUCLEOTIDE IDENTITY %	ACCESSION NUMBER
6106b	*Mucor irregularis*	84	MF991235
6106	*Fusarium oxysporum*	100	MF974394
73A	*Aspergillus niger*	100	MF974575
74B	*Penicillium janthinellum*	99	MF974569
6102B	*Fusarium oxysporum*	92	MF974393
6102	*Fusarium oxysporum*	92	MF977405
6100	*Aspergillus parasiticus*	100	MF983813
6101b	*Meyerozyma caribbica*	99	MF983800
3106b	*Enterobacter asburiae*	99	MF979777
3106br	*Enterobacter asburiae*	99	MF979662
77A	*Klebsiella pneumoniae*	99	MF979635
72A	*Enterococcus casseliflavus*	99	MF979558
75A	*Pseudomonas putida*	99	MF979809
2106b	*Enterobacter cloacae*	99	MF979810
2106A	*Enterobacter cloacae*	97	MF979821
2105	*Enterobacter sp.*	91	MF979964
2104-2	*Enterobacter sp.*	99	MF979876
2104-1	*Enterobacter cloacae*	99	MF979885
2103	*Enterobacter sp.*	99	MF980152
2103-2	*Enterobacter sp.*	99	MF980711
2101	*Enterobacter sp.*	99	MF980718
3106B	*Enterobacter sp.*	98	MF980912
2107b	*Pantoea agglomerans,*	96	MF980788
2100a	*Enterobacter cloacae*	99	MF980882
2100B	*Enterobacter sp.*	99	MF980911
3103	*Enterobacter asburiae*	98	MF980919
3100 a	*Enterobacter cloacae*	99	MF980916
3100 b	*Klebsiella pneumoniae*	99	MF980917
3106 r	*Enterobacter asburiae*	99	MF980922
3104 b	*Klebsiella pneumoniae*	98	MF980921
3102	*Serratia marcescens*	98	MF980918
3104 a	*Pseudomonas aeruginosa*	93	MF980920
1104	*Enterobacter cloacae*	99	MG031167
1107	*Achromobacter sp*	88	MG031169
1103	*Enterobacter aerogenes*	99	MG031163
1105	*Enterobacter tabaci*	88	MG031168

These are results of isolates identified by Megablast (Optimize for highly similar sequences) using 16S rRNA and 18S rRNA sequences and their GenBank deposit accession numbers.

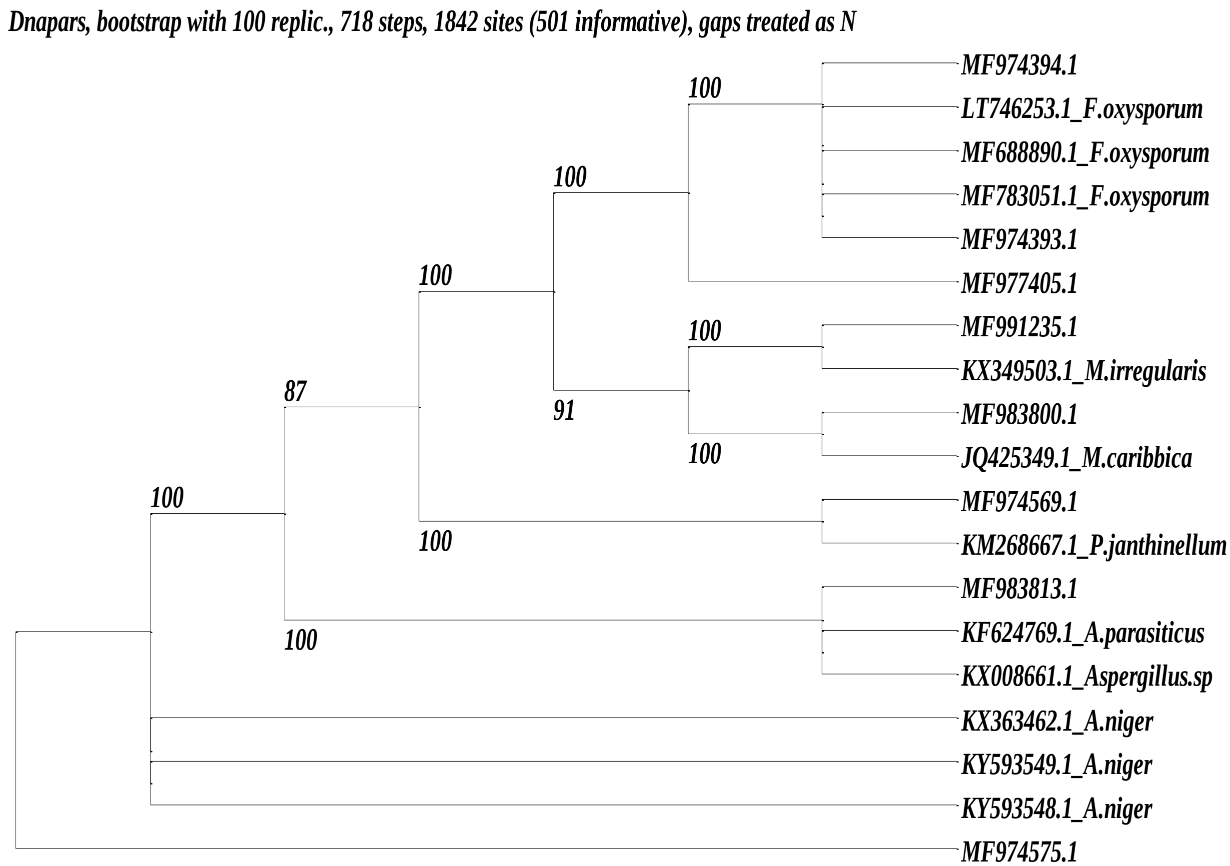

Figure 9: Phylogenetic tree based on 18S rRNA gene sequence showing the position of phosphate solubilising and pesticide degrading fungus isolated from selected agro ecological zones in Malawi compared with those available in GenBank of NCBI. The phylogenetic tree was constructed using maximum likelihood method using Seaview software; Bootstrap values analysis with Kimura 2-parameter model. The accession numbers MF991235, MF974394, MF974575, MF974569, MF974393, MF977405 and MF983813 are from different agro ecological zones of Malawi while the corresponding ones attached to the name are obtained from GenBank. Phylogenetic tree show diversity of *Fusarium oxysporum* based on an outgroup isolate MF977405 which was also observed in pesticide utilisation pattern as indicated on table 3.

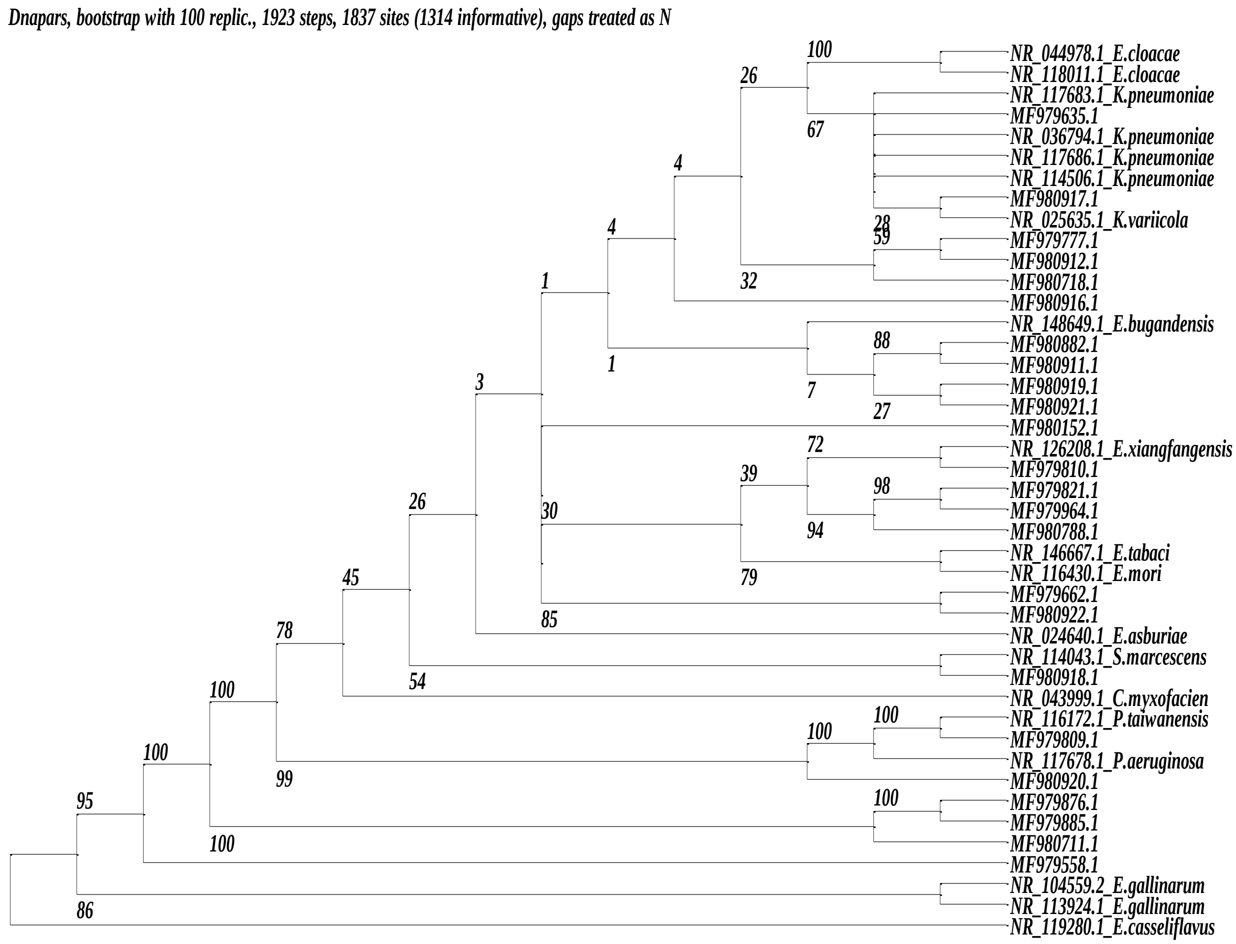

Figure 10: Phylogenetic tree based on 16S rRNA gene sequence showing the position of phosphate solubilising bacteria and pesticide degrading bacteria isolated from selected agro ecological zones of Malawi and those of NCBI. The phylogenetic tree was constructed using maximum likelihood method using Seaview software; Bootstrap values analysis with Kimura 2-parameter model. The accession numbers with no names attached are from different agro ecological zones of Malawi as indicated on table 3 and 4 while the corresponding ones attached to the name are obtained from GenBank. Phylogenetic tree shows diversity of isolates from Malawi forming unique clades separate from those of GenBank of NCBI. Many isolates formed single outgroup clades like isolate MF979558 or as group outgroup clade MF979876, MF979885 and MF980711. More than 95% of isolate unique clades were formed by those degrading Acetochlor herbicide based on agro ecological zone indicating that they have distant relationship.

CHAPTER FOUR

4.0 Discussion

The microbes isolated in this study have been designated as PSM on the basis of possessing capability to solubilise the insoluble inorganic P in agreement with other studies (Rossetti *et al.*, 2016; Investigación *et al.*, 2015; Aarab *et al.*, 2008). The capability of solubilising insoluble P was observed by appearance of visible halos which had solubilisation index of more than 1.5 on PVK agar plates (figure 3 and table 1) as described by Mendes *et al.* (2014a). The observation was also made in-vitro experiment using soil and rock phosphate through increase in available P as shown in figure 4. In the present study, periodical evaluation of P in different media revealed the potential of the isolates in releasing P from insoluble phosphate sources (figure 5A). Superiority was designated on the basis of SI value. The recorded observations indicated that every isolates, have unique SI which is in agreement with other studies (Investigación *et al.*, 2015). *A. niger* showed higher solubilisation index of 3.5 similar to other reported studies (Elias *et al.*, 2016a).

There was a strong statistically significant difference in phosphate solubilising efficiency of the isolated strains in Pikovskaya's broth, soil and RP ($P \leq 0.01$) indicating that solubilisation is dependent on strain and inorganic P source (figure 4 and table 2) and this is in agreement with other studies (Sahoo and Gupta, 2016; Sane and Mehta, 2015; Menon and Mohan, 2007). Commercial strains showed a strong statistically significant difference in solubilisation between all the groups ($P \leq 0.01$) indicating that the strain is not related to the other strains in table 2. This could be as a result of abiotic factors which favoured indigenous microbes besides general efficacy of isolated strains as reported in other studies (García-Fraile *et al.*, 2015). Some of the isolated strains increased immobilisation as shown by decrease in soluble P after 6 days while other values fluctuated as shown in figure 5. Various studies have reported different maximum P solubilisation efficiency at different incubation period because of biotic and abiotic factors that impacted the evolution of strains (Nosrati *et al.*, 2014). Strains performed differently on different media regardless of their higher PSI on PVK media as shown by statistical significance ($P \leq 0.02$). This indicates that standard method for measuring solubilisation cannot be a sole test for P solubilisation. This study showed that P solubilisation efficiency and rate is based on strain and correlates with other studies (Sahoo and Gupta, 2016). In contrast study by Sumatera, 2016 found that fungal microbes are the best solubilisers of phosphates.

The PSM exhibited a strong capacity to reduce pH of soil, rock phosphate and other medium and the activity was significant in all experiments as shown in tables in appendices. More than 80% of isolates had a significant negative correlation of ($r = -0.8$; $p < 0.04$) between the available P and pH values a finding consistent with a previous study (Aarab *et al.*, 2008). This findings is also in agreement with other studies that found that the major mechanism for the microbial dissolution of inorganic P is acid production (Liu *et al.*, 2016). The production of gluconic acid is the most frequent agent of P solubilisation regardless of other acid production mechanisms such as nitric, sulphuric, and carbonic (Stella and Halimi, 2015). Excretions of metabolites for P solubilisation are influenced by both biotic and abiotic factors (Liu *et al.*, 2016).

The study showed potential significance of inoculating RP with *A. niger* and *E. casseliflavus* while others work better under co-inoculation (figure 4A) a finding consistent with other studies that recommends *A. niger* (Ahemad and Kibret, 2014; Mendes *et al.*, 2014a). These PSM showed halo-zone on PVK media in contrast with other studies that reported microbes that solubilise RP without a halo-zone (Hamdali *et al.*, 2012). Isolated indigenous microbes had higher P solubilisation compared to commercial ones previously isolated in Asia. This could have been due to adaptation of isolates to local edaphic conditions besides general efficacy of strains (RP and soil). The use of natural phosphate bearing materials such as RP as fertilizer for P deficient soils has received attention because deposits of cheaper and low grade RP are locally available even in many parts of Malawi. RP is chemically processed by costly and environmental hazard process by reacting with sulphuric acid or phosphoric acid to produce partially acidulated RP. Biofertilisers are cheap and convenient alternative for reclamation of exhausted soil (Investigación *et al.*, 2015). Thus, PSM may play acritical role in natural P cycle and improve the agronomic value of rock phosphate, which is underutilised by smallholder farmers (Sane and Mehta, 2015).

Different PSM isolates solubilised the insoluble P sources such as tri calcium phosphate, soil and RP with solubilisation increasing at different incubation period time. These results are in agreement with other finding by Elias *et al.* (2016) and Zhu *et al.* (2011). These studies reported gradual increase in mobilised P by PMS. The decrease in phosphate solubilisation observed at the end of incubation time (figure 5) could be attributed to sufficient availability of soluble P that has an inhibitory effect on solubilisation. Alternatively, carbon source may be depleted limiting both the production of organic acids and microbial activity (Elias *et al.*, 2016; Zhu *et al.*, 2011).

There was an increase in P solubilisation by synergistic effect of co-inoculation of rhizobium with some PSM mainly on soil medium except *K. pneumoniae*. The increased solubilisation implies that strains, which solubilise P and degrades pesticide, have low compatibility with other strains (figure 4). Microbes with high solubilisation effect after co-inoculation showed no s significant difference in P solubilisation as compared to two strains of rhizobium. The increase in available P after co-inoculation could be as a result of solubilisation by synergistic nitrogen fixing rhizobium (Abd-alla *et al.*, 2014). The potential of the genus rhizobium as a phosphate solubilising bacterium besides nitrogen fixing has been previously described (Pilar *et al.*, 2013; López-Ortega *et al.*, 2013). The studies have shown that biofertilisers with compatible effective strains can replace inorganic fertiliser to reduce production cost and prevent environmental pollution (Abd-alla *et al.*, 2014). This current study was done to assess synergistic effects of co-inoculation because its known fact that soluble phosphorous availability is one of determining factor for the uptake of nitrogen and its utilisation by crops (Li *et al.*, 2013). Therefore, the co-inoculation of compatible effective strains could be considered as an appropriate substitute for all inorganic fertiliser and sustainable agricultural systems.

The evaluated microbes in the study were isolated from different agro ecological zones, therefore, it is likely that some factors such as temperature, pH and redox potential, may have affect P solubilisation (López-ortega *et al.*, 2013). These microbes were isolated from different crops and some contrary to what other studies reported indicating that these microbes are not crop specific. For example *K. pneumoniae* has been isolated in okra while other studies have isolated them in grass, wheat and maize (Sarathambal and Ilamurugu, 2014; Pilar *et al.*, 2013; Sachdev *et al.*, 2009).

The use of microorganisms for pesticide degradation requires integrated understanding of all biochemical, physiological, ecological, microbiological, and molecular aspects involved in pollutant degradation (Singh *et al.*, 2014). The study found out that some microbes can utilise pesticides as sole carbon or P source or both, which is in agreement with other reports (Shamsuddeen and Inuwa, 2013; Lim, 2011). The isolated microbes had no in-vitro quantitative analysis because real degradation is factored by several synergistic biotic and abiotic complications (Yang *et al.*, 2013). In this study cypermethrin and acetochlor were degraded by bacteria only while glyphosate was degraded by both fungal and bacteria. Dimethoate was not degraded by any of the isolated microbes. In contrast, *Enterobacter asburiae* degraded both cypermethrin and acetochlor.

The study also found that natural selection is responsible for diversity of xenobiotic degrading microbes as shown by remarkable diversity in Nkhozo and Khongoloni farms, which have long history of pesticide application compared to Chasatha farm with a year of pesticide application (Neumann *et al.*, 2014). Regular aerial application of pesticide may impact on non-target sites as shown by genetic diversity of microbes responsible for degrading xenobiotics outside the farm that does not apply pesticide. Genus *Enterobacter* domination in bioremediation is in line with other studies (Kryuchkova *et al.*, 2014; Ogot *et al.*, 2013; Thatheyus and Selvam, 2013). The study suggest that pest infestation in fields where pesticide application occurs is a result of abundance of xenobiotic degrading microbes. The abundance is due to natural selection pressure not pesticide resistance. In this case more diversity was observed in Mulanje and Rumphi than Karonga. This is the first study to isolate microbe that can degrade cypermethrin and also solubilise inorganic P in different ecological zones in Malawi.

Micro-organisms in soil, responsible for the degradation of glyphosate follow two different chemical pathways. One pathway produces a compound known as aminomethylphosphonic acid (AMPA) which is mildly toxic to plant growth while the second pathway produces the compound sarcosine (Foley *et al.*, 2008). The microbes use enzymes to breakdown glyphosate, to obtain a source of phosphorus, nitrogen and carbon. Genetic diversity of isolated strains that utilise glyphosate as sole carbon or P source, is in agreement with study by Weaver *et al.* (2007). Five fungi and four bacteria degraded glyphosate. The fungi were *Aspergillus parasiticus, Meyerozyma caribbica, 2 strains of Fusarium oxysporum, Mucor irregularis* while the bacteria were *Bordetella and 3 strains of Enterobacter*. This study found genetic diversity among *Fusarium oxysporum* species with regard to utilisation of glyphosate as carbon and P sources. The findings concur with other studies which associate glyphosate with increased severity or re-emergence of crop diseases caused by *Fusarium oxysporum* strains. Glyphosate use may result in alteration of communities of rhizosphere microbes involved in nutrient transformation, thereby shifting the balance between micro-organisms that are beneficial those that are detrimental to plant health (Johal and Huber, 2009). These findings are consistent with other studies which found that different *Enterobacter* strains, *Aspergillus* and *Fusarium* degrade glyphosate (Rohilla and Salar, 2012; Ogot *et al.*, 2013). This is in contrast with the other studies which reported *Pseudomonas sp* as best biodegrading microbe (Zhao *et al.*, 2015; Yunda, 2010). The findings of this study add some unique strains of glyphosate degrading microbes from tropical soils that may be used in further studies like *Meyerozyma caribbica*. These results indicate that bioremediation can be done using bioargumentation if microorganisms used are not pathogenic.

Synthetic pyrethroid are not usually leached in soil because they are highly hydrophobic strongly adsorbed in soil. The half-life of this pesticide vary from 4 days to 8 weeks and is significantly affected by soil characteristics or microbial activity (Bhosle *et al.*, 2013). The major degradation pathway of cypermethrin is 3-phenoxy benzyl alcohol and 3-phenoxy benzoic acid and this occur by hydrolysis via cleavage, of an ester linkage. The presence of separated layers in degrading tubes indicated 3-phenoxy benzoic. Nine bacteria strains were isolated and were capable of degrading cypermethrin. These isolates included *Serratia marcescens, Pseudomonas, Leclercia sp*., 2 strains of *K. pneumoniae* and 4 strains of *Enterobacter* from the 3 sites. Several microbes were found to degrade cypermethrin by utilisation of the compound as the sole carbon source consistent with other studies (Bhosle and Nasreen, 2013; Shamsuddeen and Inuwa, 2013). The microbes included *S. marcescens* and *Pseudomonas* (Rani *et al.*, 2008; Malatova and Morrill, 2005) and *Enterobacter* (Roy and Subbaiah, 2017; Thatheyus and Selvam, 2013; Massiha and Issazadeh, 2012). However other microbes such as *Leclercia sp and K. pneumoniae* are not known to degrade cypermethrin. These findings may provide a basis for designing a multi-resistant bacterium that can be used to reverse the altered environment (Jabeen *et al.*, 2017)

Although, there are reports that some of above isolated microbe can degrade glyphosate and other compounds, these strains isolated here only degraded cypermethrin (Zhao *et al.*, 2015; Rehman *et al.*, 2010). The study also found that not all microbes utilise cypermethrin as sole P source. A possible explanation for this is that P is not found in cypermethrin molecule. This study adds *Leclercia sp*, and *K. pneumoniae* to the list of cypermethrin degrading microbes.

Acetochlor (2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6methylphenyl) acetamide) is a widely used early post-emergent and pre-emergent chloroacetanilide pesticide in corn fields. The herbicide prevents the growth of broadleaf weeds and annual grasses by affecting the photosynthetic electron transport however, its environmental fate of residue remains unknown (Bai *et al.*, 2013). Strong mobility of acetochlor poses an environmental risk to arable land, ground and surface water (Borowik and Kucharski, 2015). Acetochlor is also suspected to be endocrine disruptor and regarded as a probable human carcinogen. Half-lives of the herbicide are 3.4 and 2.8 days in the bulk soil and rhizosphere respectively. But its residue of 0.02–0.07 μg/g can still be detected 40 days after application in the soil (Bai *et al.*, 2013). Studies have shown that cyanobacterial mat and *E. asburiae* have been involved bioremediation of acetochlor (El-nahhal *et al.*, 2013; Martins *et al.*, 2011).   In this study eleven strains of bacteria were isolated to degrade acetochlor *(Pantoea agglomeran,* and 10 strains of *Enterobacter*) from 3 sites. The genetic diversity of the genus *Enterobacter* is well known for degradation of acetochlor (Martins *et al.*, 2011). Microbes isolated in this study degraded acetochlor by utilisation of the herbicide as sole carbon source a finding consistent other related studies (Bhosle and Nasreen, 2013; Shamsuddeen and Inuwa, 2013). One strain namely *E. asburiae* degraded both acetochlor and cypermethrin detected in 2 sites a finding that supports a previous study that link the microbe with degradation of acetochlor (Martins *et al.*, 2011). The study also found that all microbes utilised acetochlor as sole P source.

Dimethoate is an organophosphorus insecticide widely used to kill mites and insects systemically and by contact. The insecticide is often detected in the environment and forms the seven metabolites after degradation. These include dimethoate carboxylic acid, 2-(hydroxy(methoxy) phosphorylthio) acetic acid, *O,O,S*-trimethyl thiophosphorothioate, *O*-methyl *O,S*-dihydrogen phosphorothioate, phosphorothioic *O*,*O*,*S*-acid, *O*,*O*,*S*-trimethylphosphorothiate and *O*,*O*,*O*-trimethyl phosphoric ester. It has been applied widely around the world on various crops. The use of this insecticide has affected many environmental matrices where it can exhibit toxic effect to target and non-target organisms' (Evgenidou *et al.*, 2005). Studies have shown that *S. marcescens* have been involved in bioremediation of acetochlor (Zmijowska and Cycon, 2014). However in the current study no strain of bacteria or fungi degraded dimethoate from the 3 sites suggesting that microbial consortia may tolerate up to about 120 mg l^{-1} of dimethoate (El-nakieb, 2008). Some studies found that *Bacillus, Enterobacter*, *Pseudomonas* and *Aeromonas* degraded dimethoate but these microbes could not utilise it as sole source C or P (Begum *et al.*, 2016). Photocatalytic oxidation of dimethoate has been studied using titanium dioxide and ZnO as catalysts (Evgenidou *et al.*, 2005).

All microbes that were able to degrade pesticides produced laccase enzyme. Laccase is a very potent enzyme with ability to act on a number of substrates. The results of the current study concurs with other studies which reported that laccase may be used for bioremediation and waste treatment such as degradation and detoxification of pollutants (EDCs, chlorophenols, PAhs, pesticides and others) (Viswanath *et al.,* 2016; Hindumathy and Gayathri 2013). Laccase also plays important roles in lignolytic degradation, detoxification studies, plant pathogenesis, odour control in decomposition of wastes and pigment production (Viswanath *et al.,* 2016). The expression of laccase is influenced by several factors such as nature and concentration of carbon source, nitrogen source, temperature and pH among others. (Viswanath *et al.,* 2016; Singh and Abraham, 2013)

Preference of fungal laccase is based on its higher redox potential of up to +800 mV compared with plants or bacterial laccases (Kunamneni *et al.*, 2007). Due to its demand, biotechnological efficiency of laccase has led to introduction of laccase-mediator systems (Kubo *et al.,* 1994; Viswanath *et al.,* 2016)**.** Laccase has several inhibitors for its enzymatic activity such as cyanide, thiocyanide, halides, fluoride, hydroxide and azide (Kunamneni *et al.*, 2007)**.** Heavy metals and xenobiotics induce laccase production because of having receptors (putative *cis*-acting responsive elements) in the promoter regions of the genes encoding for laccase (Castilho *et al.*, 2009). Increase in concentration of certain inducers can lead to production of new isoforms of the enzyme which may be beneficial to remediation (Kunamneni *et al.*, 2007). The presence of laccase gene is an indicator that the microorganism is able to degrade the xenobiotics present in the environment.

It is therefore probable that these indigenous PSM and pesticide degrading microbes may be used as biofertilisers to support growth and development of crops because of production of multiple PGRT like IAA, siderophore, and catalase amongst others as shown in table 1 and 3 table (Asnawati *et al.*, 2016). Some of these PSM such as *K. pneumonia* has been documented to have antifungal activity towards *F. oxysporum, Sclerotium rolfsii, Alterneria alternatae and Macrophomina phasiolina* (Jahangir *et al.,* 2016) while some might also enhance drought tolerance in plant and promote bioremediation of contaminated soil by heavy metal. Diagnosis of other traits, beside P solubilisation and xenobiotics degrading, like IAA siderophore catalyse and nitrogen fixation was done to identify the most efficient PGPR isolate.

Isolates with multiple PGRT are effective in improving the plant growth parameters since they are recommended for inoculants (Sharma *et al.,* 2013a). These may be a viable approach for replacing inorganic fertilisers. In addition the isolated PSM may enhance the growth and productivity of commercially grown crops under local agro-climatic conditions as reported in other studies (García-Fraile *et al.*, 2015). Production of IAA has an effect on root system where it results in increase in size and number of adventitious roots thus increasing large surface area for absorption of plant macro and micro nutrients (Jog *et al.,* 2012). IAA production by PGPR is also influenced by the type of species, strain and by both biotic and abiotic factors. The production of IAA is one of effective tool for screening beneficial microbes and previous studies have reported that PSM is associated with IAA production (Hashem *et al.,* 2016; Kavamura *et al.,* 2013).

Previous studies have shown that if microbes may improve plant growth if they produce siderophores which are basically low molecular weight iron chelating compounds. The Fe sequestered by microbial siderophores cannot be scavenged by pathogens. Siderophore producing microorganisms protect crops either by limiting the growth of pathogenic microbes or by manipulating plant's defensive metabolism. These microbes have exhibited traits, which have been reported by other researchers (Jog *et al.,* 2012). Some of these PSM produce organic acids, which solubilise mineral K, an example include *Pseudomonas putida* (Sarikhani, 2016).

Phylogenetic analysis based on the NJ and ML methods revealed that diversified divergent genera and species are involved in P solubilisation and degradation of pesticide. The genus *Enterobacter* is dominated in terms of genetic diversity at species and strain level in degradation of pesticide. The isolated strain MF974575 (*Aspergillus niger*) from Lilongwe is an outgroup to those *A. niger* already in database as well as to other isolated species. This may be due to the fact that it is from different agro ecological zone and may be P solubilisers while the others are glyphosate degrading fungi.

F. oxysporum strains that solubilised P and degraded pesticide were genetically diverse based on an outgroup MF977405 in the phylogenetic tree. This was also shown by their diversity to utilize glyphosate as sole phosphorous as well as carbon sources simultaneously and independently. The findings contradicts those of the isolate MF974393 and MF974394 which utilized glyphosate as P and C source independently. This indicates that these strains are of different phylotypes because they are from different ecological zones and effect of the pesticide selection pressure may explain the genetic diversity of microbes demonstrated in the phylogenetic tree.

For P solubilising and pesticide degrading bacteria genetic diversity was observed by formation of unique clades separate from the strains retrieved from NCBI. Many isolates formed single outgroup clades i.e. MF979558 or as a group clade MF979876, MF979885 and MF980711. More than 95% of isolate unique clades were formed by those degrading acetochlor based on agro ecological zones indicating that they have distant relationship.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion this study have shown that

1. Soil in selected agro ecological zones in Malawi contain indigenous PSM which may be used as biofertilisers
2. The indigenous microbes may contribute to biodegradation of cypermethrin, glyphosate and acetochlor residues present in soil in the agro ecological zones
3. PSM and pesticide degrading microbes isolated in selected agro ecological zones in Malawi are genetically diverse and some possess PGRT

5.2 Recommendation

The study needs further investigations in details of isolates to confirm their ability in field and also whole genome sequencing. Studies should focus on bioargumentation for pesticide degrading microbes and also characterisation of specific genes involved in solubilisation and biodegradation.

REFERENCE

Aarab, S., Ollero, F. J., Megías, M., Laglaoui, A., Bakkali, M. and Arakrak, A. (2008) ‘Isolation and Identification of Potential Phosphate Solubilizing Bacteria from the Rhizosphere of Lupinus hirsutus L . in the north of Morocco Materials & Methods’, pp. 1–7.

Abbas, Z., Zia, M. A., Ali, S., Abbas, Z., Waheed, A., Bahadur, A., Hameed, T., Iqbal, A., Muhammad, I., Roomi, S., Ahmad, M. Z. and Sultan, T. (2013) ‘Integrated Effect of Plant Growth Promoting Rhizobacteria , Phosphate Solubilizing Bacteria and Chemical Fertilizers on Growth of Maize Original Research Article’, pp. 913–921.

Abd-alla, M. H., El-enany, A. E., Nafady, N. A., Khalaf, D. M. and Morsy, F. M. (2014) ‘Synergistic interaction of Rhizobium leguminosarum bv . viciae and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (Vicia faba L .) in alkaline soil □’, *Microbiological Research*. Elsevier GmbH., 169(1), pp. 49–58. doi: 10.1016/j.micres.2013.07.007.

Adhya, T. K., Kumar, N., Reddy, G., Podile, A. R., Bee, H. and Samantaray, B. (2015) ‘Microbial mobilization of soil phosphorus and sustainable P management in agricultural soils’, *Current Science*, 108(7).

Aferi, N. K. (2014) ‘Isolation of phosphate solubilizing bacteria from tropical soil’, 3(1), pp. 8–15.

Ahemad, M. and Kibret, M. (2014) ‘Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective’, *Journal of King Saud University - Science*. doi: 10.1016/j.jksus.2013.05.001.

Ahmad, F., Ã, I. A. and Khan, M. S. (2008) ‘Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities’, 163. doi: 10.1016/j.micres.2006.04.001.

Ahuja, S. K., Ferreira, G. M., Moreira, A. R., Ahuja, S. K., Ferreira, G. M. and Utilization, A. R. M. (2016) *Critical Reviews in Biotechnology Utilization of Enzymes for Environmental Applications Utilization of Enzymes for Environmental*. doi: 10.1080/07388550490493726.

Akbar, S., Sultan, S. and Kertesz, M. (2015) 'Determination of Cypermethrin Degradation Potential of Soil Bacteria Along with Plant Growth-Promoting Characteristics', pp. 75–84. doi: 10.1007/s00284-014-0684-7.

Almaghrabi, O. A., Massoud, S. I. and Abdelmoneim, T. S. (2013) 'Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions', *Saudi Journal of Biological Sciences*. King Saud University, 20(1), pp. 57–61. doi: 10.1016/j.sjbs.2012.10.004.

Anderson, I. C., Campbell, C. D. and Prosser, J. I. (2003) 'Potential bias of fungal 18S rDNA and internal transcribed spacer polymerase chain reaction primers for estimating fungal biodiversity in soil', 5, pp. 36–47.

Annibale, A. D., Rosetto, F., Leonardi, V., Federici, F. and Petruccioli, M. (2006) 'Role of Autochthonous Filamentous Fungi in Bioremediation of a Soil Historically Contaminated with Aromatic Hydrocarbons', 72(1), pp. 28–36. doi: 10.1128/AEM.72.1.28.

Asnawati, F., Citra, H., Handayani, N. I., Junda, M., Ali, A., Hala, Y. and Jumadi, O. (2016) 'Ability of ammonium excretion , indol acetic acid production , and phosphate solubilization of nitrogen-fixing bacteria isolated from crop rhizosphere and their effect on plant growth', 11(19), pp. 11735–11741.

Bai, Z., Xu, H., He, H. and Zheng, L. (2013) 'Alterations of microbial populations and composition in the rhizosphere and bulk soil as affected by residual acetochlor', pp. 369–379. doi: 10.1007/s11356-012-1061-3.

Barroso, C. B. and Nahas, E. (2013) 'Enhanced Solubilization of Iron and Calcium phosphates by Aspergillus niger by the Addition of Alcohols', 56(April), pp. 181–189.

Begum, S. F. M., Rajesh, G. and Narendran, R. R. (2016) 'Isolation , Characterization and Identification of Dimethoate Degrading Bacteria from Soil Series of Tamil Nadu', 3(6), pp. 220–230.

Beneduzi, A., Ambrosini, A. and Passaglia, L. M. P. (2012) 'Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents', *Genetics and*

Molecular Biology, 35(4 SUPPL.), pp. 1044–1051. doi: 10.1590/S1415-47572012000600020.

Bhardwaj, D., Ansari, M. W., Sahoo, R. K. and Tuteja, N. (2014) 'Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity.', *Microbial cell factories*, 13(1). doi: 10.1186/1475-2859-13-66.

Bhattacharya, S., Das, A., Bhardwaj, S. and Rajan, S. S. (2015) 'Phosphate solubilizing potential of Aspergillus niger MPF-8 isolated from Muthupettai mangrove', 74(September), pp. 499–503.

Bhawana, P. and Fulekar, M. H. (2012) 'Bioremediation of Dyestuff Compounds using Indigenous Microorganism in a Bioreactor', *APCBEE Procedia*, 1(January), pp. 27–33. doi: 10.1016/j.apcbee.2012.03.006.

Bhosle, N. P., Khan, Z. S., Nasreen, S. and Road, C. (2013) 'In-vitro degradation of Cypermethrin through microorganisms by scale up technique Nilesh', 1(2320), pp. 229–238.

Bhosle, N. P. and Nasreen, S. (2013) 'Remediation of Cypermethrin-25 EC by Microorganisms Siddharth Art , Commerce and Science College Jafrabad , Dist Jalna', 3(1), pp. 144–152.

Borowik, A. and Kucharski, J. (2015) 'Microbial and enzymatic activity of soil contaminated with a mixture of diflufenican + mesosulfuron-methyl +', pp. 643–656. doi: 10.1007/s11356-014-3395-5.

Carvalho, F. P. (2017) 'Pesticides , environment , and food safety'. doi: 10.1002/fes3.108.

Castilho, F. J. D., Torres, R. A., Barbosa, A. M., Dekker, R. F. H. and Garcia, J. E. (2009) 'On the diversity of the laccase gene: A phylogenetic perspective from botryosphaeria rhodina (Ascomycota: Fungi) and other related taxa', *Biochemical Genetics*, 47(1–2), pp. 80–91. doi: 10.1007/s10528-008-9208-0.

Castro-Sowinski, S., Herschkovitz, Y., Okon, Y. and Jurkevitch, E. (2007) 'Effects of inoculation with plant growth-promoting rhizobacteria on resident rhizosphere microorganisms', *FEMS Microbiology Letters*, 276(1), pp. 1–11. doi: 10.1111/j.1574-6968.2007.00878.x.

Chitraselvi R, P. E., Kalidass, S. and Kant, R. (2015) 'Efficiency of Rhizosphere Bacteria in Production of Indole Acetic Acid , Siderophore and Phosphate Solubilization', 7(6), pp. 2557–

2564.

Chotchutima, S., Tudsri, S., Kangvansaichol, K. and Sripichitt, P. (2016) 'Effects of sulfur and phosphorus application on the growth, biomass yield and fuel properties of leucaena (Leucaena leucocephala (Lam.) de Wit.) as bioenergy crop on sandy infertile soil', *Agriculture and Natural Resources*. Elsevier Ltd, 50(1), pp. 54–59. doi: 10.1016/j.anres.2015.09.002.

DARS.MOAFS. Department of Agricultural Research and Services of Ministry of agriculture and Food Security, DARS Statistical Databases. Agriculture Data. (2015) DARS Lilongwe, Malawi. accessed 20 October 2016. (Available) (2016).

Dinesh, R., Anandaraj, M., Kumar, A., Kundil, Y., Purayil, K. and Aravind, R. (2015) 'Isolation , characterization , and evaluation of multi-trait plant growth promoting rhizobacteria for their growth promoting and disease suppressing effects on ginger', *Microbiological Research*. Elsevier GmbH., 173, pp. 34–43. doi: 10.1016/j.micres.2015.01.014.

El-nahhal, Y., Awad, Y. and Safi, J. (2013) 'Bioremediation of Acetochlor in Soil and Water Systems by Cyanobacterial Mat', 2013(July), pp. 880–890.

El-nakieb, A. A. F. A. (2008) 'Bioremediation of Dimethoate by Effective Microorganisms in Water', pp. 2–5.

Elias, F., Woyessa, D. and Muleta, D. (2016a) 'Phosphate Solubilization Potential of Rhizosphere Fungi Isolated from Plants in Jimma Zone , Southwest Ethiopia'. Hindawi Publishing Corporation, 2016(1). doi: 10.1155/2016/5472601.

Eman, A., Abdel-megeed, A., Suliman, A. A. and Sadik, M. W. (2013) 'Biodegradation of Glyphosate by fungal strains isolated from herbicides polluted-soils in Riyadh area', 2(8), pp. 359–381.

Evgenidou, E., Fytianos, K. and Poulios, I. (2005) 'Photocatalytic oxidation of dimethoate in aqueous solutions', 175, pp. 29–38. doi: 10.1016/j.jphotochem.2005.04.021.

Foley, M. E., Sigler, V. and Gruden, C. L. (2008) 'A multiphasic characterization of the impact of the herbicide acetochlor on freshwater bacterial communities', pp. 56–66. doi: 10.1038/ismej.2007.99.

García-fraile, P., Menéndez, E. and Rivas, R. (2015) ‘Role of bacterial biofertilizers in agriculture and forestry’, 2(3), pp. 183–205. doi: 10.3934/bioeng.2015.3.183.

Goudjal, Y., Toumatia, O., Yekkour, A. and Sabaou, N. (2014) ‘Biocontrol of Rhizoctonia solani damping-off and promotion of tomato plant growth by endophytic actinomycetes isolated from native plants of Algerian Sahara □’, *Microbiological Research*. Elsevier GmbH., 169(1), pp. 59–65. doi: 10.1016/j.micres.2013.06.014.

Guermouche, A. M., Bensalah, F., Gury, J. and Duran, R. (2015) ‘Isolation and characterization of different bacterial strains for bioremediation of n -alkanes and polycyclic aromatic hydrocarbons’, pp. 15332–15346. doi: 10.1007/s11356-015-4343-8.

Gupta, G., Parihar, S. S., Ahirwar, N. K., Snehi, S. K. and Singh, V. (2015) ‘Microbial & Biochemical Technology Plant Growth Promoting Rhizobacteria (PGPR): Current and Future Prospects for Development of Sustainable Agriculture’, *J Microb Biochem Technol*, 7(7), pp. 96–102. doi: 10.4172/1948-5948.1000188.

Gupta, M., Kiran, S., Gulati, A., Singh, B. and Tewari, R. (2012) ‘Isolation and identification of phosphate solubilizing bacteria able to enhance the growth and aloin-A biosynthesis of Aloe barbadensis Miller’, *Microbiological Research*. Elsevier GmbH., 167(6), pp. 358–363. doi: 10.1016/j.micres.2012.02.004.

Hamdali, H., Moursalou, K., Tchangbedji, G. and Ouhdouch, Y. (2012) ‘Isolation and characterization of rock phosphate solubilizing actinobacteria from a Togolese phosphate mine’, 11(2), pp. 312–320. doi: 10.5897/AJB11.774.

Hameed, S. (2015) ‘Isolation and characterization of a β -propeller gene containing phosphobacterium Bacillus subtilis strain KPS-11 for growth promotion of potato (Solanum tuberosum L .)’, 6(June), pp. 1–12. doi: 10.3389/fmicb.2015.00583.

Harms, H., Schlosser, D. and Wick, L. Y. (2011a) ‘Untapped potential : exploiting fungi in bioremediation of hazardous chemicals’, (February). doi: 10.1038/nrmicro2519.

Harms, H., Schlosser, D. and Wick, L. Y. (2011b) ‘Untapped potential : exploiting fungi in bioremediation of hazardous chemicals’, *Nature Publishing Group*. Nature Publishing Group, 9(3), pp. 177–192. doi: 10.1038/nrmicro2519.

Hashem, A., Allah, E. F. A., Alqarawi, A. A. and Al-huqail, A. A. (2016) 'The Interaction between Arbuscular Mycorrhizal Fungi and Endophytic Bacteria Enhances Plant Growth of Acacia gerrardii under Salt Stress', 7(July), pp. 1–15. doi: 10.3389/fmicb.2016.01089.

Hayes, T. B., Case, P., Chui, S., Chung, D., Haeffele, C., Haston, K., Lee, M., Mai, V. P., Marjuoa, Y., Parker, J. and Tsui, M. (2006) 'Monograph Pesticide Mixtures , Endocrine Disruption , and Amphibian Declines : Are We Underestimating the Impact ?', 40(April). doi: 10.1289/ehp.8051.

Hejazi, M. A., Barzegari, A., Gharajeh, N. H. and Hejazi, M. S. (2010) 'Introduction of a novel 18S rDNA gene arrangement along with distinct ITS region in the saline water microalga Dunaliella', pp. 1–11.

Hindumathy Ck, and Gayathri, V. (2013) 'Effect of Pesticide (Chlorpyrifos) on Soil Microbial Flora and Pesticide Degradation by Strains Isolated from Contaminated Soil', 4(2), pp. 2–7. doi: 10.4172/2155-6199.1000178.

Hundey, E. J., Russell, S. D., Longstaffe, F. J. and Moser, K. A. (2016) 'alpine lakes', *Nature Communications*. Nature Publishing Group, 7, pp. 1–9. doi: 10.1038/ncomms10571.

Investigación, A. D. E., Patricia, Á., Quevedo, M., Walter, N., Vega, O., Augusto, O. and Murillo, G. (2015) 'In vitro dissolution of acidulated rock phosphate by phosphate solubilizing microorganisms Disolución in vitro de rocas fosfóricas aciduladas por microorganismos solubilizadores de fósforo', 20(2), pp. 65–71.

Jabeen, F., Ahmed, M., Ahmed, F., Sarwar, M. B. and Akhtar, S. (2017) 'Characterization of cypermethrin degrading bacteria: A hidden micro flora for biogeochemical cycling of xenobiotics', 4(3).

Jahangir, G. Z., Sadiq, M., Hassan, N., Nasir, I. A., Saleem, M. Z. and Iqbal, M. (2016) 'The effectiveness of phosphate solubalizing bacteria as biocontrol agents', 26(5), pp. 1313–1319.

Jhala, A. J., Malik, M. S. and Willis, J. B. (2015) 'Weed control and crop tolerance of micro-encapsulated acetochlor applied sequentially in glyphosate-resistant soybean'. doi:

10.4141/CJPS-2014-422.

Jog, R., Nareshkumar, G. and Rajkumar, S. (2012) ‘Plant growth promoting potential and soil enzyme production of the most abundant Streptomyces spp . from wheat rhizosphere’. doi: 10.1111/j.1365-2672.2012.05417.x.

Johal, G. S. and Huber, D. M. (2009) ‘Glyphosate effects on diseases of plants’. doi: 10.1016/j.eja.2009.04.004.

Jonathan, K. (2016) ‘degrading fungus Phlebia radiata’, 3(1985).

Karpagam, T. and Nagalakshmi, P. K. (2014) ‘Isolation and characterization of Phosphate Solubilizing Microbes from Agricultural soil’, 3(3), pp. 601–614.

Katholm, C. L. (2016) ‘Effects of Roundup (glyphosate) on gut microorganisms of farm animals Effekter af Roundup (glyfosat) på mikroorganismer fra husdyr’, (May 2015).

Kavamura, V. N., Santos, S. N., Silva, J. L. da, Parma, M. M., Ávila, L. A., Visconti, A., Zucchi, T. D., Taketani, R. G., Andreote, F. D. and Melo, I. S. de (2013) ‘Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought’, *Microbiological Research*, 168(4). doi: 10.1016/j.micres.2012.12.002.

Khan, A. A., Jilani, G., Akhtar, M. S., Saqlan, S. M. and Rasheed, M. (2009) ‘Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production’, 1(1), pp. 48–58.

Khan, I.A., Hassan, G., Malik, N., Khan, R., Khan, H., and Khan, S.A. (2016) ‘Effect of herbicides on yield and yield components of hybrid maize’, pp. 729–736. doi: 10.1590/S0100-83582016340400013.

Khan, M. S., Zaidi, A., Wani, P. A., Khan, M. S., Zaidi, A. and Wani, P. A. (2007) ‘Role of phosphate-solubilizing microorganisms in sustainable agriculture - A review To cite this version : Review article Role of phosphate-solubilizing microorganisms in sustainable agriculture – A review’. doi: 10.1051/agro.

Kikuchi T. and Tanaka, S. (2016) ‘Biological Removal and Recovery of Toxic Heavy Metals in

Water Environment', (June). doi: 10.1080/10643389.2011.651343.

Krishnaveni, M. S. (2010) 'Studies on Phosphate Solubilizing Bacteria (PSB) in Rhizosphere and Non-Rhizosphere Soils in Different Varieties of Foxtail Millet (Setaria italica)', 1(1), pp. 23–39.

Kryuchkova, Y. V, Burygin, G. L., Gogoleva, N. E., Gogolev, Y. V, Chernyshova, M. P., Makarov, O. E., Fedorov, E. E. and Turkovskaya, O. V (2014) 'Isolation and characterization of a glyphosate-degrading rhizosphere strain , Enterobacter cloacae K7 □', *Microbiological Research*. Elsevier GmbH., 169(1), pp. 99–105. doi: 10.1016/j.micres.2013.03.002.

Kuan, K. B., Othman, R. and Rahim, K. A. (2016) 'Plant Growth-Promoting Rhizobacteria Inoculation to Enhance Vegetative Growth , Nitrogen Fixation and Nitrogen Remobilisation of Maize under Greenhouse Conditions', pp. 1–19. doi: 10.1371/journal.pone.0152478.

Kubo, M., Okajima, J. and Hasumi, F. (1994) 'Isolation and Characterization of Soybean Waste-Degrading Microorganisms and Analysis of Fertilizer Effects of the Degraded Products', 60(1), pp. 243–247.

Kucharski, M., Dziągwa, M. and Sadowski, J. (2014) 'Monitoring of acetochlor residues in soil and maize grain supported by the laboratory study', 60(11), pp. 496–500.

Kunamneni, A., Ballesteros, A., Plou, F. J. and Alcalde, M. (2007) 'Fungal laccase – a versatile enzyme for biotechnological applications', pp. 233–245.

Li, J., Zhang, S., Huo, P., Shi, S. and Miao, Y. (2013) 'Effect of phosphate solubilizing rhizobium and nitrogen fixing bacteria on growth of alfalfa seedlings', 45(5), pp. 1557–1562.

Liang, C., Chai, Q., Lemke, R. L., Campbell, C. A., Zentner, R. P. and Gan, Y. (2014) 'Improving farming practices reduces the carbon footprint of spring wheat production', *Nature Communications*. Nature Publishing Group, 5(May), pp. 1–13. doi: 10.1038/ncomms6012.

Lim, C. J. (2011) 'Glyphosate Utilization as the Source of Carbon : Isolation and Identification of new Bacteria', 8(4), pp. 1582–1587.

Liu, M., Liu, X., Cheng, B., Ma, X., Lyu, X., Zhao, X., Ju, Y. and Min, Z. (2016) 'Selection and evaluation of phosphate-solubilizing bacteria from grapevine rhizospheres for use as biofertilizers', 14(4).

López-ortega, M. P., Criollo-campos, P. J. and Gómez-vargas, R. M. (2013) 'Characterization of diazotrophic phosphate solubilizing bacteria as growth promoters of maize plants Caracterización de bacterias diazotróficas solublizadoras de fosfato como promotoras de crecimiento en plantas de maíz', di(2), pp. 115–123.

Maisnam, S. D. and Abhik, G. (2014) 'Acute Toxicity of deltamethrin and permethrin and their Sublethal effects on Growth and Feeding in Anabas testudineus', 3(4), pp. 18–22.

Malatova, K. and Morrill, T. (2005) 'Isolation and characterization of hydrocarbon degrading bacteria from environmental habitats in Western New York State'.

Martins, P. F., Carvalho, G., Gratão, P. L., Dourado, M. N., Pileggi, M., Araújo, W. L. and Azevedo, R. A. (2011) 'Effects of the herbicides acetochlor and metolachlor on antioxidant enzymes in soil bacteria', *Process Biochemistry*. Elsevier Ltd, 46(5), pp. 1186–1195. doi: 10.1016/j.procbio.2011.02.014.

Massiha, A. and Issazadeh, K. (2012) 'Biodegradation of Cypermethrin by using Indigenous Bacteria Isolated from Surface Soil', 35, pp. 71–76.

Mehrvarz, S. and Chaichi, M. R. (2008) 'Effect of Phosphate Solubilizing Microorganisms and Phosphorus Chemical Fertilizer on Forage and Grain Quality of Barely (Hordeum vulgare L .)', 3(6), pp. 855–860.

Mendes, G. D. O., Luiz, A. and Freitas, M. De (2014a) 'Mechanisms of phosphate solubilization by fungal isolates when exposed to different P sources', pp. 239–249. doi: 10.1007/s13213-013-0656-3.

Mendes, G. D. O., Silva, N. M. R. M., Duarte, J. L., Costa, D., Silva, I. R. and Roge, M. (2014b) 'Fluoride-Tolerant Mutants of Aspergillus niger Show Enhanced Phosphate Solubilization Capacity', 9(10), pp. 1–9. doi: 10.1371/journal.pone.0110246.

Menon, S. and Mohan, V. (2007) 'Isolation of Potential Phosphate Solubilizing Bacteria from the Rhizosphere of Fast Growing Native Tree Species', pp. 98–105.

Mikkelsen, R. (2004) 'Managing phosphorus for maximum alfalfa yield and quality', ... , *National Alfalfa Symposium. UC Cooperative …*. Available at: http://alfalfa.ucdavis.edu/+symposium/proceedings/2004/04-173.pdf.

Mohamed, T., Mohamed, A. and El-naggar, M. A. H. (2013) 'Diazinon decomposition by soil bacteria and identification of degradation products by GC-MS', 32(2), pp. 96–102.

Nadu, T., Ranjan, A., Mahalakshmi, M. R. and Sridevi, M. (2013) 'Isolation and characterization of phosphate-solubilizing bacterial species from different crop fields of Salem ', 3(1), pp. 29–33. doi: 10.4103/2231-0738.106982.

Nasir, M., Hashim, R., Sulaiman, O. and Nordin, A. (2015) 'Laccase , an Emerging Tool to Fabricate Green Composites : A Review', 10(3), pp. 6262–6284.

Neumann, D., Heuer, A., Hemkemeyer, M., Martens, R. and Tebbe, C. C. (2014) 'Importance of soil organic matter for the diversity of microorganisms involved in the degradation of organic pollutants', *The ISME Journal*. Nature Publishing Group, 8(6), pp. 1289–1300. doi: 10.1038/ismej.2013.233.

Nosrati, R., Owlia, P., Saderi, H., Rasooli, I. and Malboobi, M. A. (2014) 'Phosphate solubilization characteristics of efficient nitrogen fixing soil Azotobacter strains', 6(4), pp. 285–295.

Ogot, H. A., Boga, H. I., Budambula, N., Tsanuo, M., Andika, D. O., Odinga, O. and Roundup, K. (2013) 'Isolation , characterization and identification of roundup degrading bacteria from the soil and gut of Macrotermes michaelseni', 1(1), pp. 31–38.

Parray, J. A., Kamili, A. N., Reshi, Z. A., Hamid, R. and Qadri, R. A. (2013) 'Screening of beneficial properties of rhizobacteria isolated from Saffron (Crocus sativus L) rhizosphere', 7(23), pp. 2905–2910. doi: 10.5897/AJMR12.2194.

Pérez-Montaño, F., Alías-Villegas, C., Bellogín, R. A., Del Cerro, P., Espuny, M. R., Jiménez-Guerrero, I., López-Baena, F. J., Ollero, F. J. and Cubo, T. (2014) 'Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production', *Microbiological Research*, 169(5–6), pp. 325–336. doi:

10.1016/j.micres.2013.09.011.

Pilar, M., Jimena, P., Milena, R. and Fernanda, M. (2013) 'Characterization of diazotrophic phosphate solubilizing bacteria as growth promoters of maize plants Caracterización de bacterias diazotróficas solublizadoras de fosfato como promotoras de crecimiento en plantas de maíz'.

Pingale, S. S. and Virkar, P. S. (2013) 'Study of influence of phosphate dissolving micro-organisms on yield and phosphate uptake by crops', 3(2), pp. 191–193.

Piotrowska-seget, Z. (2016) 'Pyrethroid-Degrading Microorganisms and Their Potential for the Bioremediation of Contaminated Soils : A Review', 7(September), pp. 1–26. doi: 10.3389/fmicb.2016.01463.

Piscitelli, A., Pezzella, C., Giardina, P., Faraco, V., Piscitelli, A., Pezzella, C., Giardina, P., Faraco, V. and Giovanni, S. (2016) 'Heterologous laccase production and its role in industrial applications Heterologous laccase production and its role in industrial applications', 1018(October). doi: 10.4161/bbug.1.4.11438.

Rani, M. S., Lakshmi, K. V., Devi, P. S., Madhuri, R. J., Aruna, S., Narasimha, G. and Venkateswarlu, K. (2008) 'Isolation and characterization of a chlorpyrifos- degrading bacterium from agricultural soil and its growth response', (2), pp. 26–31.

Rehman, A., Rehman, A., Butt, S. A. and Hasnain, S. (2010) 'Isolation and characterization of arsenite oxidizing Pseudomonas lubricans and its potential use in bioremediation of wastewater Isolation and characterization of arsenite oxidizing Pseudomonas lubricans and its potential use in bioremediation of wastewat', (April). doi: 10.5897/AJB09.1663.

Rohilla, S. K. and Salar, R. K. (2012) 'Isolation and Characterization of Various Fungal Strains from Agricultural Soil Contaminated with Pesticides', 1, pp. 297–303.

Rossetti, J., Lacerda, M. De, Freitas, T., Vollú, R. E., Marques, J. M. and Seldin, L. (2016) 'Generally recognized as safe (GRAS) Lactococcus lactis strains associated with Lippia sidoides Cham . are able to solubilize / mineralize phosphate', *SpringerPlus*. Springer International Publishing. doi: 10.1186/s40064-016-2596-4.

Roy, O. and Subbaiah, U. M. (2017) 'Optimization of Cypermethrin Degradation By Bacterial

Cultures Isolated From Soil', 02(01), pp. 299–305.

Sachdev, D. P., Chaudhari, H. G., Kasture, V. M., Dhavale, D. D. and Chopade, B. A. (2009) 'Isolation and characterization of indole acetic acid (IAA) producing Klebsiella pneumoniae strains from rhizosphere of wheat (Triticum aestivum) and their effect on plant growth', 47(December), pp. 993–1000.

Sahoo, H. R. and Gupta, N. (2016) 'Variation in rock phosphate solubilization by three isolates of Aspergillus niger van Tieghem grown in liquid media supplemented with different carbon and nitrogen sources', 3(April), pp. 243–248.

Sane, S. A. and Mehta, S. K. (2015) 'Journal of Fertilizers & Pesticides Isolation and Evaluation of Rock Phosphate Solubilizing Fungi as Potential Bio- fertilizer', 6(2). doi: 10.4172/2471-2728.1000156.

Saraf, M., Pandya, U. and Thakkar, A. (2014) 'Role of allelochemicals in plant growth promoting rhizobacteria for biocontrol of phytopathogens', *Microbiological Research*, 169(1). doi: 10.1016/j.micres.2013.08.009.

Sarathambal, C. and Ilamurugu, K. (2014) 'Phosphate solubilising diazotrophic bacteria associated with rhizosphere of weedy grasses', 46(4), pp. 364–369.

Sarikhani, M. R. (2016) 'Increasing potassium (K) release from K-containing minerals in the presence of insoluble phosphate by bacteria', 4(16), pp. 87–96.

Savci, S. (2012) 'Investigation of Effect of Chemical Fertilizers on Environment', *APCBEE Procedia*, 1(January), pp. 287–292. doi: 10.1016/j.apcbee.2012.03.047.

Shamsuddeen, U. and Inuwa, A. B. (2013) 'Utilization of cypermethrin by bacteria isolated from irrigated soils', 6(2), pp. 19–22.

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H. and Gobi, T. A. (2013a) 'Phosphate solubilizing microbes : sustainable approach for managing phosphorus deficiency in agricultural soils', 2(1), p. 1. doi: 10.1186/2193-1801-2-587.

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H. and Gobi, T. A. (2013b) 'Phosphate solubilizing microbes : sustainable approach for managing phosphorus deficiency in agricultural soils', (Richardson 1994), pp. 1–14.

Shobha, G. and Kumudini, B. S. (2012) 'Antagonistic effect of the newly isolated PGPR Bacillus spp . on Fusarium oxysporum', 1(3), pp. 463–474. doi: 10.6088/ijaser.0020101047.

Shubhamsingh, T. and Tejashree, S. (2014) 'Effect of Neurotoxic Insecticide Dimethoate on Unicellular Freshwater Protozoan Ciliate Paramecium sp .', 3(5), pp. 62–66.

Singh, N. and Abraham, J. (2013) 'Isolation of laccase producing fungus from compost soil and partial characterization of laccase', 4(5), pp. 91–98.

Singh, R., Singh, P. and Sharma, R. (2014) 'Microorganism as a tool of bioremediation technology for cleaning environment : A review', 4(1), pp. 1–6.

Song, O., Lee, S., Lee, Y., Lee, S., Kim, K. and Choi, Y. (2008) 'DA23 isolated from cultivated soil', pp. 151–156.

Souza, R.D, Ambrosini, A. and Passaglia, L.M.P. (2015) 'Plant growth-promoting bacteria as inoculants in agricultural soils', 419, pp. 401–419.

Srinivasan, R., Yandigeri, M. S., Kashyap, S. and Alagawadi, A. R. (2012) 'Effect of salt on survival and P-solubilization potential of phosphate solubilizing microorganisms from salt affected soils', *Saudi Journal of Biological Sciences*. King Saud University, 19(4), pp. 427–434. doi: 10.1016/j.sjbs.2012.05.004.

Stella, M. and Halimi, M. S. (2015) 'Gluconic acid production by bacteria to liberate phosphorus from insoluble phosphate complexes', 43(1), pp. 41–53.

Sumatera, N. (2016) 'Effect of Microbes Phosphate Solubilizing and Organic Matter to Status the Phosphate on Andisol Impacted by Mount Sinabung', (4).

Tahir, M., L, M. Z., Tah, M., Ra, M., Tanveer, A., N, M. A. and W, A. (2017) 'Effect of Different Herbicides on Weeds , Growth and Yield of Spring Planted Maize (Zea mays L .)', (January 2009).

Thatheyus, A. J. and Selvam, A. D. G. (2013) 'Synthetic Pyrethroids : Toxicity and

Biodegradation', 1(3), pp. 33–36. doi: 10.12691/aees-1-3-2.

Thimmappa, L. S., Kumar, N., Jayanna, K. and Basaiah, T. (2016) 'Screening of efficient phosphate solubilizing fungi from mine soil and effect of phosphofungi on seed germination and vigour index of ground nut (arachis hypogaea L .) and green gram (vigna radiata L .)', 4(2), pp. 288–294. doi: 10.14419/ijbr.v4i2.6760.

Tortella, G. R., Rubilar, O., Cea, M., Wulff, C. and Diez, M. C. (2010) 'Biostimulation of agricultural biobeds with npk fertilizer on chlorpyrifos degradation', 10(4), pp. 464–475.

Vicia faba., (2012) 'Effects of microbial and chemical fertilization on yield and seed quality of faba bean, 19(2), pp. 417–422.

Viswanath, B., Rajesh, B. and Janardhan, A. (2016) 'Fungal Laccases and Their Applications in Bioremediation Fungal Laccases and Their Applications in Bioremediation', (October). doi: 10.1155/2014/163242.

Wang, S., Chen, H. Y. H., Tan, Y., Fan, H. and Ruan, H. (2016) 'Fertilizer regime impacts on abundance and diversity of soil fauna across a poplar plantation chronosequence in coastal Eastern China', *Nature Publishing Group*. Nature Publishing Group, (January), pp. 1–10. doi: 10.1038/srep20816.

Weaver, M. A., Krutz, L. J., Zablotowicz, R. M. and Reddy, K. N. (2007) 'Effects of glyphosate on soil microbial communities and its mineralization in a Mississippi soil †', 393(August 2006), pp. 388–393. doi: 10.1002/ps.

Yadav, H., Gothwal, R. K., Solanki, P. S., Nehra, S., Ghosh, P., Gothwal, R. K., Solanki, P. S., Nehra, S. and Ghosh, P. (2015) 'Isolation and Characterization of Thermo-tolerant Phosphate-Solubilizing Bacteria From a Phosphate Mine and Their Rock Phosphate Solubilizing Abilities', 0451(October 2016). doi: 10.1080/01490451.2014.943856.

Yang, B., Wang, Y. and Qian, P. (2016) 'Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis', *BMC Bioinformatics*. BMC Bioinformatics, pp. 1–8. doi: 10.1186/s12859-016-0992-y.

Yang, C., Shen, S., Wang, M. and Li, J. (2013) 'Journal of Environmental Biology Mild salinization stimulated glyphosate degradation and microbial activities in a riparian soil from Chongming Island ', 34(April), pp. 367–373.

Yunda, A. L. De (2010) 'C-glyphosate mineralization and follow up of the dynamics of Pseudomonas sp . populations in three soils under different uses in Tolima (Colombia)', 28(3), pp. 413–420.

Zhao, H., Tao, K., Zhu, J., Liu, S., Gao, H. and Zhou, X. (2015) 'Bioremediation potential of glyphosate-degrading Pseudomonas spp . strains', 170, pp. 165–170. doi: 10.2323/jgam.61.165.

Zhu, F., Qu, L., Hong, X. and Sun, X. (2011) 'Isolation and Characterization of a Phosphate-Solubilizing Halophilic Bacterium Kushneria sp . YCWA18 from Daqiao Saltern on the Coast of Yellow Sea of China', 2011. doi: 10.1155/2011/615032.

Zmijowska, A. and Cycon, M. (2014) 'Enhancement of deltamethrin degradation by soil bioaugmentation with two different strains of Serratia marcescens', pp. 1305–1316. doi: 10.1007/s13762-013-0322-0.

APPENDICES

Descriptive Statistics

	Bacterial strain	Mean	Std. Deviation	N
Null soil	No innoculation	12.0737	2.96920	2
	73A	46.2983	1.41422	2
	73B	135.1449	15.77322	2
	74A	160.2064	25.45586	2
	75A	170.2387	2.06145	2
	77A	119.3667	16.88611	2
	72A	36.4820	2.72352	2
	COM	45.2753	1.25988	2
	Total	90.6357	60.78843	16
Null rock phosphate	No innoculation	116.9655	5.11457	2
	73A	220.5350	15.60845	2
	73B	100.2095	4.69840	2
	74A	88.0207	1.85652	2
	75A	50.7868	8.76341	2
	77A	144.6033	.38773	2
	72A	160.9680	.03595	2
	COM	37.0181	1.38885	2
	Total	114.8884	58.27413	16
Soil BJ	No innoculation	31.9392	.08936	2
	73A	93.9021	1.55203	2
	73B	3.6133	.95541	2
	74A	176.2889	.55005	2
	75A	158.1838	1.67463	2
	77A	148.4954	.39925	2
	72A	119.8096	.34916	2
	COM	70.7799	1.41739	2
	Total	100.3765	59.73998	16
BJ plus rock phosphate	No innoculation	163.5188	1.32467	2
	73A	79.9655	4.82070	2
	73B	149.7216	6.99590	2
	74A	122.0358	6.22047	2
	75A	160.4600	.76337	2
	77A	49.5733	2.38372	2
	72A	74.6825	.27504	2
	COM	29.7851	1.87634	2
	Total	103.7178	50.48282	16
Ra plus soil	No innoculation	23.4816	.41451	2
	73A	141.9554	1.00138	2
	73B	14.8664	1.60408	2
	74A	157.1769	4.50981	2
	75A	198.1065	5.80921	2
	77A	146.7867	3.96426	2
	72A	75.9432	1.31389	2
	COM	24.5149	.68827	2
	Total	97.8540	69.49714	16
Ra plus rock phosphate	No innoculation	140.5206	.68952	2
	73A	21.3502	.01711	2

	73B	145.8144	2.29984	2
	74A	52.7842	1.11224	2
	75A	124.0554	6.24038	2
	77A	123.1559	1.20320	2
	72A	57.6754	1.28576	2
	COM	40.8553	2.94403	2
	Total	88.2764	48.29028	16
Picks media	No innoculation	23.0737	.64968	2
	73A	146.9830	.89762	2
	73B	225.4945	6.35939	2
	74A	167.5668	3.29273	2
	75A	120.2387	4.64523	2
	77A	119.3667	5.34278	2
	72A	44.7277	1.46758	2
	COM	78.5568	81.98867	2
	Total	115.7510	67.49562	16

Multivariate Tests

Effect		Value	F	Hypothesis df	Error df	Sig.
Medium	Pillai's Trace	.993	75.376[b]	6.000	3.000	.002
	Wilks' Lambda	.007	75.376[b]	6.000	3.000	.002
	Hotelling's Trace	150.753	75.376[b]	6.000	3.000	.002
	Roy's Largest Root	150.753	75.376[b]	6.000	3.000	.002
Medium * Bacteria	Pillai's Trace	5.177	7.186	42.000	48.000	.000
	Wilks' Lambda	.000	356.042	42.000	17.523	.000
	Hotelling's Trace	13104.174	416.006	42.000	8.000	.000
	Roy's Largest Root	10439.931	11931.350[c]	7.000	8.000	.000

a. Design: Intercept + Bacteria
Within Subjects Design: Medium
b. Exact statistic
c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Mauchly's Test of Sphericity

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Medium	.000	81.675	20	.000	.254	.577	.167

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix.
a. Design: Intercept + Bacteria
Within Subjects Design: Medium
b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Tests of Within-Subjects Effects

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Medium	Sphericity Assumed	11113.043	6	1852.174	11.975	.000
	Greenhouse-Geisser	11113.043	1.526	7282.618	11.975	.002
	Huynh-Feldt	11113.043	3.462	3209.664	11.975	.000
	Lower-bound	11113.043	1.000	11113.043	11.975	.009
Medium * Bacteria	Sphericity Assumed	264804.039	42	6304.858	40.763	.000
	Greenhouse-Geisser	264804.039	10.682	24790.261	40.763	.000
	Huynh-Feldt	264804.039	24.237	10925.798	40.763	.000
	Lower-bound	264804.039	7.000	37829.148	40.763	.000
Error(Medium)	Sphericity Assumed	7424.146	48	154.670		
	Greenhouse-Geisser	7424.146	12.208	608.150		
	Huynh-Feldt	7424.146	27.699	268.030		
	Lower-bound	7424.146	8.000	928.018		

Source	Medium	Type III Sum of Squares	df	Mean Square	F	Sig.
Medium	Linear	219.503	1	219.503	.629	.451
	Quadratic	95.324	1	95.324	.336	.578
	Cubic	7848.089	1	7848.089	41.362	.000
	Order 4	31.978	1	31.978	.491	.503
	Order 5	2695.074	1	2695.074	218.431	.000
	Order 6	223.075	1	223.075	7.773	.024
Medium * Bacteria	Linear	14748.282	7	2106.897	6.040	.011
	Quadratic	50301.428	7	7185.918	25.367	.000
	Cubic	33954.582	7	4850.655	25.564	.000
	Order 4	45150.935	7	6450.134	99.037	.000
	Order 5	31282.958	7	4468.994	362.204	.000
	Order 6	89365.853	7	12766.550	444.866	.000
Error(Medium)	Linear	2790.630	8	348.829		
	Quadratic	2266.253	8	283.282		
	Cubic	1517.949	8	189.744		
	Order 4	521.027	8	65.128		
	Order 5	98.707	8	12.338		
	Order 6	229.580	8	28.697		

Tests of Between-Subjects Effects

Measure:
Transformed Variable: Average

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	1157101.803	1	1157101.803	7419.386	.000
Bacteria	100413.422	7	14344.775	91.979	.000
Error	1247.652	8	155.957		

Table 6: Tests of Between-Subjects Effects: Multiple Comparisons

Results of phosphate solubilization

	Null soil	Null rock phosphate	soil BJ	Bj + rock phosphate	Ra soil	Ra rock phosphate	PICKS MEDIA	Ph.
No Inoculation	12.07367587	116.965529	31.93919839	163.5187576	23.48162686	140.5205795	23.07367556	6.6
73A	46.29829683	220.5350483	93.90210098	79.96552898	141.955354	21.35024308	146.9829683	4.4
3100 a	135.1449453	100.209547	3.613260403	149.7215813	14.86641187	145.8144474	225.4945332	4
74A	160.2063761	88.0207022	176.2889413	122.0357975	157.1769419	52.78423415	167.5667761	3.5
75A	170.2386635	50.78677596	158.1837507	160.4600487	198.1065388	124.0553786	120.2386635	3.5
77A	119.3666677	144.6033201	148.4954414	49.57333424	146.7867271	123.1559104	119.3666677	3.5
72A	36.48198373	160.9679569	119.8096461	74.6824619	75.94323528	57.67538678	44.7276622	4.6
COM	45.27528682	37.01814498	70.77989435	29.7851183	24.51487468	40.85534476	98.55681541	4.3

Isolates sequences obtained from GenBank together with their respective accession number.

>**MF991235.1 Mucor irregularis isolate 6106b small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**

AAGGATCATTAAATAATTTGAGTATGCTTCTCAGCATATTTCTACTTTACTGTGAACTGTTACTGTTTAC
CGTCCCTGAGGGACTGCCTAAAGATTATAGGGACCCCTCTTTTCGATGTTAACCTATTAAACTCAGGATT
ACCCTGGATCCCTAATTCATTATTTACCAAAAGAATGCATTTAATTATTGAAACATAAGCGAAAAGACTT
ATAAAACAACTTTTAACAACGGATCTCTTGGACTCGCCTCGATGAACACTTGCGCCCGCAGATAACTGGC
TGAATTGCATATTCAAKGAATCATCGACCCTTTGAACGCATCTTGCGCTCAATACTCTTCCATTGAGCAC
TCTGTTTCTGATCAACATCAACCCACATCTACCATTTTGTTGTGAATGGACCCTCGATATGGACACAAAT
TGACCTCTTTAAAACTCTCAATCTGAACTGTTGTACTCTTCCTGAACGTTTACCCTTATAAAGGAATGAT
CTATAAAAAAAGACTATCTTGGGGGCCTCCCAAATAAATCACTTTTTAAACTTGATCTGAAATCAGGTGG
GATTACCCGCTG

>**MF974394.1 Fusarium oxysporum isolate 6106 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**

CGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATACCACTTGTTGCCTCGGCGGATC
AGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTG
AGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAG
CAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCC
GCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACT
CGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAGTAAAACCCT
CGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGG
AATACCCGCTGAACTTAAGCATA

>**MF974575.1 Aspergillus niger isolate 73a internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**

CGGAAGGATCATTACCGAGTGCGGGTCCTTTGGGCCCAACCTCCCATCCGTGTCTATTGTACCCTGTTGC
TTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGCGCCTCTGCCCCCCGGGCCCGTGCCCGCCGGAGA
CCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAAC
AATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAAT
TCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGC
GTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGACGGGCCCGAA
AGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACATGCTCTGTAGGATTGGCCG
GCGCCTGCCGACGTTTTCCAACCATTCTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACT
TAAGCATATCA

>**MF974569.1 Penicillium janthinellum isolate 74b internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**

GAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACCTCCCACCCGTGTTTATCGTACCTTGTTGCTT
CGGCGGGCCCGCCGTYCAGGCCGCCGGGGGGCATCCGCCCCCGGGCCCGCGCCCGCCGAAGACACCATTG
AACGCTGTCTGAAGATTGCAGTCTGAGCGATTAGCTAAATCAGTTAAAACTTTCAACAACGGATCTCTTG
GTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATC
GAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCC
TCAAGCACGGCTTGTGTGTTGGGCCCCGCCCCCCGGCTACCGGGGGGCGGGCCCGAAAGGCAGCGGCGGC
ACCGCGTCCGGTCCTCGAGCGTATGGGGCTTCGTCACCCGCTCTGTAGGCCCGGCCGGCGCCCGCCGGCG
ACCCCCCTCAATCTTTCTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAAC

>**MF974393.1 Fusarium oxysporum isolate 6102b internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**
GCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATACCACTTGTTGCCTCGGCGGAT
CAGCCCGCTCCCGGTAAAACGGGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCT
GAGTAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA
GCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCC
CGCCAGTATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGAC
TCGCGTTAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAGTAAAACCC
TCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAG
GAATACCCGCTGAACTT

>**MF977405.1 Fusarium oxysporum isolate 6102 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**
CCCTGTGAACATTCACCACTTGTTGCCTAACGGCGGATCAGCGCCGACTCCCGGTAACAACGGGTTTACG
GCTCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAGTAAAACCATAAATAAATCAAAA
CTTTCAACAACAGGATCTGCTTGAGGGTTGAACTGGCGATCGATGAAGCAAGCCCGCCAGCAAAATGGCG
ATAAGTAATGTGAATTGCAGAGATTCAGTGAATCATCGAATCTTTGCAACGCACATTGCTGCCCGCCAGT
ATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGAAGGACTCGCG
TTATATATCGCGTTCCTCAGAAGTTAGATATGGCGGTCAGCGTCGAGGCTTCCTATAGCGTAGTAGTAAA
ACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCTGAATGTTGACCTCGGATCAG
GTAGTTGAATACCCGCTGAACTTAAGCCTCCGCA

>**MF983813.1 Aspergillus parasiticus isolate 6100 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**
TGCGGAAGGATCATTACCGAGTGTAGGGTTCCTAGCGAGCCCAACCTCCCACCCGTGTTTACTGTACCTT
AGTTGCTTCGGCGGGCCCGCCGTCATGGCCGCCGGGGGCGTCAGCCCCGGGCCCGCGCCCGCCGGAGACA
CCACGAACTCTGTCTGATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATG
GATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCG
TGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCA
TTGCTGCCCATCAAGCACGGCTTGTGTGTTGGGTCGTCGTCCCCTCTCCGGGGGGGACGGGCCCCAAAGG
CAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCG
CTTGCCGAACGCAAAACAACCATTTTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTA
AGCATAT

>**MF983800.1 Meyerozyma caribbica isolate 6101B internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence**
GGAAGGATCATTACAGTATTCTTTTGCCAGCGCTTTACTGCGCGGCGAAAAACCTTACACACAGTGTCTT
TTTGATACAGAACTCTTGCTTTGGTTTGGCCTAGAGATAGGTTGGGCCAGAGGTTTAACAAAACACAATT
TAATTATTTTTATTGATAGTCAAATTTTGAATTAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCT
CGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATATGAATTGCAGATTTTCGTGAATCATCGAATC
TTTGAACGCACATTGCGCCCTCTGGTATTCCAGAGGGCATGCCTGTTTGAGCGTCATTTCTCTCTCAAAC
CCCCGGGTTTGGTATTGAGTGATACTCTTAGTCGAACTAGGCGTTTGCTTGAAAAGTATTGGCATGGGTA
GTACTGGATAGTGCTGTCGACCTCTCAATGTATTAGGTTTATCCAACTCGTTGAATGGTGTGGCGGGATA
TTTCTGGTATTGTTGGCCCGGCCTTACAACAACCAAACAAGTTTGACCTCAAATCAGGTAGGAATACCCG
CTGAACTTAAGCATATCAAAAGCCGGAGGAA

>**MF979777.1 Enterobacter asburiae strain 3106b 16S ribosomal RNA gene, partial sequence**
ACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGC
ATAACGTCGCAAGACCAAAGAGGGGGACCTAGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCT
AGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGG
AACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGA
TGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAATACTTTCAGCGGGGAGGAAGGTGGGAG

GTTAATAACCTTGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAAT
ACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATG
TGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAG
AATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGAC
AAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA
AACGGGGGACTTGGAGGTTGACCCTTGAGAGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGG
AGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTCGATGCAACGCGAAGAACCTTACCGCTCTTGACATCCGAGAACTTTCCAGAGATGGATTGGTGCCTT
CGGGAACATGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCA
ACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAA
CTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTAGTAGGGCTACACACGTGCTACAATGGCG
CATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGTGTCGTAGTCCGGATTGGAGTC
TGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCC
GGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAACCTTCGG
GAG

>**MF979662.1 Enterobacter asburiae strain 3106br 16S ribosomal RNA gene, partial sequence**
CAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGCACAGGGAGCTTGCTCTTGGGT
GACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGT
AGCTAATACCGCATAATGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCA
GATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGA
CCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATG
GGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGG
GAGGAAGGTGTTGAGGTTAATAACCTTGTCGATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGT
GCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGC
GGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAG
TCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGC
GAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC
CTGGTAGTCCACGCCGTAAACGATGTGGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAAC
GCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCAC
AAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAAC
TTTGCAGAGATGGTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTT
GTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTGGGCCGGGAA
CTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGA
GTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCAT
AAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAG
ATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTG
CAAAAGAAGTAGGTAGCTTAACCTTCGGGA

>**MF979635.1 Klebsiella pneumoniae strain 77a 16S ribosomal RNA gene, partial sequence**
AACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGACGAGC
GGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAAT
ACCGCATAATGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGATGGGA
TTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCA
CACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAA
GCCTGATGCAGCCATGCCGCGTGTGTGAAGAGAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAA
GGCGTTAAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGC
AGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTG
TCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGT
AGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGG
CGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGT
AGTCCACGCCGTAAACGATGTGGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTT
AAATCGACCCGCATGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGC
GGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTTC
CAGAGATGGTTTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGA

AATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCA
AAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAG
GGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAG
TATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCA
GAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAA
AGAAGTAGGTAGCTTAACCTTCGGGAGGG

>**MF979558.1 Enterococcus casseliflavus strain 72b 16S ribosomal RNA gene, partial sequence**
AAAAGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCCATCAGAAGGGGATAACACTTGGAAACA
GGTGCTAATACCGTATAACACTATTTTCCGCATGGAAGAAAGTTGAAAGGCGCTTTTGCGTCACTGATGG
ATGGACCCGCGGTGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCATAGCCGACCGG
AGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTTCGGGAGGCAGCAGTAGGGAATCT
TCGGCAATGGGCGAAAGTCTGGCCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAAATTT
GTTGTTAGAGAAGAACAAGGATGAGAGTTAAATGTTCATCCCTTGACGGTATCTAACCAGAAAAGCCACG
GCTAACTACGTGCCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAA
GGGAGCGCAGGCGGTTTTTTAAGTTTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAAC
TGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAG
GAACACCAGTGGCGAAGGCGGCTTTTTGGTCTGTAACTGACGCTGAGGCTTGAAAGCGTGGGGAGCGAAC
AGGATTAGATTCCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTGGAGGGTTTCCGCCCTTCA
GTGCTGCAGCAAACGCATAAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTG
ACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTT
GACATCCTTTGACCACTCTAGAGATAGAGCTTCCCCTTCGGGGGCAAAGTGACAGGTGGTGCATGGTTGT
CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTGTTAGTTGCCATC
ATTTAGTTGGGCACTCTAGCGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATC
ATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGAAGTACAACGAGTTGCGAAGTCGCGAGGCT
AAGCTAATCTCTTAAAGCTTCTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAAGCCGGAATCG
CTAGTAATCGCGGATCAGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCA
CGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTT

>**MF979809.1 Pseudomonas putida strain 75A 16S ribosomal RNA gene, partial sequence**
CTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGATGACGGGATCTTGCTCCTTGAT
TCAGCGGCGGACGGGTGAGTAATGTCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACG
CTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGG
TCGGATTAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATC
AGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGG
CGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGA
GGAAGGGCAGTAAGTTAATACCTTGATGTTTTGACGTTACCGGCAGAATAAGCACCGGCTAACTCTGTGC
CAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGG
TTTGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGGCAAGCTAGAGTA
CGGTAGAGGGTGGTGGAATTTCATGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCGGTGGCGA
AGGCGGCCACCTGGACTGATACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCT
GGTAGTCCACGCCGTAAACGATGTCAATTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAACGC
ATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAA
GCGGTGGAGCATGTGGTTTAATTTGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAACTT
TCCAGAGATGGATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGT
GAGATGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTATGGTGGGCAC
TCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGC
CTGGGCTACACACGTGCTACAATGGTCGGTACAGAGGGTTGCCAAGCCGCGAGGTGGAGCTAATCTCACA
AAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGAATCGCTAGTAATCGCGAA
TCAGAATGTCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGC
ACCAGAAGTAGCTAGTCTAACCTT

>**MF979810.1 Enterobacter sp. strain 2106b 16S ribosomal RNA gene, partial sequence**
CGCTGGCGGCAGGCCTAACACATGCAAGTCGAACGGTAACAGGAAGCAGCTTGCTGCTTCGCTGACGAGT

GGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAAT
ACCGCATAAYGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGA
TTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCA
CACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAA
GCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAG
GTGTTGAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCA
GCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGT
CAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTA
GAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCG
GCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAG
TCCACGCCGTAAACGATGTGGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAA
GTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGT
GGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTTCCAG
AGATGGTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAAT
GTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAG
GAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGC
TACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGC
GTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAA
TGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGA
AGTAGGTAGCTTAACCTTC

>**MF979821.1 Enterobacter cloacae strain 2106a 16S ribosomal RNA gene, partial sequence**

ACATGCAAGTCGAACTGTAGCAGGAAGCAGCTTGCTGCTTTTCTGTTGAGTGGCGGACGGGTGAGTAATG
TCTGGGAATCTGCCTGATGGAGGGGGATAACTTTTGGAAACGGTAGCTAATACCGCATAATGTCGCAAGA
CCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTA
ACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGG
TCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCG
CGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGCGATTAGGTTAATAACCT
TGGTCGATTGGACGTTACCCGCAGAATAAGCACCGGCTAACTCTGTGCCTAGCAGCCGCGGTAATACGGG
AGGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGCAGGCGGTTTGTCAAGTCGGATGTGA
AATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAAT
TTCAGGTGTAGCGGTGAAATGCGTAGAGATCTGTAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACTAA
GACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAAC
GATGTCGACTTGGAGGTTGTGCCCTTGAGGTGTGGTTTCCGGAGTTAACGCGTTAAGTGGACCGCCTGGG
GAGTACGGCCGCAAGGTTAAAATTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT
AATTGGATGCAACGGGAAGACCTTTACCTACTTTTGACATCCAGAGAACTTTCCAGAGATGGTTTGGTGC
TTTGGGGAATTTTGAGACAGGTGCTGCATGGCTGTGGTCAGTTGGTGTTGTGAAATGTTGGGTTAATTCC
CGCAAGGAGCGCAACCTTTATCTTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGCCAGTGA
TAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTAC
AATGGTGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGTCCGGAT
TGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTATTCGTGGATCAGAATGTCACGGTGAATA
CGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTTAA
CCTTC

>**MF979964.1 Enterobacter sp. strain 2105 16S ribosomal RNA gene, partial sequence**

TGGAAGGGGTAGCTAATACCGCATAGTGTCGCAAGAGCAAAGAGGGGGTCTTTCGGGCCTGTTGCCATCA
GATGTGCTCAGTTGGAATTAGCTAGTAGGTGGGGTAACGGCTCACATAGGGGAGGATCCTTAGGTGTTTT
GAGAGGATGTCCAGCCACATTGGAATTGAGACACGGTCCAGATTCTTACGGGAGGCAGCATTGGGGAATA
TTGCACAATGGGGGCAAGCTTGATGCAGCCATACAGAGTGTATGAAGAAGCCCTTTGGTTTGTAAAGTAT
TTTCAGGGGGGAGGAAGGTGTTTAGGTTAATAACATAGGCAATTGACGTTACTCGCAGAATAAGCACCGG
CTAACTCTGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGC
GCGCGCAGGCGGTTTGTTAAGTCAGGATTGTGAAATCCCCGGGGCTCAACTTGGGAATTGCATTCGATAC
TTGGCAGGCTAGAGTGTTGTAGAGGGTGGTTAGAATTCCCAGGTGTAGCGGTGAAATGCGTAGAGATCTG
GAGGAATACCGGTGGCGAAGGCGGCCATCTGGACTAAGACTGACGCTGAGGTGCGAAAGCGTGGGGAGCA
AACAGGATTAGATACCCTGGTAGTCCATGCCGTAAACGATGTCTACTTGGAGTTTGTTCCTTTGAGGATT

TGGTGGCGGAGCTAACGCGTTAATTGGACCCCTTGGGGAGTACGTCGGCAAGGTTAAAATTCAAATGAAT
TGAGGGGGGCCGGCCCAAGCGGTGGACCATGTGGTTTAATTGGATGCAACGGGAAGACCTTTCCTTGCTT
TTGCCATCCAGAGAACTTTCCAGAAATGGTTTGGTCCTTTGGGGAATTATGAGCCAGGTGTTCCATGGCT
GTGGTCAGTTGGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGGCCAACCTTTATCTTTTTTTGCCA
GCGGTTAGGCCGGGAACTCTAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTC
ATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGTGCATACAAAGGGTAGCTACCTAGCGAG
AGCAAGCTGACCTCATAAAGTGGGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAA
TCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCGGCCCTTGTACACACCGCCCTTCC

>**MF979876.1 Enterobacter sp. strain 2104-2 16S ribosomal RNA gene, partial sequence**

CCGAAGGTTAAGCTACCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCC
GGGAACGTATTCACCGTAGCATTCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCA
GACTCCAATCCGGACTACGACGCACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATG
CGCCATTGTAGCACGTGTGTAGCCCTGGTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTC
CAGTTTATCACTGGCAGTCTCCTTTGAGTTCCCGGCCTAACCGCTGGCAACAAAGGATAAGGGTTGCGCT
CGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCACAGTT
CCCGAAGGCACCAAACCATCTCTGCAAAGTTCTGTGGATGTCAAGACCAGGTAAGGTTCTTCGCGTTGCA
TCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGC
CGTACTCCCCAGGCGGTCTATTTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAAT
ACACCTCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGC
GTCAGTCTTTGTCCAGGAGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTAC
ACCTGGAATTCTACCYCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAGTTCCCAGGTTGAGCCC
GGGGATTTCACATCCGACTTGACAGACCGCCTGCGTGCGCTTTACGCCCAGTAATTCCGATTAACGCTTG
CACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATTAC
CGAGGTTATTAACCACAACACCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACA
CGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTG
GACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCC
GTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCAAGAGGCCCGAAGGTCCCCCTCTTT
GGTCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCA
GACATTACTCACCCGTCCGCCACTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGTTCGACTTGCATGT
GTTAGGCCTGCCGCCAGCGTTCAATATGA

>**MF979885.1 Enterobacter sp. strain 2104-1 16S ribosomal RNA gene, partial sequence**

AAGGTTAAGCTACCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGG
AACGTATTCACCGTAGCATTCTGATCTACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGAC
TCCAATCCGGACTACGACGCACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGC
CATTGTAGCACGTGTGTAGCCCTGGTCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCAG
TTTATCACTGGCAGTCTCCTTTGAGTTCCCGGCCTAACCGCTGGCAACAAAGGATAAGGGTTGCGCTCGT
TGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCACAGTTCCC
GAAGGCACCAAACCATCTCTGCAAAGTTCTGTGGATGTCAAGACCAGGTAAGGTTCTTCGCGTTGCATCG
AATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGT
ACTCCCCAGGCGGTCGATTTAACGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAATCGA
CATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTC
AGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACC
TGGAATTCTACCCCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAGTTCCCAGGTTGAGCCCGGG
GATTTCACATCCGACTTGACAAACCGCCTGCGTGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCAC
CCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATTGCTGC
GGTTATTAACCACAACACCTTCCTCCCCGCTGAAAGTAACTTTACAACCCGAAGGCCTTCTTCATACACG
CGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGA
CCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCGT
TACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCAAGAGGCCCGAAGGTCCCCCTCTTTGG
TCTTGCGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGATTCCCAGA
CATTACTCACCCGTCCGCCACTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGATCGACTTGCATGAGT
TAGGCCTGCCGCCAGCGTTCAATCT

>**MF980152.1 Klebsiella aerogenes strain 2103 16S ribosomal RNA gene, partial sequence**

ACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTA
GCTAATACCGCATAATGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAG
ATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGAC
CAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGG
GCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGG
AGGAAGGTGTTGAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTG
CCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCG
GTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGT
CTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCG
AAGGCGGCCCCCTGGMCAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCC
TGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACG
CGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACA
AGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACT
TTCCAGAGATGGATTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTG
TGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAAC
TCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAG
TAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATA
AAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGA
TCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGC
AAAAGAAGTAGGTAGCTTAACCTTCGGG

>**MF980711.1 Enterobacter sp. strain 2103-2 16S ribosomal RNA gene, partial sequence**

AGTGGGGTTAGCGCTCTCCCGTGCGGTTAGACTACCTACTCCTGTAGAAACCAATTCCATGGGGGGGAGG
GGGGGGGTGAACAGGGCCCGGGAACGTATTCCCCGCGACATTCTGATTACGATTTCTAGCGATTCCGACT
TCATGGAGTCGAGTTGCAGACTGCAATCCGGACTACGATCGGTTTTATGAGGTTAGCTTGATCTCGCTAG
GTAGCTACCCTTTGTATGCACCATTGTAGCACGTGTGTAGCCCTGCTCGTAAGGGCCATGATGACTTGAC
GTCATCCCCACCTTCCTCCAGTTTGTCACTGGCAGTCTCCTTAGAGTTCCCACCATAACCCGTTGCAAAA
TAAGGAAAAGGGTGGCCTTCTTGCCGGAATTTAACCCAACTTTTCACAACACAACTTGACAACAGCCATG
CACAACCGGCTTTCAGTTTCCCAAAGGACCCATCCATTCTCTGAAAGGTTTCTGCATTGCAAAAGCAAGG
AAAGGTCCTTCCCTTTGCTTCAAATAAAACAACTGGCTCAACCGCTGGGGCGGCCCCCCTCAAATCAATT
TAATTTTAAACTTGGCGACGGAATTCCCAAGGCGGCCAACTAATCCCGTAACTTGCGTTAGCTCCGCCAC
CAAAACCTCAAGGACACAAACTCCAAGTAGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTG
GTTTGCTCCCCACGCTTTCGCACCTCAAGCGTCAGTATTAGTCCAGGTGGCCGCCTTCGCCACCGGTATT
CCTCCATATATCTACGCATTTCACCGCTACACCCTGAAATTCTACCACCCCTCTACAACACTCTAGCCAG
CCAGTTACGAATGCAATCTCCCAGGTTGAGCCCGGGGATTTCACATCCAACTTAACAAACCCGCCTACGC
GCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCCGTATTACCGCGGCTGCTGGCACAAAGT
TAGCCGGTGCTTATTCTGCGGGTAACGTCAATTAACTAAGGTATTAACCTACTGACCTTCCTCCCCCCTT
AAAGTACTTTACAAACCGAAGGCCTTCTTCATACACCCGGCATGGCTGGATCAAGCTTGCGCCCATTGTG
CAATATTCCCCACTGCTGCCTCCCGTAAGAATCTGGACCGTGTCTCAATTCCAGTGGGGCTGGACATCCT
CTCAGACCACCTAAGGATCGTCGCCTAGGTGAGCCCTTACCCCACCCACTAACTAATCCCATCTGAGCAC
ATCTGATGGCAAGAGGCCCGAAGGACCCCCTCTTTGCTCTTGCGACATTATGCGGTATTAGCTATCCTTT
CCAAAAGTTATCCCCCTCCACCAAGCAGAATCCCAGACATTACTCACCCGTCCGC

>**MF980718.1 Enterobacter sp. strain 2101 16S ribosomal RNA gene, partial sequence**

GATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGCACAGAGAGCTTGCTCTYGGGTGA
CGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAG
CTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGA
TGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACC
AGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGG
CGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGA
GGAAGGTGGTGAGGTTAATAACCTTGTTGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGC
CAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGG
TCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTC

TTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGA
AGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCT
GGTAGTCCACGCCGTAAACGATGTGGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGC
GTTAAGTCGACCCGTCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACA
AGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCAGAGAACT
TTCCAGAGATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTG
TGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAAC
TCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAG
TAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATA
AAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTTGA
TCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGC
AAAAGAAGTAGGTAGCTTAACCTTCGG

>**MF980912.1 Enterobacter asburiae strain 3106b 16S ribosomal RNA gene, partial sequence**

CGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATAC
CGCATAACGTCGCAAGACCAAAGAGGGGGACCTTGGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATT
AGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACA
CTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGC
CTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGT
GGTGAGGTTAATAACCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGC
CGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCA
AGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGA
GGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGC
CCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC
CACGCCGTAAACGATGTGGACTTGGAGGTTGTTCCCTTGAGGGGTGGCTTCCGGAGCTAACGCGTTAAGT
CGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGG
AGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGCTCTTGACATCCAGAGAACTTTCCAGAG
ATGGATTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGT
TGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTGGGCCGGGAACTCAAAGGA
GACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTA
CACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGTGT
CGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATG
CTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAG
TAGGTAGCTTAACCTTCGGGAG

>**MF980788.1 Raoultella ornithinolytica strain 2107b 16S ribosomal RNA gene, partial sequence**

GGAGCCTAACGCGTTAAGTCGACCGCCTGGGAAGTACGGCCGCAAGGTTAAAATTCAAATGAATGACGGG
GGCCCGCACAAGCGGTGGAGCATTTGTTTTAATTGGATGCAACGGGAAGAACCTTACCTACTTTTGACAT
CCAGAGAACTTACCAGAGATGGTTTGGTGCCTTCGGGAATTTTGAGACAGGTGCTGCATGGCTGTGTTCA
GCTGGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCTTTATCCTTTTTTGCCAGCGGTTC
GGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGG
CCCTTACGAGTAGGGCTACACACGTGCTACAATGGCGTATACAAAGAGAAGCGACCTCGCGAGAGCAAGC
GGACCTCATAAAGTGTGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAG
TAATCGTAGATCAGAATGCTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACAATGGG
GTTGGGTTGCMMAAGAAGTGGGTAGC

>**MF980882.1 Enterobacter cloacae strain 2100a 16S ribosomal RNA gene, partial sequence**

ATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTAGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGAC
GAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGC
TAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGAT
GGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCA
GCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGC
GCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAATGTACTTACAGCGGGGAG
CAAGGTGATGAGCTGAGTATCATCGTCGATTGACGTGACCCGCAGAAGAAGCACCGGCTAACTCCGTGCC

AGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGT
CTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCT
TGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAA
GGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG
GTAGTCCACGCCGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCG
TTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAG
CGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTRCTCTTGACATCCAGAGAACTTW
CCAGAGATGGWTTGGTGCCTTCGGGAACTSTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTG
AAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTYMGGCCGGGAACTC
AAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGYA
GGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAA
GTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATC
AGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAA
AAGAAGTAGGTAGCTTAACCTTCGGG

>**MF980911.1 Enterobacter sp. strain 2100b 16S ribosomal RNA gene, partial sequence**

ATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTAGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGAC
GAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGC
TAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGAT
GGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCA
GCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGC
GCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCCGGGTAGTAAAGTACTTTCAGCGGGCAGTA
AGCYGATGAGCTGATTAACTTCGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAG
CAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCT
GTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTG
TAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGG
CGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGT
AGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTT
AAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCG
GTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTTCC
AGAGATGGATTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAA
ATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCAA
AGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGG
GCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGT
GCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAG
AATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAA
GAAGTAGGTAGCTTAACCTTCGGGA

>**MF980919.1 Enterobacter asburiae strain 3103 16S ribosomal RNA gene, partial sequence**

CGGCAGGCATAACACATGCATGTAGATCGGTAGCACAGAGAGATTGTTCTCGGGTGATGAGCGGCGGACG
GGTGAGTAATGTCTGGGAATCTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATA
ATGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTAG
TAGGTGGGGTAAAGGCTCACCTAGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAA
CTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATG
CAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGTGATGAG
GTTAATAMCCTTGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGT
AATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTCAAGTTGG
ATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGG
TGGAATTTCAGGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAATACCGGTGGCGAAGGCGGCCCCCTG
GACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC
GTAAACGATGTAGACTGTGAGGTTGTGCCCTTGAGGTGTGGGTTCCGGAGCTAACGCGTTAAGTCGACCG
CCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATG
TGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGCTCTTGACATCCAGAGAACTTTCCAGAGATGTGT
TGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTT
AAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTCAAAGGAGACTGC

CAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACG
TGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGTAGT
CCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGG
TGAATACGTCCGGGGCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGC
TTAACC

>**MF980916.1 Enterobacter sp. strain 3100a 16S ribosomal RNA gene, partial sequence**
CAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGCACAGAGAGCTTGCTCTCGGGT
GACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGT
AGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCA
GATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGA
CCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATG
GGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGG
GAGGAAGGTGTTGAGGTTAATATACCTTAGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCC
GTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAG
GCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAG
AGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTG
GCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
CCCTGGTAGTCCACGCCGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTA
ACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGC
ACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCAGAGA
ACTTTGCAGAGATGGTTTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTG
TTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGG
AACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTAC
GAGTAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTC
ATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGT
AGATCAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGT
TGCAAAAGAAGTAGGTAGCT

>**MF980917.1 Klebsiella variicola strain 3100b 16S ribosomal RNA gene, partial sequence**
AGATTAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGCACAGAGAGCTTGCTCTCGGGTGA
CGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAG
CTAATACCGCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCAGA
TGGGATTAGCTRGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACC
AGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGG
CGCAAGCCTGATGCAGCCATGCCGCGTGGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAG
GAAGGCGGTGAGGTTAATAACCTCGTCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCC
AGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGT
CTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCT
TGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAA
GGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG
GTAGTCCACGCTGTAAACGATGTGGATTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCG
TTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAG
CGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACAGAACTTT
GCAGAGATGGTTTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTG
AAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTGGGCCGGGAACTC
AAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCA
GGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAA
GTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATC
AGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAA
AAGAAGTAGGTAGCTTAACCTTCGG

>**MF980922.1 Enterobacter asburiae strain 3106d 16S ribosomal RNA gene, partial sequence**
CAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGCACAGGAGCTTGCTTTGGGTGA

CGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAG
CTAATACCGCATAAYGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAGA
TGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACC
AGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGG
CGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGA
GGAAGGTGTGAGGTTAATAACCTTGTCGATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCC
AGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGT
CTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCT
TGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAA
GGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTG
GTAGTCCACGCCGTAAACGTGTGGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGT
TAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGC
GGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTTG
CAGAGATGGTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGA
AATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTMGGCCGGGAACTCA
AAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAG
GGCTACACACGTGCTACAATGGCRCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAG
TRCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCA
GAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAA
AGAAGTAGGTAGCTTAACCTTCGGGAGGGC

>**MF980921.1 Klebsiella pneumoniae strain 3104b 16S ribosomal RNA gene, partial sequence**

TCAGATTGAACGCTGGCGGCAGGCATAACTCATGCAAGTAGATCGGTAGCACAGAGAGCTTGCTCTCGGG
TGWCGAGCGGCGGACGGGTGAGTAATGTCTGGGAATCTGCCTGATGGAGGGGGATAACTACTGGAAACGG
TAGCTAATACCGCATAACGTCGCAAGACCAAATGGGGGACCTTCGGGCCTTTGCCATCAGATGTGCCCAG
ATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGAC
CAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGG
GCGCAAGCCTGATGCAGCCATGCCGCGTGTRTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGGGG
AGGAAGGGGGTGAGGTTAATAACCTTATCGATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTYCGTG
CCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCG
GTTTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGT
CTTGTAGAGGGGGGTAGAATTTCAGGTGTAGCGGTGAAATGCGTAGAGAATGGAGGAAYACCGGTGGCGA
AGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCT
GGTAGTCCACGCTGTAAACGATGTCGATTTGGAGGTTGTGCCCTTGAGGTGTGGCTTCCGGAGCTAACGC
GTTAARTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAA
GCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCAGAGAACTT
TCCAGAGATGGTTTGGTGCCTTCGGGAACTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGT
GAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGCCGGGAACT
CAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGT
AGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAA
AGTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGAT
CAGAATGCTACGGTGAATACGTTCCCGGSCCTTGTACACACCGCCCGTMACACCATGGGAGTGGGTTGCA
AAAGAAGTAGGTA

>**MF980918.1 Klebsiella oxytoca strain 3102 16S ribosomal RNA gene, partial sequence**

ACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTA
GCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCAGATGTGCCCAG
ATGGGATTAGCTAGTAGGTGGGGTAATGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGAC
CAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGG
GCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGG
AGGAAGGTGGTGAGGTTAATAGCTTCATCGATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTG
CCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCG
GTTTGTTAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTTGAAACTGGCAGGCTGGAGT
CTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCG
AAGGCGGCCCCCTGGACRAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCC

TGGTAGTCCACGCTGTAAACGATGTGGATTTGGAGGTTGTTCCCTTGAGGAGTGGCTTCCGGAGCTAACG
CGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACA
AGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACT
TTGCAGAGATGGTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTG
TGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTAGGYCGGGAACT
CAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGY
AGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAA
AGTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGAT
CAGAATGCTACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCMGTCACACCATGGGAGTGGGTTGCA
AAAGAAGTAGGTAGCTT

>**MF980920.1 Pseudomonas aeruginosa strain 3104a 16S ribosomal RNA gene, partial sequence**

CGGCGGACGGGTGAGTAATGTCTAGGAATCTGCCTGGTGTGGGGGATAACTTCTGGAAACGGTAGCTAAT
ACCGCATAAGTTCTGAGGGAGAAAGTGGGGGATCTTCGGGCCTCATGCTATCAGATGTGCTAGGGGGATT
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AGTTCCCGGGCCCTGGTCCCCCCCCCCCTCCCCCCCAGGGAATTGGTTTGTACAAAAAGAAGGAATC