UNIVERSITY OF GONDAR

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

DEPARTMENT OF BIOLOGY, MSc IN MICROBIOLOGY

Isolation, characterization and identification of salt tolerant and DDT degrading *Bacillus* species from low land (saline) soil of Metema North west Ethiopia

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June, 2017

Gondar, Ethiopia

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Thesis submitted to Department of Biology, College of Natural and Computational Sciences, University of Gondar for partial fulfillment of the requirements for the degree of Master of Science in Microbiology

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June, 2017

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Acknowledgement

I am most grateful to my advisors, Dr Samuel Sahile and Dr.Zewdu Teshome their immeasurable guidance. I am also grateful to the entire staff at the Department of Biology, College of Natural and Computational Science, University of Gondar. And also I would like to say thanks UOG Mega project from "Sustainable production of healthy seedling systems for rice, pepper and tomato for small scale farmers in northern west Ethiopia project "which helped me in financial Support to accomplish the task. Lastly, I would like to appreciate the support of all my friends and family members for their continuous support throughout the period.

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Abbreviations

DDT-Dichloro diphenyl trichloroethane

PGPB-Plant Growth Promoting Bacteria

PGPR-Plant Growth Promoting Rhizobacteria

EPS-Exopolysaccride

PEB-Poly ethylene bag

RNA-ribosomal deoxynucleic acid

ANOVA-Analysis of variance

NAM-Nutrient agar medium

DNA-deoxy ribo nucleic acid

PCR-polymerase chain reaction

DNBA-dilute nutrient broth agar

PFEGE-Pulsed field gel electrophoresis

MEE-Multi locus enzyme electrophoresis

MM4-Matisse Gel Medium No.4

PPM-Parts Per Million

ACC- 1-AminoCyclopropane-1-Carboxylic acid

AAS-Atomic Absorption Spectroscopy.

Abstract

Members of the *Bacillus* genus are generally found in soil and represent a wide range of physiological abilities, allowing the organism to grow in every environment due to its capability to form extremely resistant spores. Soil salinity affects agricultural production of crops, soil physico-chemical properties and ecological balance of the area. To mitigate the problem *Bacillu*s species that can grow in every harsh environment is the best option. The objective of this study was to asses salt tolerant *Bacillus* from saline soil around Metema area, west of Gondar and to test their biodegradation ability of pesticides such as DDT. The study design is purposeful randomized laboratory based experimental design with 3 replications. The research was conducted from January, 2017-May, 2017. Randomized Purposive sampling technique was used to collect the soil sample from the area, and then bulked to make a composite sample. Isolation of *Bacillus* species using serial dilution and pure culture method was done. Biochemical, morphological and cellular characterization of the isolates were done. Then the isolates were further grown on different salt concentration and temperature for further identification. The isolates nearest *Bacillus* species include *B.thuringiensis*, *B.subtilis*, *B.mycoides*, and *B.cereus* and *B.pumilis*. The salt tolerant ability and bioremediation potential of the isolates was checked. Three isolates have a potential to tolerate salt stress of 9%, 16% and 18% NaCl these are isolates nearest relative to B6(*B.thuringiensis*), B3(*B.subtilis*) and B5(*B.pumilus*). Three isolates *B.subtilis, B.mycoides* and *B.cereus* grown and used DDT as a carbon source at concentration of 0ppm, 25ppm, 50ppm and 100ppm were identified. Each isolate have different optical density under different DDT concentration. The maximum optical density of 0.51(B3), 0.75(B4), 0.50(B10). This study showed that there are many *Bacillus* species in the saline soil which have better potential to use xenophobic chemicals as a carbon source and partially degraded them.

Keywords: - Antibiotic, *Bacillus*, bioremediation, characterization, halophilic, isolation, and saline soil.

1. INTRODUCTION

Soil microorganisms play a great role in balancing the environment via recycling materials as they determine the mineralization of soil organic matter and energy flow. They also influence above-ground ecosystems by contributing to plant nutrition, plant health, soil structure and soil fertility (Wafula *et al*. , 2014).

Bacillus species are described as Gram-positive, endospore forming, chemo heterotrophic rod-shaped soil inhabiting bacteria (Waites *et al*., 2008). Members of the *Bacillus* genus are generally found in soil and represent a wide range of physiological abilities, spore formation allowing the organism to grow in every environment and antagonistic effect with other organisms within the environment (Kuta, 2008).

Extreme environmental factors such as recalcitrant wastes (DDT) , agrochemicals and salinity are affecting the physiological activities of microbes and affects almost all aspects of plant development including: germination, vegetative growth and reproductive development. Salinity of the soil results on ion toxicity, osmotic stress, nutrient scarcity and oxidative stress on plants, and thus limits water absorption from soil. However some microorganisms particularly bacteria, known as halophiles can survive at environments with high salt concentrations by creating different defense mechanisms (Munns, 2002).

Microbes have the potential to break down xenobiotics; scientists have been exploring the microbial diversity, particularly of contaminated areas in search of organisms that can degrade a wide range of pollutants. Hence, biotransformation of organic contaminants in the natural environment has been extensively studied to understand microbial ecology, physiology and evolution due to their bioremediation potential (Mishra *et al*., 2001). Thus, microorganisms provide a potential wealth in biodegradation. The ability of these organisms to reduce the concentration of xenobiotics is directly linked to their long-term adaptation to environments where these compounds exist. Moreover, genetic engineering may be used to enhance the performance of such microorganisms that have the preferred properties, essential for biodegradation (Schroll *et al*., 2004).

Most halophiles within the domain bacteria are moderate rather than extreme halophiles. Rodriguez-Valera (1988) cited in(Arora, 2014) stated that “there was an abundance of halophilic bacteria in saline soil from those the dominant group is the genus *Bacillus* is one of

them". Garabito *et al*. (1998) cited in(Arora *et al*,2014 and Nieto *et al*.,1989) isolated and studied 71 halotolerant Gram-positive endospore forming rods from saline soils and sediments of salterns located in different areas of Spain. These isolates were tentatively assigned to the genus *Bacillus*, and the majority of them were classified as extremely halo tolerant microorganisms, being able to grow in most cases in up to 20 or 25% salts (Arora ,2014).

The applications of salt loving bacteria include recovery of salt affected soils by directly supporting the growth of vegetation thus indirectly increasing crop yields in salt affected soils. Halophilic microbes are also found to remove salt from saline soils (Bhuva *et al*., 2013).There are reports that potential salt tolerant bacteria isolated from soil or plant tissues and having plant growth promotion trait, helps to alleviate salt stress by promoting seedling growth and increased biomass of crop plants grown under salinity stress (Chakraborty *et al*., 2011). Endophytic salt tolerant bacteria residing within plant tissues have been reported to be promoting the plant growth directly or indirectly through production of phytohormones, bio-control of host plant diseases and improvement of plant nutritional status (Arora *et al*., 2014).

They also possess the capacity to solubilize phosphates. Plant-microbe interaction is beneficial association between plants and microorganisms and also a more efficient method used for the reclamation of salt affected soils. Bacteria are the most commonly used microbes in this technique. However, archaea, fungi, actinomycetes, etc that may be found in environments with high salt concentrations can also be effective. Thus, there is high potential for bio-remediation applications for salt affected soils using halophilic microbe (Garcia, 2004; Ventossa *et al*., 2005; Yumoto *et al*., 2005).

Salinity of soil increases from time to time because various reasons such as using of artificial fertilizers, in adequate irrigation management and weathering of minerals in the soil. Soil salinity is a major problem for agriculture under irrigation. Salinity not only decreases the agricultural production of most crops, but also, affects soil physicochemical properties, and ecological balance of the area. Salinity effects are the results of complex interactions among morphological, physiological, and biochemical processes including seed germination, plant growth, and water and nutrient uptake (Akbarimoghaddam *et al*., 2011).

Various studies have been undertaken to isolate and characterize of *Bacillus* from soil. Much

of the recent studies have focused on isolation and characterization of soil microorganisms without considering their specific environment. This research is focused on the isolation, characterization and identification of *Bacillus* species from saline soil and their biodegrading ability of pesticide such as DDT.

2. REVIEW OF LITERATURE

2.1. The Genus *Bacillus* and its importance

The unique characteristics of genus *Bacillus* is aerobic growth of spore forming rods. Species of genus *Bacillus* are Gram-positive rods, motile (some non- motile) and non-acid-fast. They produce spores under aerobic conditions when exposed to high temperature. Some species are facultative anaerobic and variable in oxidase but catalase positive (Vargas *et al*., 2004)

The genus *Bacillus* has been divided into major groups. First group involves *B. thuringiensis, B. sphaericus, B. subtilis, B. anthracis and B. cereus*. This group contains a large number of soil-inhabiting species. Some of these species are so closely related as to form separate subgroups within group one. For example the *B. cereus* subgroup consists of *B. cereus*, *B. thuringiensis* and *B. mycoides*. and considered that the two latter species to be subspecies of *B. cereus* (Saadeldin and Osman, 2007).

The majority of the *Bacillus* species with validly published names are phylogenetically grouped into sub clusters within this genus. However, some validly named species of *Bacillus* are not phylogenetically related to the type species, *B. subtilis*, and are more closely related to other genera. The phylogenetic sub clusters within the genus *Bacillus* are: *Bacillus subtilis, amyloliquefaciens, atrophaeus, mojavensis, licheniformis, sonorensis, vallismortis,* including the very likely misclassified *Paenibacillus popilliae*; *Bacillus farraginis, fordii, fortis, lentus, galactosidilyticus*c; *Bacillus asahii, bataviensis, benzoevorans, circulans, cohnii, firmus, flexus, fumarioli, infernus, jeotgali, luciferensis, megaterium, methanolicus, niacini, novalis, psychrosaccharolyticus, simplex, soli, vireti*; *Bacillus anthracis, cereus, mycoides, thuringiensis, weihenstephanensis* ; *Bacillus aquimaris, marisflavi* ; *Bacillus badius, coagulans, thermoamylovorans, acidicola, oleronius, sporothermodurans* ; *Bacillus alcalophilus, arsenicoselenatis, clausii, gibsonii, halodurans, horikoshii, krulwichiae, okhensis, okuhidensis, pseudoalcaliphilus, pseudofirmus* ; *Bacillus arsenicus, barnaricus, gelatini, decolorationis,* ; *Bacillus carboniphilus, endophyticus, smithii,* ; *Bacillus pallidus, ;Bacillus funiculus, panaciterrae* (Devos ,2009).

2.2. Taxonomy and Classification of *Bacillus* and General Description

Genus *Bacillus* belongs to Kingdom: *Eubacteria,* Phylum: *Firmcutes,* Class: *Bacilli*, Order: *Bacillales*: Family: *Bacillaceae* and Genus: *Bacillus* (Saadeldin and Osman, 2007).

The cell of *Bacillus* ranges from 0.5-1.2 µm in width to 2.5-10 µm in length. The organisms usually grow well on blood agar, producing large, spreading, gray white colonies, with irregular margins. Many clinical isolates are β-hemolytic, helpful characteristic in differentiating various *Bacillus* species from *B. anthracis*, which is non-hemolytic. Catalase is produced by most species and sporulation is not inhibited by most incubation temperatures, positive characteristics that aid in distinguishing genus *Bacillus* from bacteria that can grow aerobically on nutrient medium at 30°C. Although the optimal temperature for growth of these bacteria, termed Mesophiles, is 30°C, they can grow at temperature ranging from 20 to 45°C. Those with an optimal incubation temperature of 15°C, are termed Psychrophiles and those with an optimal incubation temperature close to 60°C, are termed Thermopiles' (Saadeldin and Osman, 2007).

The most effective approach to *Bacillus* taxonomy may be analysis of 16S rRNA molecules by oligo-nucleotide sequencing. That technique holds much promise for leading microbial taxonomy into natural phylo-genetic relationships. However, traditional taxonomists may be dismayed to find that *Bacillus* species show kinship with non spore forming species. In a recent study 16S rRNA cataloging showed that *B. subtilis* and other ellipsoidal- spore forming species, *B. cereus*, *B. megaterium*, and *B. pumilus*, formed a coherent cluster, while the round-spore forming species, *B. sphaericus*, *B. globisporus*, and *"B. aminovorans*" did not cluster (Slepecky and Hemphill, 2006).

In a more recent 16S rRNA sequencing survey, three major *Bacillus* taxonomic cluster groups were defined. This was accomplished by determining complete or partial sequences of 16S RNA on 35 recognized neo type reference strains or type species by the technique of Lane *et al*. 1985. The partial sequences analyzed typically exceeded 1,100 nucleotides. Phylogenetic analyses were performed using three different approaches which showed three major groupings of *Bacillus* species hereinafter referred to as clusters I, II, and III (Slepecky and Hemphill, 2006).

The 16S rRNA *Bacillus* cluster groups were quite different from those previously noted by (Stackebrandt *et al*., 1987, cited in Slepecky & Hemphill., 2006). This is revealed by direct comparison to the commonly used morphological groupings (see Table 7.,Slepecky and Hemphill., 2006). Except for morphological group II and the un assigned Subgroup 2E, all strains sequenced fell into the *B. subtilis* cluster I grouping. *Bacillus* strains of morphological

group II fell into all three 16S rRNA cluster groups and *B. macquariensis*, unlike other psychrophiles, fell into the *B. alvei* cluster II group (Slepecky and Hemphill ,2006).

Comparative16S rRNA analyses on thermophilic and psychrophilic *Bacillus* strains showed that the thermophiles, *B. stearothermophilus*, *B. thermodenitrificans* and *B. caldotenax* formed a subgroup within the *B. subtilis* cluster but separate from both the "thermotolerant" mesophilic, *B. subtilis* and *B. licheniformis* strains, and the moderate thermophile, *B. coagulans*. The psychrophilic strains, *B. psychrophilus* and *B. insolitus*, fell into cluster I while *B. macquariensis* fell into cluster II. Because several species were included in the current study that had previously been examined by 16S rRNA oligo-nucleotide cataloging, it is possible to compare the two data sets directly. As a result, it is possible to augment the membership of cluster I to include *B. fastidiosus*, *B. firmus*, *B.badius* and *B. pasteurii* (C. B. Woese, personal communication cited in (Slepecky and Hemphill, 2006).

2.3. Cultural and microscopic characteristics of important *Bacillus* species.

Colonies of endospore-forming bacteria on blood agar and nutrient agar after 24–36 h at 37 °C. (a)*Bacillus anthracis*: circular to irregular colonies with entire to undulate, crenate and fimbriate edges, and granular surface textures; (b) *Bacillus cereus*: irregular, with undulate, crenate and fimbriate edges, and matt or granular textures; (c) *Bacillus megaterium*: glossy, round to irregular colonies with entire to undulate margins; (d) *Bacillus mycoides*: rhizoid or hairy-looking, adherent colonies which may readily cover the whole agar surface; (e) *Bacillus pumilus*: wrinkled, irregular colonies with undulate margins; (f) *Bacillus sphaericus*: smooth, glossy, round to irregular colonies with entire to undulate margins; (g) *Bacillus subtilis*: irregular colonies that may give the appearance of a mixed culture. They range in consistency from moist through butyrous or mucoid to membranous, with an underlying mucoid matrix (with or without mucoid beading at the surface), and become rough and crusty in appearance as they dry. Margins vary from undulate to fimbriate; (h) *Bacillus thuringiensis*: circular to irregular colonies with entire or undulate edges, and matt to granular surface textures; (i) Motile, spreading microcolonies sometimes seen in strains that were previously assigned to *Bacillus circulans* (Devos *et al*.,2009).

2.4. Halophilic *Bacillus* species

Several alkaliphilic *Bacillus* species have been isolated from soil samples and it showed

halophilic characteristics. *Bacillus krulwichiae,* a facultative anaerobic, isolated in Tsukuba, Japan, is a straight rod with peritrichous flagella that produces ellipsoidal spores. These have ability to utilize benzoate or *m*-hydro-xybenzoate as the sole carbon source. *Bacillus patagoniensis* was isolated from the rhizosphere of the perennial shrub *Atriplex lampa* in north-eastern Patagonia. Another is *Bacillus oshimensis* (Arora *et al.*,2014).

2.5. Impact of soil salinization

Salinization and soil degradation occur in areas where saline ground waters are elevated to where they approach the ground surface and evaporation exceeds precipitation. In irrigated areas, where the water table approaches the ground surface, salt accumulation occurs in areas known as discharge zones. Capillary rise of saline groundwater causes the direct precipitation of evaporate minerals at the surface in these zones. Salinization may also occur when salts are concentrated in soils by the evaporation of free standing irrigation water (Dehaan, 2002).

Soil salinity is an enormous problem for agriculture under irrigation. In the hot and dry regions of the world the soils are frequently saline with low agricultural potential. In these areas most crops are grown under irrigation, and to exacerbate the problem, inadequate irrigation management leads to secondary salinization that affects 20% of irrigated land world-wide (Glick *et al*., 2007). Irrigated agriculture is a major human activity, which often leads to secondary salinization of land and water resources in arid and semi-arid conditions. Salts in the soil occur as ions (electrically charged forms of atoms or compounds). Ions are released from weathering minerals in the soil (Shrivastava and Kumar, 2015).

They may also be applied through irrigation water or as fertilizers, or sometimes migrate upward in the soil from shallow groundwater. When precipitation is insufficient to leach ions from the soil profile, salts accumulate in the soil resulting from soil salinity. Plants absorb essential nutrients in the form of soluble salts, but excessive accumulation strongly suppresses the plant growth. During the last century, physical, chemical and/or biological land degradation processes have resulted in serious consequences to global natural resources (e.g. compaction, inorganic/organic contamination, and diminished microbial activity/diversity). The area under the affected soils continues to increase each year due to introduction of irrigation in new areas (Shrivastava and Kumar., 2015). Salinity is recognized as the main threats to environmental resources and human health in many countries, affecting almost 1 billion ha worldwide/globally representing about 7% of earth's

continental extent, approximately 10 times the size of a country like Venezuela or 20 times the size of France. It has been estimated that an approximate area of 7 million hectares of land is covered by saline soil in India (Shrivastava and Kumar., 2015).

Salinity causes unfavorable environment and hydrological situation that restrict the normal crop production throughout the year. The factors which contribute significantly to the development of saline soil are, tidal flooding during wet season, direct inundation by saline water, and upward or lateral movement of saline ground water during dry season. It affects crops depending on degree of salinity at the critical stages of growth, which reduces yield and in severe cases total yield is lost. Soil reaction values (pH) in coastal regions range from 6.0-8.4. The organic matter content of the soils is also pretty low (1.0-1.5%). Nutrient deficiencies of N and P are quite dominant in saline soils. Micronutrients, such as Cu and Zn are widespread. During the wet monsoon the severity of salt injury is reduced due to dilution of the salt in the root-zone of the standing crop (Haque, 2006)

2.6. Application of Salt tolerant or halophilic *Bacillus*

All halophilic microorganisms contain potent transport mechanisms, generally based on Na^+ /H^+ antiporters, to expel sodium ions from the interior of the cell. Also, some halophiles express ACC deaminase activity that removes stress, ethylene from the rhizosphere and some produce auxins that promote root growth (Oren, 2002).

2.6.1. Environmental application

The application of halophilic bacteria in environmental biotechnology is possible for (1) recovery of saline soil, (2) decontamination of saline or alkaline industrial wastewater, and (3) the degradation of toxic compounds in hypersaline environments. The use of halophilic bacteria in the recovery of saline soils is based on the hypothesis that microbial activities in saline soil may favor the growth of plants under salt stress. The second hypothesis is based on the utilization of these bacteria as bio-indicators in saline wells. Indicator microorganisms can be selected by their abilities to grow at different salt concentrations (Arora *et al*., 2014 and Ventossa *et al*.,2002).

These organisms could indicated that well water could be used for producing low saline contamination of plants or soils which could be alleviated desertification of soil .Last hypothesis is the application of halophilic bacterium genes using a genetic manipulation

technique to assist wild type plants to adapt to grow in saline soil by giving them the genes for crucial enzymes that are taken from halophiles (Olivera *et al*.,2005). Compatible solutes are low-molecular weight organic compounds such as polyols, amino acids, sugars and betaines that the halophilic and halotolerant bacteria accumulate intracellular to achieve osmotic balance. On the other hand, halophilic bacteria tolerant to heavy metals could be used as bioassay indicator organisms in saline- polluted environments. Several halotolerant and halophilic bacteria isolated from hypersaline soils tolerate high concentrations of different metals such as Co, Ni, Cd, or Cr. In the biological treatment, the micro-organisms conventionally used show only poor degradative efficacy due to the highly saline conditions. The potential of halophilic organisms in effluent treatment offers the promise of innovative research. Other than that, halophilic is also used to recover saline soil by directly supporting the growth of vegetation, thus indirectly increasing crop yields in saline soil (Arora *et al*., 2014).

Bacteria are an important part of the soil micro flora because of their abundance (up to 109 cells per gram of soil, their species diversity (a minimum of 4 000–7 000 different bacterial genomes per gram of soil and the multiplicity of their metabolic activities. They play a key role in the biogeochemical cycles of the main elements (carbon, nitrogen, sulfur, etc.) and of trace elements (iron, nickel, mercury, etc.) and are therefore heavily implicated in energy and nutrient exchanges within the soil. They also have the potential to reflect the past history of a given environment. It is therefore essential to understand the interrelationships between bacteria and their environment by studying the structural and functional diversity of soil bacterial communities and how they respond to various natural or manmade disturbances (Ranjard *et al*., 2000).

2.6.2. Degradation of some toxic compound (biodegradation).

2.6.2.1. Degradation of DDT

DDT is the one of the effective pesticide in killing insects in indoor environments and it's low cost, persistence and relatively safety to humans. However, DDT is severely reduced and restricted to indoor residual spraying, due to its persistence in the environment and ability to bioconcentrate in the food chain (Mwangi *et al*., 2010).

To mitigate the problem biodegradation is the best option with significant impact on the fate of

DDT in the environment is biodegradation. Biodegradation is removal or conversion of pesticides from the environment through microorganisms. A successful bioremediation technique requires an efficient microbial strain that can degrade largest pollutant to minimum level .The rate of biodegradation in soil depends on four variables: (i) Availability of pesticide or metabolite to the microorganisms (ii) Physiological status of the microorganisms (iii) Survival and proliferation of pesticide degrading microorganisms at contaminated site and (iv) Sustainable population of the microorganisms. Therefore, to attain an achievable bioremediation, it requires the creation of unique niche or microhabitats for desired microbes, so they can be successfully exploited (Mwangi *et al*., 2010).

Currently, using of microorganisms to remove such complex and xenobiotic compounds from the environment has no side effect relative to the other methods. For example removal of crude oil pollution contaminated sites as bioremediation studies was considered by scientists because other methods such as surfactant washing and incineration lead to production of more toxic compounds and they are non-economic. Fifteen crude oil degrading *Bacillus* species were isolated from contaminated sites. Two isolated showed best growth in liquid media with 1-3% (v/v) crude oil and mineral salt medium, then studied for enzymatic activities on tested media. The results showed maximal increase in optical densities and total viable count concomitant with decrease in pH on fifth day of experimental period for *Bacillus* S6. Typical generation time on mineral salt with 1% crude oil is varying between 18-20h, 25-26h respectively for *Bacillus* S6 and S35. Total protein was monitored at determined time intervals as biodegradation indices. Increasing of protein concentration during the incubation period reveals that isolated *Bacillus* can degrade crude oil and increase microbial biomass. This *Bacillus* spp. reduced surface tension from 60 (mN/m) to 31 and 38 (mN/m). It means that this *Bacillus* spp. can produce sufficient surfactant and have good potential of emulsification capacity. This study tells us *Bacillu*s species can use crude oil as a carbon and energy source (Sepahi *et al*., 2008).

2.7. *Bacillus* as biological control of plant pathogens and insect pests.

Bacillus species have special characteristics that make them good candidates as biological control agents. First, they are well known as antibiotic producers with antagonistic activity against fungal and some bacterial pathogens. Second, they form spores that can be easily formulated, and have high viability compared with vegetative cells. Third, they are commonly found in soils. *B. thuringiensis* is a Gram positive, spore forming, soil bacterium

and is the most successful biological control agent that produces distinctly shaped crystals during sporulation. These crystals are composed of proteins, known as insecticidal crystal proteins (ICPs), and also called Cry proteins, which are selectively toxic to different species of several invertebrate phyla (Sansinenea and Ortiz, 2011).

Several *Bacillus* strains belonging to the *B. subtilis*/*amyloliquefacien*s group isolated from soil by FZB Biotechnik GmbH, Berlin, were shown to have remarkable effects in biological activities against some soil-borne fungal plant diseases and in plant growth-promoting actions for higher yields after colonizing plant root. A commercial formulation based on spores of *Bacillus amyloliquefaciens* FZB 24 has recently been developed and has found application in agriculture and horticulture. Despite extensive studies, the molecular mechanism responsible for observed plant growth-promoting effects exerted by Gram positive rhizobacteria and especially for *Bacilli* is poorly understood. Several possible mechanisms were proposed including production of plant growth regulating substances as indole-3-acetic acid and gibberellins (Elsorra *et al*.,2004).

Biological control of plants by microorganisms is a very promising alternative to an extended use of pesticides, which are often expensive and accumulate in plants or soil, having adverse effects on humans. Nonpathogenic soil bacteria living in association with roots of higher plants enhance their adaptive potential and, moreover, they can be beneficial for their growth. Here, we present the current status of the use of *Bacillus subtilis* in biocontrol. This prevalent inhabitant of soil is widely recognized as a powerful biocontrol agent. Naturally present in the immediate vicinity of plant roots, *B. subtilis* is able to maintain stable contact with higher plants and promote their growth. In addition, due to its broad host range, its ability to form endospores and produce different biologically active compounds with a broad spectrum of activity, *B. subtilis* as well as other *Bacilli* are potentially useful as biocontrol agents (Nagórska *et al*., 2007).

Mixtures of biocontrol strains can show enhanced levels of disease control compared with that of individual strains. Separate nutritional niches of mixture components, limited overlap of the physical environmental optima of mixture component strains, or heightened biocontrol activity of a mixture component due to the presence of a second mixture component offer possible explanations of increased biocontrol success with formulations of microbial mixtures. Despite taking precautions to enhance the survival of a biocontrol strain, severe

environmental conditions may drastically limit biocontrol agent establishment on a host target site (Schisler *et al*., 2004).

In the case of attempting to protect aerial plant parts with biocontrol agents, a formulation solution could take the form of replacing lost biocontrol inoculum or protecting the inoculum initially delivered to the host surface from deleterious environmental conditions. While significant improvements still are needed, controlled release formulations of *Bacillus spp*. for insect control show considerable promise in providing *Bacillus* spores and toxin crystals over time from one application event and represent a virtually unexplored area for *Bacillus*-based products for use in reducing plant diseases (Schisler *et al*., 2004).

2.8. Secondary Metabolites of soil *Bacillus* spp.

Bacillus species produce secondary metabolites that are the object of natural product chemistry studies. The wide structural variability of these compounds has attracted the curiosity of chemists and their biological activities have inspired the pharmaceutical industry to search for lead structures in microbial extracts. Screening of microbial extracts reveals the large structural diversity of natural compounds with broad biological activities, such as antimicrobial, antiviral, immunosuppressive, and antitumor activities, that enable the bacterium to survive in its natural environment (Sansinenea and Ortiz, 2011).

Bacillus spp. appears to be a relatively abundant source of antimicrobials, since many species of this genus synthesize antimicrobial peptides. The possibility of screening for a new *Bacillus* bacteriocin producer is considered to be one of the major interests in bacteriocin research. *B. subtilis*, the model system for Gram-positive organisms, is able to produce more than two dozen antibiotics with a variety of structures. Several bacteriocins have been reported such as lichenin produced by the *B. licheniformis* 26-103RA strain , megacin produced by strains of *B. megaterium*, antilisterial coagulin produced by *B. coagulans*, *polyfermenticin* SCD produced by *B. polyfermenticus* , and cerein produced by strains of *B. cereus*. Moreover, some of these bacteriocins have been indicated as potential biopreservatives in food systems and beverages, as agents for biological control of phytopathogens, and as antibiotic precursors(Sansinenea and Ortiz, 2011).

Bacteriocin production and secretion was observed in stationary phase, after 10–16 h of bacterial population growth in broth at30^0C. As is well known, bacteriocins of lactic acid bacteria, particularly lantibiotics, are usually produced in the midgrowth phase. The

production of bacteriocins takes place successfully in both broth media and on solid media. The biopreservation capacity of a bacteriocin could be achieved either by using a bacteriocin producing starter culture or by applying the bacteriocin itself as a food additive. Therefore, a thorough study of the essential parameters affecting the inhibitory activity, followed by optimization of production that is usually dependent on multiple strain-specific factors is necessarily required for introducing a bacteriocin into foods as a potent biopreservative. For this purpose Khalil *et al*., (2009) described the influence of cultural and physical conditions such as nutrients, nutrient concentrations, nutrient combinations/interactions, aeration, and other physical factors, including heat, UV, pH, and storage conditions, to obtain better and more stable bacteriocin activity from a newly isolated strain of *B. megaterium* (Sansinenea and Ortiz, 2011).

Peptide antibiotics with an inter-residual thioether bonds as unique feature are called lantibiotics. The best-characterized is subtilin, a 32-amino-acid pentacyclic lantibiotic of *B. subtilis*. Subtilin production is controlled both by culture density in a quorumsensing mechanism in which subtilin plays a pheromone type role and in response to the growth phase. There are some other lantibiotics, such as ericin, mersacidin, sublancin and subtilolisin A, that are produced by *B. subtilis* (Sansinenea and Ortiz., 2011).

B. amyloliquefaciens FZB42 capable to produce a vast array of secondary metabolites aimed to suppress competitive bacteria and fungi within the plant rhizosphere. Interestingly, five of the nine giant gene clusters involved in non-ribosomal synthesis of secondary metabolites are located in close vicinity of the *ter* site in which the bidirectional replication fork meets together (Alexandra *et al*., 2009).

2.8.1. Secondary metabolites of *Bacillus thuringiensis*

Bacillus thuringiensis is the best known insect pathogen from all *Bacillus* species. It can produce a protein called parasporal crystals, which are selectively toxic to different species of several invertebrate phyla. Many isolates of *B. thuringiensis* also produce an assortment of various other virulence factors that are secreted into the culture medium. These factors include the vegetative insecticidal proteins, and b-exotoxinI13, also called thuringiensin 13 a non proteinaceous toxin. Unlike the Vip and Cry toxins, b-exotoxin I 13 is not specific and thus, may have detrimental effects on non target organisms; it is particularly active against dipterans' species, but it is also active against coleopteran, lepidopteran, and some nematode

species. (Sansinenea and Ortiz, 2011).

2.9. Statement of the problem

Salinity of soil increases from time to time because various reasons such as using of artificial fertilizers, in adequate irrigation management and weathering of minerals in the soil. Soil salinity is a major problem for agriculture under irrigation. In the hot and dry regions of the rift valley soils are frequently saline with low agricultural potential. In these areas most crops are grown under irrigation, and to exacerbate the problem, inadequate irrigation management leads to secondary salinization that affects 20% of irrigated land worldwide Salinity not only decreases the agricultural production of most crops, but also, affects soil physicochemical properties, and ecological balance of the area. Salinity effects are the results of complex interactions among morphological, physiological, and biochemical processes including seed germination, plant growth, and water and nutrient uptake. To mitigate such a problem salt tolerant three *Bacillus* species have been isolated and identified that may enhance the growth and production of agricultural crops under harsh saline condition.

2.10. Significance of the study

To solve the problem of salinization by introducing salt tolerant *Bacillus* species to the agricultural soil that affected by salinity. The most important *Bacillus* species that can enable adapt saline soil and also the *Bacillus* species which can degrade pesticides such as DDT were determined that has a great significant for agriculture and to protect the environment from pollution. *Bacillus* is the frequently soil inhabiting genus in the rhizosphere of soil, are naturally occurring soil bacteria that highly colonize plant roots and benefit plants by providing growth promotion. *Bacillus subtilis, B.pumilus and B.thuringiensis* able to survive in high temperatures and salt concentrations and thus confer on them potential competitive advantage to survive in arid and saline soils such as calcisol. Using of these *Bacillus* species to solve such a problem is very important because they are natural found in the soil, less costly and environmental friendly way of approach to solve a problems.

2.11. Objective

2.11.1. General objective

- The general objective of the study was to asses salt tolerant *Bacillus* species that can be used for bioremediation purpose from saline soil around Metema area, Gondar.

2.11.2. Specific objectives

- To isolate, characterize and identify salt tolerant *Bacillu*s species.
- To evaluate salt tolerant ability of *Bacillu*s species.
- To assess bioremediation potentials of the isolated *Bacillus* species on pesticide degradation ability.

3. MATERIALS AND METHODS

3.1. Collection of Soil Samples

Soil samples were collected from low land areas specially Metema, North of Ethiopia. Metema is located at latitude and longitude of 12.95 and 36.16, respectively. The soil samples were collected from a depth of 0-20cm and 20-40cm and mixed or bulked thoroughly to constitute composite samples it was then transported intact at 4 °C in sealed polyethylene bags to the laboratory for processing. One gram of soil was serial diluted in normal saline (Garbeva *et al*., 2003).

3.2. Study design and period

Purposeful Laboratory based experimental design was used to perform the task. Each treatment had had three replications and the experiment was repeated twice. The research was conducted from January 1, 1, 2017-May 30, 5, 2017.

3.3. Physical and chemical enrichment

The soil samples were air dried, heat treated under water bath at 80°C for 30 minutes. Antifungal antibiotics such as amphoteracin B (250µg/ml) was incorporated into the media to enhance the selection of members of the bacteria (Zhang, 2005).

3.4. Media preparation and isolation of *Bacillus*

Nutrient agar and nutrient broth were used for the cultivation of microorganisms were prepared for cultivation of the microbes. One gram of the NA(Difco) was taken and mixed in one liter of water to which the antifungal antibiotic amphotericin B(250µg/ml) was incorporated(Zhang, 2005). The media was then dispensed into 90-mm-diameter polystyrene sterile plastic petridishes. The samples were transferred into 100ml of water in 250ml Erlenmeyer Flasks and heated in water bath at 80°C for 30 minutes. The soil suspension was then diluted to appropriate dilutions (10^{-6}-10^{-7}) from which 0.1ml was transferred into three petridishes containing the medium. The plates were incubated at 30°C for 2-3 days. The isolated and different colonies were picked up and repeatedly sub cultured to obtain pure colonies. The pure colonies were then preserved on NA slants at 4°C for further study (Garbeva *et al*., 2003).

3.5. Inoculation and incubation

The bacterial colonies were inoculated in 5ml nutrient broth and NA containing plate and then incubated at 30^0C overnight (Bai *et al*., 2002).

3.6. Characterization and identification of isolates

Characterization of the isolates was performed using morphological and cultural characteristics. The cultural characteristics were colony color, diameter, texture and elevation (Wafula *et al*.,2014).

3.6.1. Biochemical tests for identification

3.6.1.1. Oxidase Test

Drops of oxidase reagent (1%tetramethyl-p-phenylenediamine dihydrochloride) were poured over sterilized strips of filter paper in plate wetted with distilled water. Using sterile forceps, strips were put on a clean slide. Colony on nutrient agar was picked with strile glass rod and rubbed on the filter paper. A dark purple pigment that developed within five to ten seconds indicates positive result (Bai *et al*., 2002).

3.6.1.2. Catalase test

A loopful of a *Bacillus* culture was transffered on a clean slide. A drop of hydrogen peroxide (H_2O_2) was dropped on to the samples. The production of gas bubbles indicated positive catalase test (Travers *et al*., 1987).

3.6.1.3. Starch Hydrolysis

The test isolates were inoculated into starch agar medium plates containing (g/L): peptone 10g, yeast extract 10g, K2HPO4 5g, starch 3g, Agar 5g and distilled water and pH approx 7.2. The plates were incubated at 30^0C for 48 hrs, and flooded with iodine solution (iodine 10g, potassium iodide 6g, distilled water 10ml and 90%ethanol 100ml), the presence of a clear zone around the colonies was indication of starch hydrolysis (Travers *et al*., 1987).

3.6.1.4. Gelatin Hydrolysis

Nutrient gelatin medium containing (g/L) lab lemco 3g peptone 5g, gelatin 120g suspended in one liter of distilled water and poured in sterile 2ml volume of Bejon bottles.This medium was inoculated with the test isolates and incubated at 30°C for 2-3 days. The inoculated tubes

were incubated at 4°C to test the stability of the gelling feature of gelatin (Pathak *et al*., 2015).

3.6.1.5. Urease Activity

The reaction of urease was shown by alkali production (ammonia) from urea solutions. Test culture was streaked on test tubes containing urea agar slope that contained (g/L) peptone 1g, dextrose 1g, NaCl 5g, Na2 PO4 1.2g, KH2 PO4 0.8g, phenol red 0.012g and Agar No.3 15g, pH approx 6.8. Exactly 2.4 grams were suspended in 95 ml of distilled water, boiled to dissolve completely, and sterilized by autoclaving at 115° for 20 min. The preparation was cooled to 50°C and 5 ml of sterile 40% urea solution was added aseptically mixed well then distributed in 10 ml volume into sterile McCartney bottles and allowed to set in a slope position after that incubated at 37°C for two days. A positive result was indicated by change of color to pink (Pathak *et al*., 2015).

3.7. Motility Test

Motility of isolates was determined through stab culturing with straight wire into semi solid agar (Luna *et al*., 2005) The nutrient broth medium (Oxoid) contained (g/l) lab lemco 1g, Yeast extract 2g peptone 5g and sodium chloride 5g and was added 0.3% agar and one liter of distilled water pH approx 7.4 sterilized by autoclaving at 121ºc for 15 min and dispensed in test tubes, then incubated in an upright position at 30°C. The tubes were examined intervally for swarming (diffuse growth) for up to four days. Cultures were regarded as motile if growth spread hazy, diffusing throughout the medium making it slightly opaque (Travers *et al*., 1987).

3.8. Microscopic Examination

Smears were prepared from the bacterial isolates used to determine the shape of cell, presence, arrangement and position of spores (Paidhungat *et al*., 2000).

3.9. Gram's Staining Method

Smears were prepared from purified colonies cultures (24 hrs old). These were dried in air and fixed by passing through a flame. The Gram positive was the one with crystal violate color (Gregersen, 1978).

3.10. Growth at different salt concentration and temperature

The growth of isolates was tested on NA medium containing 6.5% and 10% NaCl and at temperature of 40^0C and 55^0C *(*Margesin and Schinner, 2001)

3.11. Salt tolerance ability

The ability of *Bacillus* species to withstand different salt concentration was done at salt concentration of 9%, 16% and 18% NaCl to evaluate salt tolerant ability (Margesin and Schinner, 2001

3.12. Bioremediation potential of the isolates on pesticides degradation

3.12.1. Degradation of DDT by the isolates

Aliquots of 1.0 ml from actively growing pure culture of each isolate was inoculated separately in MM4 medium in which 0ppm,25ppm 50ppm and 100 ppm DDT were added and incubated for 31 days at 30°C. The initial concentration of DDT used was 5000 ug/ ml and dilutions in sterile media were made to a final concentration of 25ppm, 50ppm and 100 ppm DDT. Uninoculated medium with DDT and inoculated medium without DDT but containing sterile mineral salt medium were used as controls. Turbidity, optical density and growth were measured by spectrophotometer at 600nm after every two days. Isolates that grow in the medium and made turbidity in the media was judged that the isolate reduced the concentration of DDT in the medium and as biodegraded DDT (Mwangi *et al*., 2010).

3.13. Data analysis

Descriptive statics, tables and histogram used to analyses the data which was obtained from the result of the study. Statistical analysis by using IBM SPSS version 20 was used to analyse mean, minimum and maximum value to compare between different test results.

4. RESULT

4.1. Identification of the isolates

4.1.1 Morphological and microscopic identification

Most isolates were able to grow within 2-3 days of incubation at 30^0C. The colony characteristics of the isolates ranged from, flat and filamentous or branching. They were smooth or rough and the color ranged from white to cream and brown (Table 1). The colony margin of the isolates includes undulate, ciliate, whipe-like and branching type. All isolates were Gram positive and have rod shaped bacteria. Among the10 selected colonies B1and B10 showed the same morphological and microscopic characteristics and also B5 and B8 have the same morphological and microscopic characteristics (Table 1) below.

Table 1: Morphological and microscopic characteristics of isolates

Isolates	Colony characterization			
	colony color	Colony form	Colony elevation	Colony margin
B1,B10	White	Irregular	Flat	Undulate
B2	Brown	Irregular	Flat	Undulate
B3	Cream	Irregular	Flat	Undulate
B4	Brown	Irregular	Flat	Ciliate
B5,B6,B8	Cream	Irregular	Flat	Ciliate
B7	White	Irregular	Flat	Whip-like
B9	Cream	Irregular	Flat	Branching

4.1.2. Biochemical identification

The ability of the isolates to excrete extracellular enzymes was tested through hydrolysis of starch, and gelatin. The ability of the isolates to excrete intracellular enzymes was determined through tests on catalase and reaction and motility and triple sugar-iron test. The isolates deferred greatly on their ability to excrete various enzymes (Table 2). All ten isolates were catalase, positive but they differed in other tests. For example from ten isolates only B4 was negative for motility test. Isolates B3, B6 and B7 are gelatinase negative the rest are positive and all isolates are urease positive except isolate B3.

Depending on the biochemical test result the isolates were grouped as follows. Isolates B1, B2, B6, B7 and B8 almost had the same biochemical reaction but with slight difference between them. B1 and B2 100% the same biochemical reaction; B6 and B7 differ from B1 ,B2 and B8 only by gelatinase negative reaction but B1 ,B2 and B8 were gelatinase positive. B8 differed from the rest B1, B2, B6 and B7 by triple iron sugar agar reaction negative but the rest were positive. Isolates B9 and B10 were 100% similar by biochemical reaction. The isolates B3, B4 and B5 were not shaped because of differences in many characteristics (Table 2).

Table 2: Biochemical characteristics of the isolates obtained from saline soil

Isolates	Biochemical tests					
	Star	Cat	Mot	Urea	Gel	Oxid
B1,B2,B8	+	+	+	+	+	-
B3	+	+	+	-	-	-
B4	+	+	-	+	+	+
B5	-	+	+	+	+	-
B6,B7	+	+	+	+	-	+
B9 ,B10	+	+	+	+	+	-

Key: (+) Positive, (-): Star: Starch, Gel: Gelatine, Cat: Catalase, Oxid: Oxidase, Mot: Motility

4.1.3. Growth under salt concentration and temperature for identification

Isolates were also tested for their ability grow at different salt concentration and temperature for further identification. All isolates were able to grow at salt concentration of 6.5%. All isolates can able to growth under salt concentration of 10%NaCl except B3 and B4. B3 was

tolerate to10% NaCl. At 40^0C all isolates show growth except B4.

Table 3: The growth of *Bacillus* isolates on salt concentration and temperature.

Salt concentration & temperature		Isolates									
		B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
NaCl conc	6.5%	+	+	+	-	+	+	+	+	+	+
	10%	+	+	+	-	+	+	+	+	+	+
Temp.	40^0C	+	+	+	-	+	+	+	+	+	+

Key :(+) growth, (-) no growth, (Temp) temperature, (conc.) concentration.

4.1.4. Identification of isolates to related Bacillus species

Depending on morphological, biochemical, microscopic characteristics and growth under different salt concentration and temperature the isolates were grouped as follows (Devos. *et al*., 2009). B1, B2, B6, B7, and B8 were closely related to *Bacillus thuringiensis* because they are positive on starch catalase, motility and negative on oxidase. That makes them the same with *B. thuringiensis.* Isolate B3 was related to *Bacillus subtilis* because it was positive on starch, urea, motility, to tolerate at 6.5%NaCl .That makes it related to *B. subtilis* rather than other *Bacillus* species. Isolate B4 was related to *Bacillus mycoides* because its non motility ,starch positive, catalase and Vp positive and negative on oxidase and not growth at 40^0C and 55^0C are similar characteristics with *B.mycoides*. Isolate B5 relates with *B.pumilus* due to its similar by the following characters such as motility, starch negative, grow 40^0C. The two isolates B9 and B10 were more related to *Bacillus cereus* because they were positive on starch, motility, urea, gelatinase, tolerated to 6.5% NaCl and negative to oxidase (Table 3).

Table 4: *Bacillus* isolates from saline soil and their nearest neighbors

Isolates	Closest *Bacillus* species	Differs from each other
B1,B2, B6 ,B7,and B8	*B. thurnigiensis*	Ox. (-)
B3	*B. subtilis*	Gel (-)
B4	*B. mycoides*	Non motile
B5	*B.pumilis*	Star.(-)
B9 and B10	*B. cereus*	Ox.(+),Gel.(+)

4.2. Salt tolerance test

From 10 selected *Bacillus* isolates all of them were grown at salt concentration of 9% NaCl except B4. At salt concentration of 16% and 18%NaCl only B1, B3 and B6 were tolerated.

Isolate B5 (*B.pumilis*) showed better growth in both cases than B3 (*B.subitilis*) and B6 (*B.thuringiensis* strain) Fig 2.

Table 5: Salt tolerance test for different Bacillus isolates.

NaCl conc.	Isolates									
	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
9%	+	+	+	-	+	+	+	+	+	+
16%	-	-	+	-	+	+	-	-	-	-
18%	-	-	+	-	+	+	-	-	-	-

4.3. Biodegradation of DDT

The growth of isolates observed at DDT concentrations of 0, 25, 50 and 100 ppm, after a period of 30 days were different from each other. The maximum growth of 0.75 OD was obtained from DDT concentration of 100ppm by isolate B4 which was related to *B.mycoides*. The minimum growth of 0.06 OD was seen at DDT concentration of 0ppm by isolate B3 which was related to *B .cereus*. The average optical density of each isolate B3 (*B.subitilis*) was 0.3075, B4, 0.575 and B10, 0.46 OD at 600nm. The isolate B4 (*B.mycoides*) showed more growth than the two isolates. The highest optical density of growth was seen from isolate B3 at DDT concentration of 50ppm. The highest growth for isolate B4 (*B.mycoides*) is at DDT concentration of 100ppm and 50ppm was for isolate B10 (*B.cereus*). Both isolates showed least growth at DDT concentration of 0ppm (Fig 1). Isolate B3(*B.subitilis*) showed optical density of 0.06, 0.26 and 0.51 at DDT concentration of 0ppm, 25ppm, 50ppm and 100ppm respectively. Isolate B4 displayed optical density of 0.29, 0.56, 0.70 and 0.75 at DDT concentration of 0 ppm, 25ppm, 50 ppm and 100ppm respectively. Isolate B10(*B.cereus*) exhibited optical density of 0.13, 0.26 .0.50 and 0.49 at DDT concentration of 0ppm, 25 ppm, 50ppm and 100ppm respectively. The highest optical density of 0.75OD was recorded at 100ppm by *B.mycoides* and the lowest OD of 0.26 has shown at 25ppm both by *B.subitilis* and *B.cereus.*

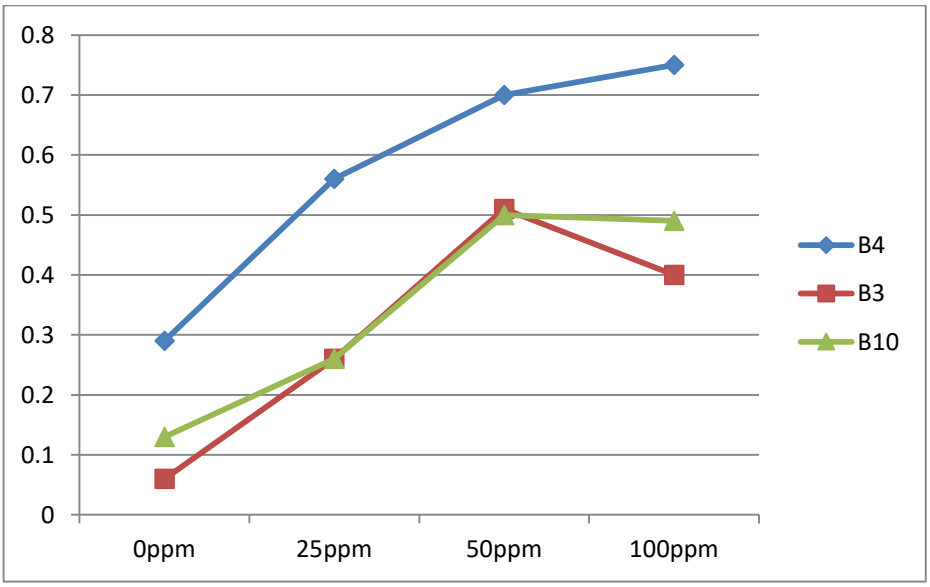

Figure 3: The growth of three isolates B3, B4 and B10 on DDT concentration

The x-axis in the figure above indicates DDT concentration and the y-axis indicates their growth (optical density).

5. DISCUSSION

Morphological, biochemical, cellular characteristics of isolates B1, B2, B6, B7, and B8, were closely related to *Bacillus thuringiensis* with starch, catalase, nitrate, citrate and VP positive; motile, oxidase negative. Isolate B3 was closely related to *Bacillus subtilis*. Isolate B4 closely related to *Bacillus mycoides* and it was a non- motile rod (Table 2). Isolate B5 was closely related to *B.pumilus* with motility, starch negative, nitrate negative, Vp positive, grow at 40^0C . The rest 2 isolates B9 and B10 were closely related to *Bacillus cereus* they are catalase, triple iron sugar agar, starch , urease , positive, liquefied gelatin, citrate positive, Voges Proskauer positive and motile and had the ability to reduce nitrate to nitrite, oxidase positive and growth at 6.5% NaCl. Most isolates were negative with indole, methyl red test and hydrogen sulphide gas production (Garbeva *et al*., 2003).

In the previous report it was stated that from the most common halophilic bacterial genera of saline soil environment the most dominant one was genus *Bacillus* Distinctions between different kinds of halophilic microorganisms were made on the basis of their level of salt requirement and salt tolerance. According to Kushner (1993) classification, microbes responsive to salt are defined under five groups: non-halophilic, (~1%) salt, slight halophiles, (~1-3%) salt, Moderate halophiles, (~3-15%) salt, Borderline extreme halophiles, (~9-23%) salt and Extreme halophiles, (~15-32%) salt (Arora *et al*., 2014). The isolates B1(*B.thuringiensis*), B3(*B.subitilis*) and B6 showed growth at salt concentration of 9% ,16% and 18%NaCl that indicates they are salt tolerant and moderate halophilic or border line extreme halophiles that can able to mitigate the problem of salinization on the environment. From the three salt tolerant isolates B3 (*B.subtilis*) shown the best growth and tolerance at salt concentration of 16% and 18% NaCl.

The potential for biodegradation of DDT by soil microorganisms through enrichment and isolation of DDT biodegrades from soils without a history of prior exposure to DDT was done. More over from both cultivated and non cultivated soils bacteria were collected and checked for biodegradation ability. The highest growth of 0.48 (O.D 600) was observed at DDT concentration of 50 ppm (Mwangi *et al*., 2010). In this study the maximum growth seen at DDT concentration of 100ppm by B4 (*B.mycoides*) is 0.75(O.D 600) which is better than the previous studies.

In this study *Bacillus s*pecies live in saline soil that can use DDT as a carbon source and partially degrade DDT. From ten isolates three isolates B3, B4 and B10 were identified as DDT degrader. If they were not able to use DDT as a carbon source there would no growth. Most of the isolates couldn't use DDT as a carbon source and did not show growth. Even the ability to degrade DDT and use it as a carbon source between the three DDT degrading isolates was also different.

6. CONCLUSION

Five *Bacillus* species were isolated from saline soil. Characterization and identification of the isolates were done by using biochemical tests, further characterization under different salt concentration and temperature were done. The isolates B1 ,B3 and B6 shown growth at salt concentration of 9% ,16% and 18%NaCl that indicates they are salt tolerant and some of them could be moderate halophilic or halophilic. This study showed that there are *Bacillus* species in the tropical saline soil that can use DDT as a carbon source and partially degrade DDT. From ten isolates three isolates B3 (*B.subitilis*), B4 (*B.mycoides*) and B10 (*B.cereus*) were capable of utilizing DDT as a carbon source. The most powerful ability to catabolite DDT as a carbon source recorded by isolate B4 (*B.mycoides*). Isolate B10 had a biochemical test result similarity with *B. cereus*. Isolates B3 had a biochemical test result similarity with *B.Subtilis* and B4 with *B.mycoides*. These isolates may have the potential to degrade other xenophobic pesticide if other testes are done.

7. RECOMMENDATION

*Bacillus s*pecies in the tropical saline soil that can use DDT as a carbon source and partially degrade DDT. Bioremediation of xenobiotics such as DDT by using extremophiles such as halophilic *Bacillus species* is better way of mitigating the problem of environmental pollution caused by the accumulation of persistence chemicals in the environment. The potential difference between the microbes to biodegrade DDT should be assessed or the reason behind why all halophilic *Bacillus* have no equal ability to use DDT as a carbon source and degrade DDT. Molecular characterization and their active compound should be a future work. *Bacillus* species isolated from saline soil can tolerate high salt concentration and may mitigate salinity problem.

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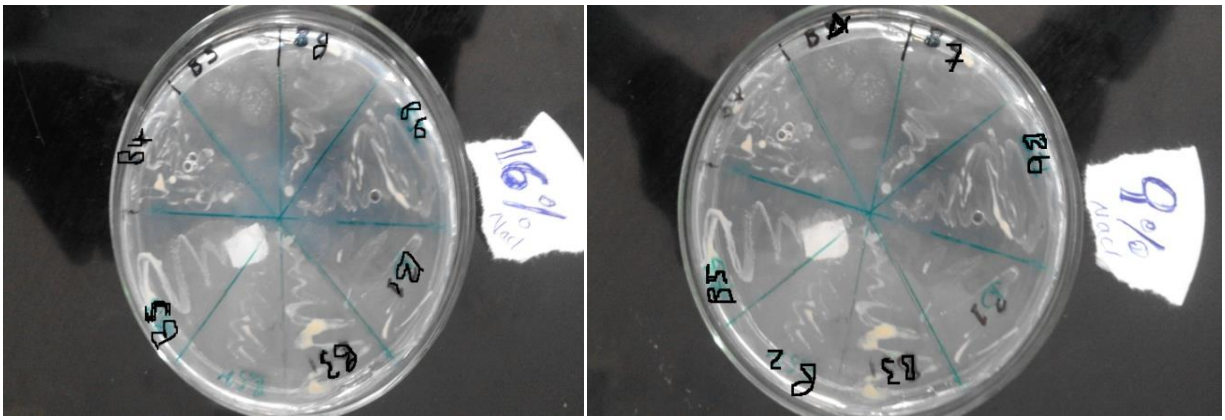
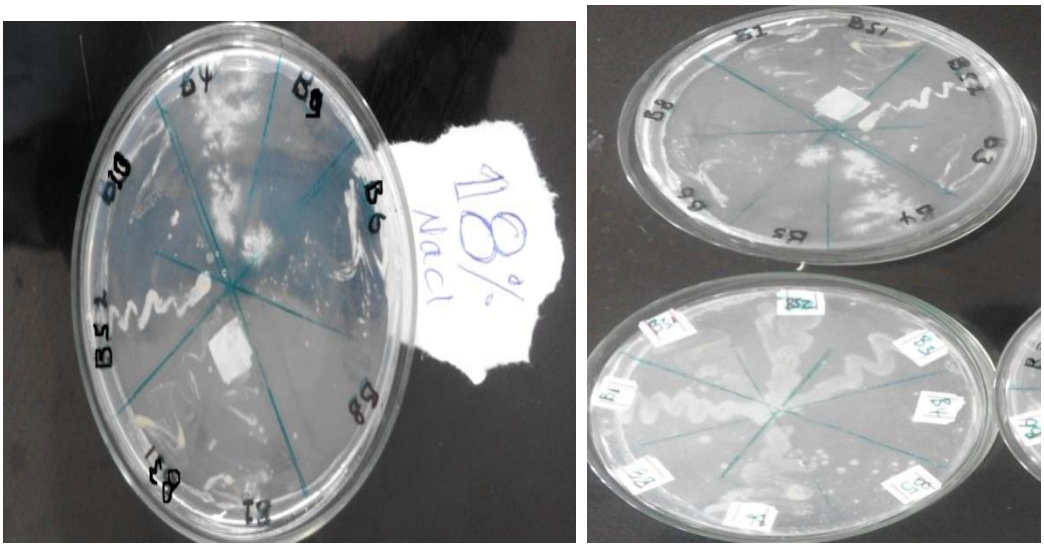
APPENDEX

Fig 1: Pure culture of *Bacillus* isolates





Figur 2: Growth of *Bacillus* isolates at high salt concentration

A.B3 (*B.subtilis*) B. B10 (*B.cereus*) C. B4 (*B.mycoides*)

Fig 4: Optical density of the isolates

Declaration

We, the undersigned, declare that this scientific research paper is our own original work. All sources of materials, ideas and grant used for this research have been duly acknowledged.

Name of student with signature and date

Name of Advisor with signature and date