Jimma University

College Of Natural Sciences,

Department Of Biology

Isolation and Characterization of Phosphate Solubilzing Rhizobacteria from Maize (*Zea maysL.*) and Sorghum (*Sorghum bicolorL.*) at Sekoru District, Jimma Zone, South West Ethiopia.

By: Shimeles T/Aregay

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Advisor; Delelegn Woyessa (Associate Professor)

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List of Abrivations

ATP-Adenosine triphosphate

MPS – Mineral phosphate solubilization

FAO- Food association organization

PGPB- Plant growth-promoting bacteria

PGPM s– Plant growth promoting micro-organisms

PSB – Phosphate solubilizing bacteria

PSMs- Phosphate solubilizing micro-organisms

PVK-Pikovskaya agar

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Abstract

Phosphorus is one of the major essential macronutrient for plants. It is applied to the soil in the form of phosphatic fertilizer. The bioavailability and solubility of inorganic and soil bound phosphorus can be improved by using beneficial microbes such as phosphate solubilising bacteria. The ability of these microbes to convert insoluble form of phosphorus to soluble form is important for the promotion of plant growth and productivity Thus the general objective of this study was to isolate and characterize phosphorus solubilising rhizobacteria from rhizospheres of maize and sorghum crops. Rhizosphere soil samples for the study were collected from eight (8) selected kebeles of the district using sterilized plastic bags. A total of 40 rhizosphere soil samples were collected from 25 plant samples of maize and 15 plant samples of sorghum. Twohundred(200) rhizosphere isolates were taken and screened on Pikovskaya agar media for phosphate solubilisation and 140(70%) were tested for positive (PSI) result, but only 60(30%) were failed and the morphological and biochemical features of the colonies were determined. The bacteria with good ability to solubilise phosphate had a high halo: colony ratio. The isolates colony diameter,halozone diameter and solubilisation index were presented.On the bases of their gram reaction, out of 140 isolates 65 (46.4%)had round shape G-ve bacteria while 57(40.7%) were G-ve rod shaped, similarily 8(5.7%) had round shaped G+ve and 8 (5.7%)were rod shaped G+ve bacteria.Growth of the isolates were tested by adjusting the pH and all showed growth above pH6 up to pH10 but no growth below pH5.On the bases of biochemical tests, *among the isolated strains showed positive result were HCN(111), NH_3(31), H_2S(14), motility (103), chitinase (104), catalase (131), urase (140) and KOH(126) repectively. The HCN produced by these bacteria were the only biocontrol mechanism. The biocontrol case often can produce HCN to synthesize antibiotics or cell wall degrading enzymes.So this study contains a potential reservoir of bacteria with phosphate solubilizing potentials.*

Keywords*: Isolate, Bioavalability, Rhizosphere, Halozone, Solubilizationindex, Rhizospher*

CHAPTER ONE

1. INTRODUCTION

1.1 Background of the study

Phosphorus(P) is one of the major essential macronutrient for plants. It is applied to the soil in the form of phosphatic fertilizer. Plants can recover only 10% to30% of phosphorus from the soil. Phopsphorus(P) is the second most essential macro-nutrient for plant growth, after Nitrogen. Phosphorus regulates the functions and activities of many enzymes and affects plant physiology. The deficiency of phosphorus is commonly an important limiting factor in agriculturalproduction. Therefore, massive amounts of phosphate fertilizer have been applied to optimize plant yields in the past century. However, a large portion of the phosphate fertilizer is unavailable to plants because large amounts of P rapidly immobilized and insoluble (Asea *et al*., 1988).

Bacteria,with a vital role for plant growth known as plant growth promoting rhizobacteria(PGPR) have proven potentialin enhancing production and productivity of crops (Frey *et al.* ,2009).The bioavailability and solubility of inorganic and soil bound phosphorus can be improved by using beneficial microbes such as phosphate solubilizing bacteria (Khan *et a*l., 2007). The ability of these microbes to convert insoluble form of Phosphorus to soluble form is important for the promotion of plant growth and productivity (Richard son, 2011).

Soil is replete with microscopic life forms including bacteria, fungi, actinomycets, protozoa, and algae. Of these different microorganisms bacteria are by far the most common i.e. 95%. Both the number and type of bacteria that are found in different soils are influenced by the soil conditions including temperature, moisture, and the presence of salt and other chemicals. In addition, bacteria are generally not evenly distributed in the soil. The concentration of bacteria found around roots of plants (rhizosphere) is typically much greater than the rest of the soil. This is because of the presence of nutrients such as sugars, amino acids, organic acids and other small molecules.Phosphate solubilizing bactria (PSB) can promote the dissolution of insoluble phosphorus (P) in soil, enhancing the availability of soluble P. Their application can reduce the consumption of fertilizer and aide in sustainable agricultural development*(*Sarig, 1990).

Several species of phosphate solubilizing bacteria have been identified so far. The bacteria are usually associated with rhizosphere, rhizoplane, or root tissues. Phosphate solubilizing micro-organisms, *Bacillus sp*. and *Pseudomonas sp.* and others release organic acids to the soil, useful to the bacteria. *Pseudomonas sp.* dominated in the rhizosphere of maize (Hafeez ,2006). Phosphate solubilizing micro-organisms have great significance on promoting plant- growth especially on maize and sorghum. So phosphorus is one of the most limiting macronutrients for agricultural production in many soils of the world as the overall efficiency of applied fertilizer can be less than 10% (Baligar *et al.,* 2001).

According to Zhang (2013) many PSB strains have been isolated at the beginning of the last century, including, *Agrobacterium, Bacillus, Pseudomonas, Erwinia, Serratia,Flavobacterium, Enterobacter, Micrococcus, Azotobacter,Bradyrhizobium, Salmonella, Alcaligenes, Chromobacterium, Arthrobacter, Streptomyces, Thiobacills, and Escherichia.* These bacteria have the capability of colonizingin the plant rhizosphere and thus play an active role inpromoting plant growth; therefore these bacteria are alsotermed as plant growth promoting rhizobacteria (PGPR) (Hungria*et al.,* 2010*).*

In Ethiopia, the effects of plant growth promoting rhizobacteria (PGPR) on growth and yield of teff were evaluated by Woyessa and Assefa (2011). Furthermore, the effect of phosphate solubilizing fungus on growth and yield of teff was studied by Hailemariam (1993). However, information on the diversity of phosphate solubilizing bacteria inhabiting various rhizospheres in this region is limited. The present study was designed to isolate and characterize the phosphate solubilizing bacteria from various rhizospheric soils.

The use of phosphate solublizing bacteria as inoculants increase P uptake by plants and crop yield strains from the genera *Pseudomonas, Bacillus,* and *Rhizobium* are among the most powerful phosphate solubilizers. So there are considerable populations of phosphate-solubilzing bacteria in soil and in plant rhizosphere. Several studies have shown that phosphate-solubilizing bacteria interact with vascular arbuscular mycorrhizae by releasing phosphate ions in the soil. It is likely that phosphate solubilized by the bacteria could be more efficiently taken up by the plant through a mycorrhizae-mediated bridge between roots and soils that allows nutrient translocation from soil to plants.

1.2 Statement of the problem

Several species of phosphate-solublizing bacteria have been identified so far includes *Pseudomonas, Bacillus, Rhizobium, Bradyrhizobium, Berkholdera.* The bacteria are usually associated with rhizosphere, rhizoplane, or root tissues. Currently, attempts have been made to identify new phosphate-solublizing bacteria with a better capacity from many sources to improve agricultural land and crop productivity. Most phosphate-solublizing bacteria are highly specific to host plant, and host specificity in bacterial colonization is an important factor for success bio-fertilization. However, some phosphate solublizing bacteria have rather wide host range. *Pseudomonas sp.* dominated in the rhizosphere of maize *(* Hafeez , 2006) .

The subsistence farmers in our local area or in Ethiopia in general depend on chemical fertilizers or inorganic fertilizers for their crops and vegetables. The cost of living by this time is very high and people are suffered with buying inorganic fertilizer with high costs. There is also lack of knowledge using animals manure or compost. So there is a big knowledge gap in our country in general and in my local area or district in particular. To fill this gap of related work in my study area needs to be researched or studied.The main objective of this research was to isolate, characterize and evaluate phosphate solubilization activity of micro-organisms in maize and sorghum rhizosphere soil to manage soil microbial communities and to select potential microbial inoculants. So many researches were done before in different areas of the world on this topic on the significance of phosphate solubilizing rhizobacteria on crops production and promoting plant-growth..

1.3 Significance of the study

Since phosphorus solubilizing rhizobacteria have these much significance for plant growth and productivity, it is important to study and isolate these micro-organisms. So this study has great significance in isolating and characterizing these phosphorus solubilzing rhizobacteria.The aim of this study was to isolate and characterize phosphate solubilizing rhizobacteria from the rhizosphere of maize and sorghum crop plants at the eight selected kebeles of Sekoru district. Collected soil samples were lebeled as JuD, Juchop, JuW, JuGeng, JuBore, JuEnk, Juchala, and JuAlga.The representative colonies showing different morphology was randomly picked up using the spreading method on nutrient agar plate. A total of 200 bacterial isolates were obtained

from the rhizosphere soil. On screening using PVK agar 140 isolates form clear zone and the SI index was calculated.

According to Khan *et al., (*2007) the bio-availability and solubility of inorganic and soil bound Phosphorus can be improved by using beneficial microbes such as phosphate-solubilizing bacteria. This research is also recommended concerned bodies for further investigations. As agriculture is the backbone of our country Ethoipia, it is the people of Ethiopia especially farmers in Jimma zone of Sekoro district will be benefitted from this research study. So by using beneficial microbes as phosphate-solubilizing bacteria , it is possible to increase the availability of nutrients in the soil.

1.4. Objectives of the Study

1.4.1 General objectives

The general objective of this study was:

- To isolate and Characterizephosphate solubilizing rhizobacteria from maize and sorghum crops at sekoru district, Jimma zone.

1.4.2 Specific Objectives

The specific objectives of this study were

- To isolate and characterize potential phosphate solubilizing bacteria from resospheres of sorghum and maize plants grown in agricultural fields of selected kebeles in sekoru district on the basis of morphological and biochemical features.
- To dentify the plant growth promoting characteristics of the isolates.
- To evaluate the phosphate solubilization characteristics of plant growth promoting rhizobacteria from sorghum and maize.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Mecchanism of phosphate solubilization

According to Chean *et al. (*2006) phosphate solubilizing bacteria solubilize inorganic soil phosphate such as Ca_3 (PO4)2, $FePO_4$, and $AlPO_4$, through the production of organic acids, siderphores, and hydroxyl. PGPB in agricultural soils is among the different sources of P in the soil. Many PSB strains have been isolated at the beginning of the last century, including *Agrobacterium, Bacillus, Pseudomonas, Erwinia, Serratia, Flavobacteria, Enterobacter, Micrococcus, Azetobacter, Bradyrhizobium, Streptomyces, Thiobacillus , and Escherichia* (Zhang, 2013). These bacteria have the capability of colonizing in the plant rhizosphere and thus play an active role in promoting plant growth; therefore these bacteria are also termed plant – growth promoting rhizobacteria (PGPR) (Hungria *et al.,* 2010).

Phosphorus is an essential nutrient for plants. It is a structural component of nucleic acids, phospholipids and adenosine triphosphate (ATP), as a key element of metabolic and biochemical pathways. Plants absorb P in two soluble forms: the monobasic ($H_2PO_4^{-}$) and the dibasic (HPO_4^{2-}). However, a large proportion of P is present in insoluble forms and is consequently not available for plant nutrition. Low levels of P reflect the high reactivity of phosphate with other soluble components (Khan *et al.* 2009*).*According to Khan *et al. (*2007) phosphate solublizing bacteria and fungi constitute approximately 1__50% and 0.1__0.5% respectively, of the total population of cultivable micro-organisms in the soil. The bacteria may affect plants in one of the three ways. The interaction between soil bacteria and plants may be beneficial, harmful or neutral.

The plant growth-promoting bacteria (PGPB) belongs to a beneficial and heterogeneous group of micro-organisms that can be found in the rhizosphere, on the root surface or associated to it , and are capable of enhancing the growth of plants and protecting them from disease and abiotic stresses (Dimpka etal. 2009a). Several phosphate solubilizing bacteria have been isolated from the roots and rhizospheric soil of various plants (Ambrosinic *et al*., 2012*).*

The mechanism by which PGPB stimulate plant growth involve the availability of nutrients originating from genetic process, such as biological nitrogen fixation and phosphate

solubilization, stress alleviation through the modulation of ACC deaminase expression, and production of phytormones and siderophores. Among several other interactions between plants and bacteria occur through symbiotic, endophytic or associative processes with distinct degrees of proximity with the roots and create a favorable environment for development and function. Non-symbiotic endophytic relationships occur within the intracellular spaces of plant tissues, which contain high levels of carbohydrates, amino acids and inorganic nutrients (Bacon *and* Hinton,2006).

The influence of bacteria in the rhizosphere of plants is largely due to the production of auxin and phytohormones (Spaepen *et al.*, 2007). Several bacterial species can produce indol-3-acetic acid (IAA), which present great physiological relevance for bacterial-plant interaction, varying from pathogenesis to phytostimulation (Spaepen *et al., 2*007).

According to Frey *et al. (*2009) the use of micro-organism is an important practice in agriculture for improving nutrients availability of plants. As phosphorus is one of the major essential macronutrients of plants, which is applied to soil in the form of phosphatic fertilizer. However, a large portion of applied phosphorus is rapidly immobilized, rendering it unavailable to plants (Glic*k*, 2010).

The plants can recover only few amount of P from the soil, while the remaining is accumulated and fixed by the formation of complexes with iron(Fe) and Aluminium (Al) in acidic soil or with calcium(Ca) and magnesium(Mg) in alkaline soils(You *et al.* , 2005). The availability of Phosphorus to plant roots and increasing P-mobilization in soil can be improved by soil micro-organisms. The ability of these microbes to convert insoluble forms of Phosphorus to soluble form is important for promotion of plant growth productivity (Richard son, 2011). With regard to plant health and promotion, the commonly found endophytes such as bacteria and fungi are very important (Neal, 2012*).*

Phosphorus is one of the major macronutrients for biological growth and development. Micro-organisms play a central role in the natural phosphorus cycle. This cycle occurs by means of cyclic oxidation reduction of phosphorus compounds. The concentration of soluble P in the soil is very low. The biggest reserves of phosphorus are rocks and other deposits. Most agricultural soils contain large reserves of phosphorus. However, a large portion of soluble inorganic phosphorus applied to soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants. A second major component of soil P is organic matter.

Organic forms of P may constitute 30%__50%, of the total phosphorus in most soil is (5%__95%). Organic P in the soil is largely in the form of inositol phosphate (soil phosphate). It is synthesized by micro-organisms and plants and is the most stable forms of P in soil.

2.1.1 Solubilization of mineral phosphates

It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms (Halder *et al.,* 1990). Production of organic acids results in acidification of the microbial cell and its surroundings. Consequently, Pi may be released from a mineral phosphate by proton substitution for Ca21 (Goldstein, 1994).

The production of organic acids by phosphate solubilizing bacteria has been well documented.

Among them, **gluconic acid** seems to be the most frequent agent of mineral phosphate solubilization. It is reported as the principal organic acid produced by phosphate solubilizing bacteria such as *Pseudomonas* sp., *Erwinia herbicola,Pseudomonascepacia* and *Burkholderia cepacia* (Rodriguez et al., unpublished results). Another organic acid identified in strains with phosphate-solubilizing ability is **2-ketogluconic acid**, which is present in *Rhizobium leguminosarum,Rhizobium meliloti,Bacillus firmus*, and other unidentified soil bacteria (Duff *and* Webley, *1959)*. Strains of *Bacillus liqueniformis* and *Bacillus amyloliquefaciens* were found to produce mixtures of lactic, isovaleric, isobutyric, and acetic acids. Other organic acids, such as glycolic, oxalic, malonic, and succinic acid, have also been identified among phosphate solubilizes (Illmer and Schinner, 1992).

There is also experimental evidence that supports the role of organic acids in mineral phosphate solubilization *(*Halder *et al.,* 1990) showed that the organic acids isolated from a culture of *Rhizobium leguminosarum* solubilized an amount of P nearly equivalent to the *H. Rodríguez, R. Fraga* that was solubilized by the whole culture. Besides this, treatment of the culture filtrates from several *Rhizobium* strains with pepsin or removal of proteins by acetone precipitation did not affect phosphate release capacity, showing that this was not an enzymatic process. However, neutralization with NaOH destroyed the solubilization activity (Halderand Chkrabartty, 1993). Based on these findings, following the cloning of mineral phosphate solubilization genes, *(*Goldstein1994 *and* Goldstein, 1995) has proposed that the direct periplasmic oxidation of glucose to gluconic acid, and often 2-ketogluconic acid, forms the metabolic basis of the mineral phosphate solubilization phenotype in some Gram negative bacteria.

Alternative possibilities other than organic acids for mineral phosphate solubilization have been proposed based on the lack of a linear correlation between PH and the amount of solubilized P (Ehrlich H,1990, Thomas , 1985,*and* Aseaa *et al.,* 1988). In addition, no significant amounts of organic acid production could be detected from a phosphate solubilizer fungus, *Penicillium* sp. (Illmer and Schinner, 1992). Studies have shown that the release of H1 to the outer surface in exchange for cation uptake or with the help of H1translocation ATPase could constitute alternative ways for solubilization of mineral phosphates.

2.1.2 Mineralization of organic phosphorus

Organic phosphate solubilization is also called mineralization of organic phosphorus, and it occurs in soil at the expense of plant and animal remains, which contain a large amount of organic phosphorus compounds. The decomposition of organic matter in soil is carried out by the action of numerous saprophytes, which produce the release of radical orthophosphate from the carbon structure of the molecule. The organo-phosphonates can equally suffer a process of mineralization when they are victims of biodegradation (McGrath *et al.* 1995). The microbial mineralization of organic phosphorus is strongly influenced by environmental parameters; in fact, moderate alkalinity favors the mineralization of organic phosphorus (Paul and Clark, 1988).

The degradability of organic phosphorous compounds depends mainly on the physicochemical and biochemical properties of their molecules, e.g. nucleic acids, phospholipids, and sugar phosphates are easily broken down, but phytic acid, polyphosphates, and phosphonates are decomposed more slowly (Ohtake *et al.,* 1996, *45* McGrath *et al.,* 1995,McGrath *et al.,* 1998).

The mineralization of these compounds is carried out by means of the action of several phosphatases (also called phosphohydrolases). These dephosphorylating reactions involve the hydrolysis of phosphoester or phosphoanhydride bonds. The phosphohydrolases are clustered in acid or alkaline. The acid phosphohydrolases, unlike alkaline phosphatases, show optimal catalytic activity at acidic to neutral pH values. Moreover, they can be further classified as specific or nonspecific acid phosphatases, in relation to their substrate specificity. Rossolini *et al.* , (1998) recently published a comprehensive review of bacterial non-specific acid phosphohydrolases. The specific phosphohydrolases with different activities include:

39-nucleotidases and 59-nucleotidases (Burns and Beacham, 1986); hexose phosphatases (Pradel and Bouquet ,1988)*; and phytases (*Cosgrove *et al.,* 1970).

2.3 Phosphate-solubilizing bacteria as plant growth promoters

Although several phosphate solubilizing bacteria occur in soil, usually their numbers are not high enough to compete with other bacteria commonly established in the rhizosphere. Thus, the amount of P liberated by them is generally not sufficient for a substantial increase in in situ plant growth. Therefore, inoculation of plants by a target microorganism at a much higher concentration than that normally found in soil is necessary to take advantage of the property of phosphate solubilization for plant yield enhancement. There have been a number of reports on plant growth promotion by bacteria that have the ability to solubilize inorganic and/or organic Phosphorus from soil after their inoculation in soil or plant seeds Kloepper *et al.*,1988,Gaur *and* Ostal,1972Subba Rao,1982,Kucey *et al.*,1989. The productions by these strains of metabolites beneficial to the plant are phytohormones, antibiotics, or siderophores.

According to Mohd and Taq*i (*2008) considerably higher concentrations of phosphate solubilizing bacteria are commonly found in the rhizosphere in comparison with non -rhzosphere soil. After screening phosphate solubilising bacterial isolates obtained, which were further screened based on the halo: colony ratio using the spot inoculation method. The halo: colony ratio is the ratio of colony diameter and clear zone diameter of bacteria in culture medium. The clear zone can appear at the surface of the medium and the bottom of the medium. The bacteria with good ability to solubilise phosphate should have a high halo: colony ratio. This criterion is generally used for preliminary screening of phosphate- solubilising micro-organisms including fungi and bacteria.

According to Raghothama, *(*200*)*inorganic phosphate is one of the major nutrients in plant nutrition. Phosphorus, the second most important macro-nutrient, next to nitrogen, plays an important role in transfer of high energy, cell division, photosynthesis, and biological oxidation, metabolism for growth, reproduction and nutrient uptake in plants. Phosphorus occurs in fully oxidized state as phosphate, but invariably forms a large number of insoluble chemical complexes with calcium, iron and aluminium, forming insoluble phosphate salts present in the soil, which indeed makes this nutrient a paradox. Certain group of higher plants evolved highly efficient mechanisms for absorbing phosphate even from very dilute solutions and achieve the maximum growth. Some other plants have adapted to phosphate limiting conditions by secreting organic acids that facilitate the release of phosphates from inorganic ion complexes. However,

this impressive capability shown only by a limited group of plants is not enough to allow maximizing agronomic productivity. It is recognized that the availability of phosphate in soils is a major factor limiting the productivity of many ecosystems (Daniels *et al.*, 2009).

Application of phosphatic fertilizers, therefore, has been considered essential for agronomic levels of crop production in most agro-ecosystems. As most of the plants are unable to utilize the phosphate in these bound forms, farmers are advised to apply four times the phosphate requirement of a particular crop. This over fertilization often leads to an imbalance of nutrients in the soil and is one of the major environmental concerns. There has been a continuous search for viable alternatives to the chemical phosphate fertilizers (Goldstein,1986*).*

2.4 Phosphate-solubilizing micro-organisms (PSMs)

Numerous soil micro floras were reported to solubilise insoluble phosphorous complexes into solution and make it possible for its use by the plant. Several groups of fungi and bacteria, popularly called as phosphate-solubilising micro-organisms (PSMs) assist the plants in mobilization of insoluble forms of phosphate. PSMs improve the solubilisation of fixed soil phosphate, resulting in higher crop yields, and therefore are used as bio-fertilizers. A significant increase in the grain yield was observed for rice, chickpea, lentil, soybean, cow pea and also an increase in the phosphate uptake in the potato tubers was observed when *Pseudomonas striata, Aspergillus awamori and Bacillus polymyxa* were used either alone or in combination. Microbial solubilisation of inorganic phosphate compounds is of great economic importance in plant nutrition (Gaur, 2002). Bacteria from genera such as *Achromobacter, Agrobacterium, Bacillus, Enterobacter, Erwinia, Escherichia, Flavobacterium, Mycobacterium, Pseudomonas and Serratia* are highly efficient in solubilizing unavailable complex phosphate into available inorganic phosphate ion. Phosphate-solubilizing bacteria use different mechanism(s) to bring about the insoluble forms of the phosphate into soluble forms. Organic acids released by the micro-organisms act as good chelators of divalent cations of Ca^{2+} accompanying release of phosphates from insoluble phosphatic compounds (Goldstein, 2001).

2.5 Products of direct oxidation of glucose

The mineral phosphate solubilzation (MPS) role in weathering and solubilisation of phosphate in soil and as a major phosphate-solubilizing compound released by rhizobacteria growing in wheat

roots supporting the role of the direct oxidation pathway in the MPS phenotype (Moghimi *et al.*, 1978).

2.6 **Fungi as plant growth promoter**

Exposure of plants to continually changing environmental conditions had adapted them to develop symbiotic associations with such microbes that can help in their protection and survival, or succumb to selective pressures such as extreme temperatures, insufficient water and toxic chemicals. Plants have thus evolved complex biochemical/genetic systems to perceive stresses, transmit stress-activated signals to different tissues and activate cellular responses to avoid detrimental effects (Rodriguez *et al.*, 2004). Most plant studies do not consider the fact that plants in natural ecosystems have symbiotic associations with fungi. These fungi are important to the structure, function, and health of plant communities (Rodriguez & Redman, 1997; Clay & Holah, 1999, Hong *et al*., 2011). In fact, symbiotic fungi contribute to and may be responsible for the adaptation of plants to environmental stresses (Morton, 2000; Redman *et al.,* 2002). These fungi express a variety of symbiotic life styles including mutualism, commensalism, and parasitism (Lewis, 1985). Endophytes constitute a major portion of fungal symbionts associated with plants, reside entirely within plant tissues, and may be associated with roots, stems and/or leaves. These fungi can act as defenders against predators (Siegel & Bush, 1997), growth promoters (Bacon & White, 2000) and competitors of microbial pathogens (Scannerini *et al.*, 2001). According to some researchers, the vegetative growth enhancement shown by many grass species in the presence of their fungal symbionts has been principally attributed to increased plant fitness (Belesky & Malinowski, 2000; Hill *et al*., 1996). However, recent studies have shown that plant growth promotion may be attributed to the secretion of plant growth promoting secondary metabolites (gibberellins, auxin, cytokinin) by the endophytic fungi in the rhizosphere (Hamayun *et al*., 2010a). During current study an effort was made to investigate the role of indigenous endophytic fungi in the growth promotion of the sand-dune flora and their possible role in any future conservation strategy.

2.7 Economic importance and nutritional value Of Maize and Sorghum

Maize

Nutritional composition:

Pollens and seeds are the nutritious and edible parts of maize. Seeds are consumed in raw and cooked form that serves as good source of carbohydrates. Corn contain

Vitamin B-complex such as B1 (thiamine), B2 (niacin), B3 (riboflavin), B5 (pantothenic acid) and B6 that makes it commendable for hair, skin, digestion, heart and brain. It contains vitamin C, A and K together with large amount of beta carotene and fair amount of selenium that helps to improve thyroid gland and play important role in proper functioning of immune system. It has higher content of protein and fat as compared to other cereals.

Medicinal value:

From the ancient time corn has been used to pacify kapha, pitta, anorexia, general debilities, emaciation and haemorrhoids. It is a potent antioxidant that guards body from harming by free radicals responsible for cellular damage and/or cancer. It has the potential to alleviate pain and possess analgesic activity as well (Owoyele et al., 2010). Helping production of sex related hormones assemble it good for sexual health especially for men with erectile dysfunctions. It is believed to improve symptoms of rheumatism as B-complex is able to improve joint motility.

Major nutrient of corn silk is potassium that is powerful diuretic. It acts as a good emollient for ulcer, wound and swelling. In some places decoction of corn silk and parched corn is extremely useful in nausea and vomiting.

Economic Importance

The oil present in corn (rich in embryo) is far and wide used for cooking and manufacture of soaps. Sticky gum contains dextrin used for sealing envelopes and labels. Corn starch is well recognized for its uses in cosmetics and pharmaceutical industries as diluents. Corn seeds are functional in making alcohol and stem fibers for manufacture of paper

Sorghum

Sorghum (*Sorghum bicolor* L.) is an important rain fed crop, grown globally on 41.9 million ha. In India, sorghum is grown in a wide range of environments. It occupies around 7.7 million hectares especially grown in semi-arid regions of the country producing 7.0 million metric tons of grain (FAO). Sorghum is the fifth most economically important cereal crop in the world after wheat, maize, and barley (Dengel, 1991).

Nutritional composition

The composition of sorghum grain is similar to that of maize or other cereal grains. However, the perceived poor nutritional and processing quality of sorghum is because of the presence of tannins and poor protein digestibility, which affects its use in food and feed. The protein quality of sorghum grain is poor because of the low content of essential amino acids such as lysine tryptophan, and threonine Sorghum is poorly digested by infant.Sorghum is a rich source of various phytochemicals including phenolic compounds, anthocyanins, phytosterols, and policosanols that are secondary plant metabolites or integral cellular components.

Economic importance

Sorghum is used for two major purposes: human food and animal feed. In the early 1960s a significant portion of the sorghum output was used directly as human food. However, consumption of sorghum for food purposes has been declining since then, whereas consumption of sorghum as animal feed has more than doubled. Sorghum is consumed in a number of forms depending on the part of the world concerned. Generally, whole grain is processed into flour, from which traditional meals are prepared. Main sorghum-based foods are: unleavened bread prepared from fermented or unfermented dough in Asia and parts of Africa; thin or thick fermented or unfermented porridge, mainly consumed in Africa; and boiled products similar to those prepared from rice or maize grits. In India, sorghum is utilized in the preparation of many traditional foods and in bakery products such as bread, cakes, and biscuits.

The demand for sorghum for feed is concentrated in the developed countries, where animal feed accounts for about 97 % of total use, and in some higher-income developing countries, especially in Latin America where 80 % of all sorghum is utilized as animal feed (FAO 1995).

Alternative uses of sorghum involve utilization of the grain and sweet stalk in food and nonfood sectors for production of commercially valued products such as alcohol, syrups ,glucose , modified starches, maltodextrins, jaggery, sorbitol, and citric acid.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Description of the study area

The study was conducted in Sekoru district, Jimma zone of Oromia regionalstate, Ethiopia. Jimma Zone is located at 353km Southwest of Addis Ababa. The total area of Jimma zone is 18415km^{2}and located between latitudes 7º18´N and 8º56´N and longitudes 35º52´E and 37º37´E.Jimma town is the capital of Jimma zone and is located 353 km far from Addis Ababa, Ethiopia. Sekoru district is one of the 'woredas' of Jimma zone and found at 100 km East of Jimma town. The latitude of the area is from 900m__2,300m, longitude 37º 16'-37º-37'E. The general climate of the ′woreda′ higher 28.3 ºc, lower 12.1ºc (ave.20.3ºc) and the annual rainfall is 1458mm. The agro-ecology of the district is: ′ woina dega′ (60%), ′dega′ (20%) and′ kola ′ (10%).

The major economic activities and the types of crops grown in the area are maize, sorghum, teff, sea sam (selit), niger seed (nug), cash crops like mango, orange, papaya, avocado, apple, coffee, are the examples. In animal husbandry cow, sheep, goat, mule, horse, donkey, chickens and so on. There is also beekeeping activities in the area using modern and traditional beehives. In addition there are also vegetables grown in different kebeles like green peppers, potatoes, tomatoes, sweet potatoes, beet roots, and carrots and so on.

3.2 Study design

A cross sectional study design was conducted for this study.

3.3 Sampling techniques

A total of 40 rhizosphere soil samples were collected from 25 plant samples of maize (*Zea mays L.)* and 15 plant samples ofSorghum(*sorghum bicolor* L.) The rhizosphere soil samples were collected from eight selected kebeles of Sekoru District (Chopa, Deneba, Welmera, Enkure, Gengeleta, Algae, Chala and Bore) agricultural fields and the reason for selecting the kebeles is as they are maize growing area of the district. Roots with adhering soils of healthy plants were collected and transferred to sterile plastic bags. Soil samples at a depth of 20 to 30cm were dug and collected from these areasat a distance of 10m to 15m distances.The study was conducted by

using cross-sectional study design and the rhizosphere soil samples were collected from each farm lands.

3.4 Soil sample collection

Rhizosphere soil samples were collected from eight selected areas or kebeles of the district using sterilized plastic bags. All of the laboratory samples were collected by the principal researcher from the different farming areas or kebeles of the woreda. The researcher asks requests farmers to give maize and sorghum plant for sample. The roots of the crops contain or harbor large numbers of microbes which solubilize nutrients in the soil. The samples were taken to the post graduate andresearch laboratory, Department of Biology, Jimma University for the isolation and characterization of phosphate solubilizing bacteria from Rhizosphere soil.

3.4.1 Isolation of Phosphate Solubilizing bacteria

Soil samples from the rhizosphere of maizeand sorghum were carefully removed and ten (10) grams of soil from each sample was suspended in 90ml of sterilized distilled water to make dilution. These were shaken manually and stirred for 30minutes to break the larger particles. Then series of dilution was made from 10^{-1}- 10^{-6} and from dilutions of 10^{-4} and 10^{-5}, 0.1ml of suspensions was transferred on petri dishes containing nutrient agar medium. The suspensions were spread uniformly on a petri dishes using glass rod spreader and incubated at 32ºc.

3.4.2 Plant growth promoting characteristics of the isolates:

Phosphate solubilization:

All the purified isolates were screened *in vitro* for their phosphate solubilization activity following the method described by Donate-Correa *et al*. (2004) on Pikovskaya agar medium.

Ingredients:	in G/L
- Yeast extract	0.500
- Dextrose or Glucose	10.000
- Calcium phosphosphate ($Ca(PO4)_2$)	5.000
- Ammonium Sulphate $(NH_4)_2SO_4$	0.500
- Potassium Chloride(KCl)	0.200
- Magnesium Sulphate(MgSO4.7H2O)	0.100
- Manganese Sulphate(MnSO4)	0.0001
- Ferrous Sulphate(FeSO4)	0.0001
- Agar	15.00
- Distilled water	1000ml

The medium was autoclaved at 121°C for 15 minutes. About 20 ml of the moltenPVK medium was poured into each Petri dish and allowed to solidify before inoculating the isolates. A 24 hr. broth culture was streaked or dotted on the Petri dishes using sterile loop and incubated at 32 ± 2°C for 5-7 days. Bacterial colonies that formed clear zones (haloes) were considered as phosphate solubilizes and clear zone diameters were measured in cm. The phosphate solubilization index was determined by the formula (Premono *et al*., 1996).

$$\text{Solubilization Index (SI)} = \frac{\text{Colony diameter + halo zone diameter}}{\text{Colony diameter}}$$

3.4.3 Characterization and identification of the isolates

3.4.3.1 Morphological and biochemical characteristics

Fluorescent pigment production

Kings B agar medium was used for this experiment (King *et a*l., 1954) containing: Mixed peptone (20g), K_2HSO_4 (1.5g), $MgSO_4.7H_2O$ (1.5g), Glycerol (10ml), Agar (10g), Distilled water (1000ml) PH (7.2). About15ml of the medium was distributed in a petri plate Autoclaved at 121 ºc and pressure of 15pi for 15min. Then each isolates were inoculated in to a cold medium and incubated for 5days. Then the diffusing green pigment was observed using UV-light adjusted at 203nm.

Gram staining

The smear was prepared from 1-2 drops of culture on clean slide and heat fixed. 1-2 drops of crystal violet solution was applied on the fixed smear for 1 min and then washed with sterile distilled water. Gram's iodine solution was applied for 1 min and then washed with 95% alcohol. Finally stain the smear with counter stain safranin for 30 seconds again washed with sterile distilled water. The smear was air dried and examined under light microscope by using oil immersion. The Gram positive bacterial cells appeared violet while gram negative bacteria turned from pink to red (Gram, 1884).

Growth of the isolates at different pH ranges

At different pH values (4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0) on nutrient agar medium was assessed and the pH was adjusted using concentrated solutions of 0.1 N HCl and 0.1 N NaOH

before autoclaving. Each of the sterile nutrient agar plate was streaked with a loop full of 24 hr old culture grown in nutrient broth and incubated at 32 ± 2°C. Finally growth was recorded as (+) or (-) for growth and no growth respectively.

3.4.3.2 Biochemical Characterization of the isolates

***Hydrogen cyanide production*:**

Hydrogen cyanide (HCN) production from glycine was tested following the procedure of Lorck (1948). Tryptic soya agar (TSA) supplemented with glycine (4.4g L^{-1}) was prepared and autoclaved. A loop full of 24hr old broth culture of the isolates was inoculated to the plates. Sterile filter paper strips soaked with 0.5% picric acid was fixed to the underside of the Petri dish lids. The plates were then sealed with parafilm and incubated for 7 days at 32±2ºc. An uninoculated plate and an inoculated plate without picric acid impregnated papers were used as controls. A change in the color of the filter strips from yellow to brown or reddish-brown was regarded as an indication of cynogenic potential.

Ammonia production

Peptone broth was prepared and bacterial culture was inoculated in it. The broth was incubated for 24 hours in test tubes. Bacterial isolates were tested for ammonia production in peptone water using Nessler's reagentcotainnig KI(50g), $HgCl_2$ (20g) and NaOH(20ml of 5N). The production of ammonia was indicated by the color change from yellow to brown precipitate. Development of brown color was a positive test for ammonia production.

Hydrogen Sulphide Production

SIM agar medium tubes were stab inoculated by *bacterial* isolatesand incubated for 24 to 48 hours at 37±2°C(Clarke, 1953).Tubes wereobserved for presence or absence of black coloration along the line of stab inoculation indicating hydrogen supplied production and their motility is observed.

Motility test

This is used to detect motility in organisms. The test organism was stabbed into a motility agar with a swab stick and incubated. Turbidity in the entire tube indicated that the organism has moved away from the stab line and thus is motile while absence of turbidity in the test tube with clear mark of the stab indicated that the organism is non motile

Production of enzymes

The isolates of *bacteria* possessing the growth promoting characteristics were further characterized biochemically through tests for production of chitinase, catalase, oxidase and urease.

Chitinase Production

For the production of chitinase, chitin agar was prepared and the enriched culture broth was inoculated on chitin agar plates containing [g\l chitin (10g), peptone ((0.5g), yeast extract ((0.5g), K_2HPO4 (0.1g) MgSO4.7H2O (0.01g), distilled water (1000ml) and incubated at 30±2ºc for 48hours. The colonies with clear zone were taken as potential chitin degrading isolates. (Buchnan and Gibbons, 1974**).**

Catalase production

The colonies were flooded with 2 to 3 drops of 3% H_2O_2 solution. Formation of bubbles of gas indicates a positive test reaction (Collins and Lyne, 1976).

Urease production

Bacteria growing naturally in an environment are exposed to the urine and with the help of enzyme Urease they decompose the urea. The plates were streaked with different bacterial cultures and incubated at 30±2°C for 4 days. The yellow to pink color in the test tubes indicated the positive result for the test.

3.5.Data Analysis

All the data gained from the laboratory results were analyzed using Microsoft excel software and results were presented in the form of tables, graphs and percentages.

CHAPTER FOUR

4. RESULTSAND DICUSSION

4.1 Results

Morphological characteristics

On the bases of their gram reaction, out of 140 isolates 65 (46.4%) had round shape G-ve bacteria while 57(40.7%) were G-ve rod shaped, similarily 8(5.7%) had round shaped G+ve and 8 (5.7%) were rod shaped G+ve bacteria.

Fluorescent pigment production

Flourescent pigment production test using Kings Bagar medium was done, then the diffusing green pigment was observed using UV- light adjusted at 203nm. A total of 98 isolates show fluorescent green pigment and only (42) of them shows non-diffuse green pigmentation (fig.1).

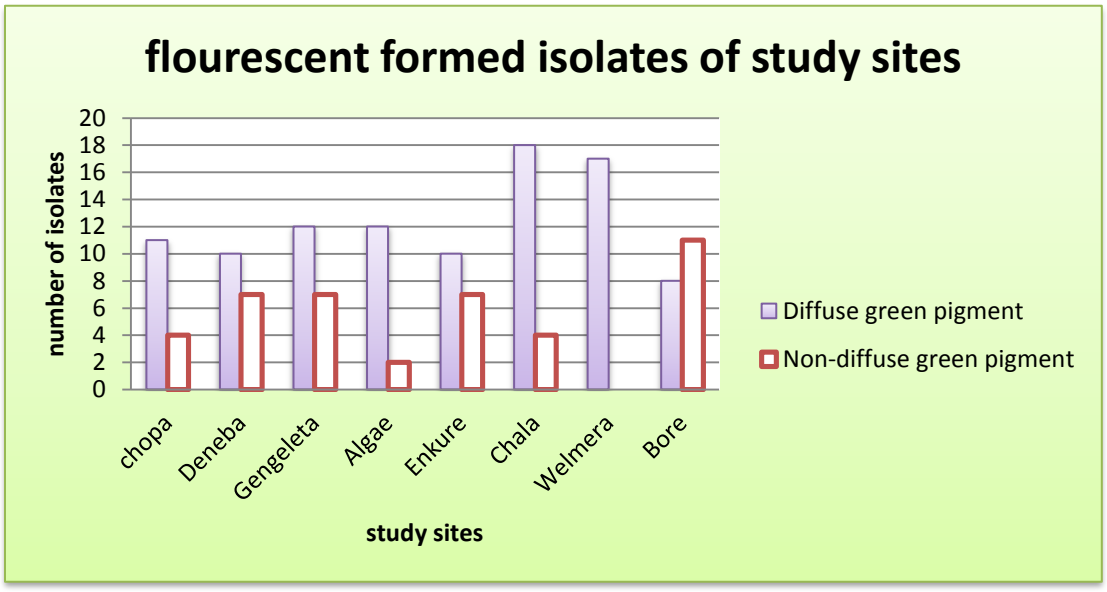
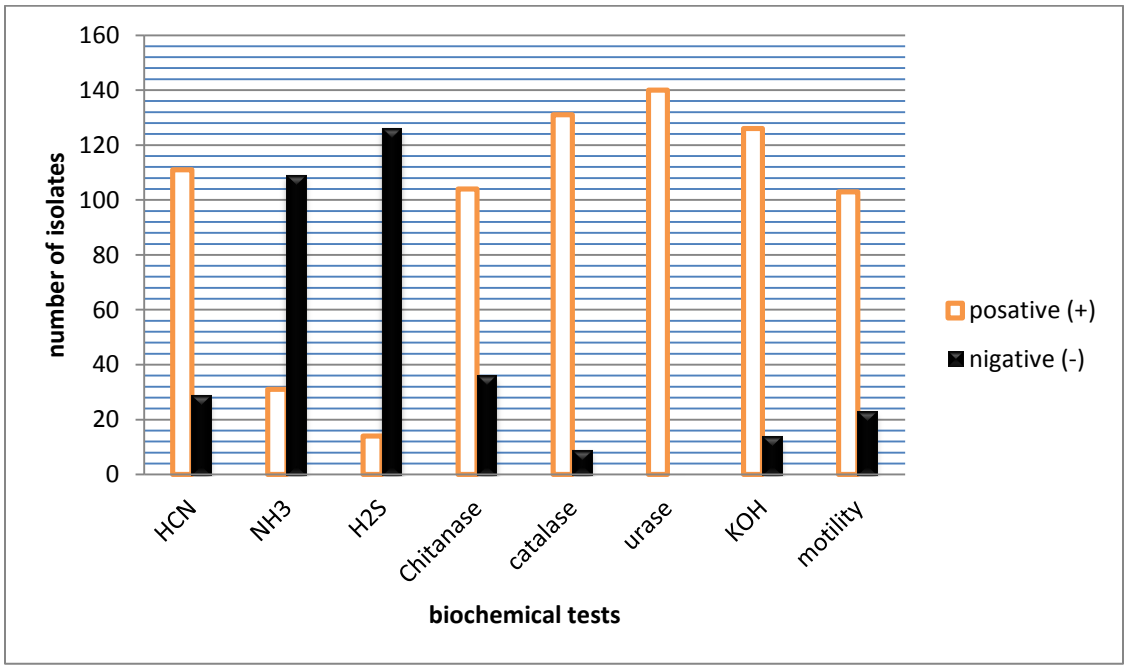


Figure 1.flourescent formed isolates of sekoru district

Biochemical characterization of the isolates

Result of qualitative assessment of the isolates for the ability to promote plant growth shows that PSB from sekoru district possess varying characteristics of plant growth promoting bacteria. Different isolates were shownthe ability to produce some PGP metabolites including; ammonia (3I), HCN(111), chitinase (104), H_2S(14), urease(140), catalase(131) , motile (103), KOH (126) fig.2.

Figure 2.Qualitative assessment of plant growth promoting traits of PSB isolated from sekoru district.



Phosphate solubilizing activity

The ability of bacterial isolates from plant rhizosphere soil to solubilize phosphorus was qualitatively enumerated and the results were presented in (Table 1)in the form of solubilization index. Out of the 200 bacteria isolated from rhizosphere soil sample, only 140(70%) showed the ability to solubilize phosphorus, thus were tagged Phosphate Solubilizing Bacteria (PSB). According to this study, the isolates exhibited varying solubilization index ranging from 1-5. Apart from the solubilization index exhibited by the isolates from Chala and Gengeleta 3 (PSI) were 12 for each, which is significantly higher indexes than other isolates. The Phosphate

Solubilization index for majorities (47.10%) of bacteria isolates were 3 (PSI) and followed by 2.5 (PSI) which accounted for (25.36%) respectively. Colony diameter, halo zone diameter and solubilization index for effective isolates were presented in (annex 2). The highest colony diameter was recorded by the isolates JUD4d, and JUEnk1d. The smallest colony diameter werealso recorded by isolates JUEnk3c, JUEnk4c and JUEnk5d. The highest halo zone diameter was recorded by the isolates JUChala4d and JUD4d, however the smallest halo zone diameter was recorded by the isolate JUEnk5d. The smallest solubilization index (1) was recorded by isolate JUD1d (annex 2).

Table 1.Results of Phosphate Solubilization Index (PSI) of bacteria isolated from sekoru district

	FREQUENCY OF ISOLATES									
Result for PSI	DENEBA	CHOPA	Algae	GENGELETA	BORE	ENKURE	CHALA	WALMERA	TOTAL NUMBER	%
1	1	0	0	0	0	0	0	0	1	0.724638
1.5	0	0	0	0	0	1	0	0	1	0.724638
2	1	0	0	0	3	4	0	0	8	5.797101
2.1	1	0	1	0	0	0	0	0	2	1.449275
2.3	2	0	0	0	1	0	1	0	4	2.898551
2.5	5	3	5	6	11	1	0	4	35	25.36232
3	4	8	6	12	4	10	12	9	65	47.10145
3.5	0	1	0	0	0	0	1	0	2	1.449275
4	2	1	0	0	0	0	3	2	8	5.797101
4.5	0	1	0	0	0	1	0	0	2	1.449275
5	0	1	2	1	1	0	5	2	12	8.695652
TOTAL	16	15	14	19	20	17	22	17	140	101.4493

The growth of the isolats at different pH was checked by adjusting the nutrient agar media in pH of 4.0,5.0,6.0,7.0, 8.0,9.0 and 10.0. All the isolates show growth in pH of 6.0, 7.0,8.0, 9.0, 10.0. But no growth below pH of 5.0 (table 2).

Table 2.Growthof the isolates at different pH vlues

Total number of isolates	pH 4.0	pH 5.0	pH 6.0	pH7.0	pH8.0	pH9.0	pH10.0
140	-	-	+++	+++	+++	+++	+++

Where; - no growth, +++ = significant growth

4.2 Discussion

In the present study, on the bases of their Gram-staining or Gram-reaction, out of 140 isolates:65(46.4%) had round shape gram-negative bacteria, while 57(40.7%) were gram-negative rod shaped similarly 8(5.7%) had round shaped gram-positive and 8(5.7%) were rod shaped gram-positive bacteria**.**

The growth of the isolats at different pH was checked by adjusting the nutrient agar media in pH of 4.0,5.0,6.0,7.0, 8.0,9.0 and 10.0. All the isolates show growth in PH of 6.0, 7.0,8.0, 9.0, 10.0. But no growth below Ph of 5.0 since as pH decreases and acidity increases. This studyshows that all the isolates (100%) shows positive result in higher PH significantly (+++) (table2).

The increase in available P in solution following inoculation of the isolates was negatively correlated with pH of the media. Such associations between decreasing pH and increasing available P concentrations that have been reported previously.

Phosphate solubilizing micro-organisms occur in nature and have the ability torender insoluble phosphate compounds soluble, such as $Ca_3(PO4)_2$, FePO4 and AlPO4, through the production of organic acids, siderophores and hydroxyl ions (Chean *et al*., 2006).

In the present study two hundred(200) isolates were isolated from the 40plant samples of maize and sorghum crop plants and phosphate solubilizing bacteria (PSB) were isolated from rhizosphere soil by using serial dilution method. On screening of the isolates using PVK agar only 140 PSB were isolated. Out of these isolates 90 isolates were from maize and 50 isolates were from sorghum. Maize isolates were from Deneba, Chopa, Gengeleta, Welmera and Chala kebeles,and sorghumisolates were from Algae, Enkure and Bore.

Phosphate-solubilizing bacterial colonies appear in addition to the other bacterial groups. However, in the present study, populations of other bacteria were not quantified. The production of clear zones around the colonies indicates the phosphate-solubilizing ability of the bacterial strain, which is able to enumerate PSB alone. Such cultures have been isolated and the extent of phosphate solubilization is determined quantitatively by biochemical methods.

In this study from the total of 200 isolates, one handred fourty (140) isolates were positive for phosphate solubilization on solid Pikovskaya media that formed halos were screened(table1). Out of the positive isolates, JUG4a isolate was effective having halo or clear zone >2.5cm. Halos or clear zones indicating phosphate dissolution by bacterial isolates were measured in cm on PVK media. Colony diameter, halo zone diameter and solubilization index for effective isolates are presented in(table1). The highest colony diameter was recorded by the isolates JUD4d, JUEnk1d. The smallest colony diameter were also recorded by isolates JUEnk3c, JUEnk4c and JUEnk5d. The highest halo zone diameter was recorded by the isolates JUChala4d and JUD4d, however the smallest halo zone diameter was recorded by the isolate JUEnk5d. The smallest solubilization index (1) was recorded by isolate JUD1d (annex 2).

The populations of PSB differ according to the sampling part of the plant not to the soil.The higher PSB populations were found in rhizosphere than non-rhizosphere soil. Reys *et al*.(2006) also found a significantly higher content of PSB population in the rhizosphere in comparison with non-rhozosphere or bulk soil. The PSB population(rhizosphere and endosphere) give better results in Pikovskaya media plates due to its composition.The isolation results from the eight selected kebeles of sekoru district were summarized in (tables 1and 2). Isolation results from maize and sorghum plants obtained were two hundred (200)bacterial isolates, out of these isolates only 140 (70%) isolates were screened using PVK agar forming clear zone and SI index is calculated and recorded (table1, annex2).This study and the present study were directly comparable.

Goldstein (1986) have demonstrated that inoculations with phosphate solubilizing micro-organisms can improve the phosphorus nutrition of plants. Al-P or Fe-P inacidic soils or Ca-bound P in alkaline soils are released in to the plant available P pool of rhozosphere due to the action of the PSMs. In this study this is also comparable in that, growth of the isolates in different pH shows that the growth of the isolates in acidic condtions decreases.

The primary mechanism involved in phosphate solubilization is acidification, due to organic acids produced by PGPR (Puente *et al*. 2004). All the 140 isolates used in this study formed clear zone or halos around colonies on pikovskayas agar plates supplemented with calcium phosphate as a source of inorganic phosphate. This confirms the solubilization of inorganic phosphate by bacteria.

Application of phosphatic fertilizers, therefore, has been considered essential for agronomic levels of crop production in most agro-ecosystems. As most of the plants are unable to utilize the phosphate in these bound forms, farmers are advised to apply four times the phosphate requirement of a particular crop. This over fertilization often leads to an imbalance of nutrients in the soil and is one of the major environmental concerns. There has been a continuous search for viable alternatives to the chemical phosphate fertilizers (Goldstein,1986*).*

PSB have a great ability to transform insoluble P in the soil in to anavailable form and have great application prospects for eco- agriculture.The secretion of organic acids and chelation are major P-solubilization mechanism of PSB. The presence of many PSB in the soil is an important index of effective promotion of crop growth and sustainable agricultural development (Fernandez *et.al,* 2012).The phosphate-solubilizing ability of the isolate would primarily depend upon the type and concentration of natural substrates, enzymes used, temperature, ionic strength and pH of metal ions (McComb et al., 1979). In general, phosphate solubilization seems to be linked with the decrease of the pH of the medium, but this pH decrease is not strictly proportional to the amount of phosphate solubilized. In the present study, a linear relationship was observed with the drop in pH of the culture and increase in inorganic phosphate concentration. This has been reported earlier by Chen et al. (2006).

Accordining toGoldstein (1951) | Asea *et al*. ,(1988) P is an important factor limiting agricultural production. Large amounts of P fertilizer added to soil not only increase the cost of agriculture but also cause environmental problems (Shahid *et a*l., 2012; Hanif et al., 2015; Majeed *et al*., 2015) and are not conducive to the sustainable development of agriculture. Application of P-containing fertilizers is common for stimulating crop yields. However, repeated applications of phosphate fertilizers affect environment, microbial diversity and can lead to loss of soil fertility and consequently lower crop yields (Gyaneshwar et al., 2002).

Lateef *et al*.(2015) have demonstrated that Strains of *B. safensis* were also found in diverse terrestrial and marine environments and are known for their plant growth promoting properties. Plant growth promotion by similar study, *B.subtilis, B. velezensis,* and *P.fluorescens,* were previously reported from acidic soils of coffee growing areas of Ethiopia (Diriba Muleta *et al*., 2009).

According to Chen et al. ,(2016) the colonization ability of micro-organisms on roots has a significant influence on establishing on interaction relationship with the host. PSB can turn insoluble P in to soluble P and promote plant growth (Rodriguez *et al.*, 2006).

Ramette et al., (2006) have demonstrated that a number of biocontrol PGPB have the ability to synthesize hydrogen cyanide (HCN). The HCN produced by these bacteria were the only biocontrolmechanism. The biocontrol case often can produce HCN to synthesize antibiotics or cell wall degrading enzymes. HCN toxicity affected its abilityto inhibit cytochrome c oxidase and other important metalloenzymes (Nandi etal.. 2017). Many bacterial genera such as *Rhizobium, Pseudomonas, Alcaligenes, Bacillus,* and *Aeromonas* have shown to be HCN producers.

The higher PSB populations were found in rhizosphere than non-rhizosphere soil. Reyes et al. (2006) also found a significantly higher content of PSB population in the rhizosphere in comparison with non rhizosphere or bulk soil.So the populations of PSB differ according to the sampling part of the plant not to the soil.

In the present study among the 140 isolated strains showed positive result were HCN(111), NH_3(31), H_2S(14), motility (103), chitinase (104), catalase (131), urase (140) and KOH(126) repectively (figure 2).

Use of chemical fertilizer is limited in many areas of the tropics due to high cost and lack of availability. In order to reduce dependence on commercial fertilizer, there is much interest to use local available farmyard manure as alternative sources. Palm *et al* (1998) the organic fertilizer cannot meet crop nutrient demand over large areas because of limited availability, low nutrient composition, and high labor requirement. Integrated soil fertility management is the most feasible options to resource poor farmers to sustain the productivity of maize/bean in intercropping system. Supplementation of farmyard manure with chemical fertilizers is the most potentially options for agronomic effectiveness of component crops in the system. Integrated

nutrient management methods, combining mineral and organic nutrient sources, offer better results than reliance on one source alone (Bekunda, 1999). Today, very little attention has been focused on the integrated use farmyard manure and NP fertilizers to fertilize maize-climbing bean intercropping system.

In the suggustions of present study, the increase in the world's population, coupled with the limitations in the world's supply of natural resources and widespread degeneration of the environment presents a major challenge to agriculturalists. Chemical fertilizer is used to give the plant nutrient requirements within a short period to get faster results. Newly improved varieties of crops need high proportions of fertilizer. But chemical fertilizer has certain limitations and entails a lot of disadvantages. No doubt, the application of chemical fertilizer provides nutrition in high concentration in the soil and plants. When chemical fertilizer is applied, the entire contents would not be absorbed by the plants and the remaining parts would react in the soil. Part of it would be washed away and would contaminate water and some part of it would evaporate to the atmosphere; there by the environment is polluted. Microorganisms are useful for bio-mineralization of bound soil and make nutrients available to their host and/or its surroundings.

CHAPTER FIVE

5. CONCLUSION AND RECOMENDATION

5.1 Conclusion

The results of this study have shown that the rhizosphere soils of maize and sorghum at different kebeles site of Jimma Zone of Sekoru wereda farm lands support a diverse group of naturallyoccurring potential phosphate solubilizing bacteria (PSB). All the screened isolates were capable of mobilizing P in PVK solid medium.In the present study, 140 bacterial isolates with phosphate solubilizing abilities were isolated for plant growth and promotion in maize and sorghum crop plants. Pikovskaya media was more effective for the isolation of PSB compared with other media plates. Isolated PSB strains were able to solubilize P, produce different metabolites. The highest PSI was obtained from sekoru districts particularly from Chala kebele. Generally,this study contains as a potential reservoir of bacteria with phosphate solubilizing potentials that also exhibit plant growth promoting traits making them potential bioinoculants.

5.2 Recommendation

Based on the current investigation the following recommendations were forwarded

- Further isolation and screening should be taken to get more efficiently PSB isolates.
- Green house experiments shall be conducted in different locations of Ethiopia with different crops.
- The interactions of PSB inoculants with other useful micro-organisms in plant growth and promotion should be investigated.
- Further education and training should be given to peasants or farmers of the district to use organic manure rather than chemical fertilizers and to add lime on their farm land and threat the farms in decreasing soil acidity.
- By using crop rotation with legumes to increase the nitrate content of the their farm lands.
- Educate the peoples of the districts of Jimma zone to conserve their environment and maintaining the ecology.
- Teaching the students in my lessons about the limmitations of the over-use of chemical fertilizers in the invironments.

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Annexe

Annexe 1. Fluorescent pigment production test showing diffuse green and non-diffuse colours

No.	Sample code	Isolates code	Isolates which show diffuse green pigment	Isolates non-diffuse green pigment
1.	JUChopa1	b,c,d,e	b,c,d,e	
2.	JUChopa2	a,b,d,e	b,d e	A
3.	JUCh0pa3	b,c,d,e	d,e	b,c
4.	JUChopa4	b	b	
5.	JUChopa5	c,d	d	C
6.	JUD1	a,c,d,e	a,c,d	d, e
7.	JUD2	a,e	a	E
8.	JUD3	a,c,d,	a,c	D
9.	JUD4	a,b,c,d	a,b,c	D
10.	JUD5	b,c,d,	c	b,d
11.	JUG1	a,b,c,d,e	a,b,e	c,d
12.	JUG2	a,b,c,d,e	a,b,e	c,d
13.	JUG3	a,b,c,d,e	a,b,d	c,e
14.	JUG4	a,b,d	a,d	b
15.	JUG5	d	d	

16.	JUAlga1	c	c	
17.	JUAlga2	a,e	a,e	
18.	JUAlga3	a,c,e	a,c,e	
19.	JUAlga4	a,b,c,d,e	a,c,d	b,e
20.	JUAlga5	a,b,d	a,b,d	

No.	Sample code	Isolate code	Diffuse green pigment	Non-diffuse green pigment
21	JUEnk1	b,c,d	b	c,d
22	JUEnk2	c,d,e	c,d	e
23	JUEnk3	a,b,c,d,e	a,b,e	c,d
24	JUEnk4	b,c	b,c	
25	JUEnk5	b,c,d,e	b,c	d,e
26	JUChala1	a,b,c,d,e	a,b,c,d,e	
27	JUChala2	a,b,c,d,e	a,b,c,d,e,	
28	JUChala3	a,b,c,d,e	c,d,e	a,b
29	JUChala4	a,b,c,d,	a,b,c,d	
30	JUChala5	a,b,e	a,b,e	
31	JUW1	b,c,d,e	b,c,d,e	
32	JUW2	b,d	b,d	

33	JUW3	a,b,d	a,b,d	
34	JUW4	a,b,c,e	a,b,c,e	
35	JUW5	a,b,c,d	a,b,c,d	
36	JUBore1	a,e		a,e
37	JUBore2	a,b		a,b
38	JUBore3	a,b,c,d,e	a,b,d	c,e
39	JUBore4	a,b,c,d,e	c,d,e	a,b
40	JUBore5	a,b,c,d,e	a,d	b,c,e

Fig.1 Growth at pH of 10.

Fig. 2 activation in the lab.work

Annex 2. Solubilization Index (SI) of the isolates calculated using the formula

Sample code	Isolate code	colony diameter	halo zone diameter	SI	Sample code	Isolate code	colony diameter	halo zone diameter	SI
JUD1	a	0.5	0.75	2.5	JUENK1	b	0.5	1	3
JUD1	c	0.5	0.55	2.5	JUENK1	c	0.5	1	3
JUD1	d	0.5	0.55	1	JUENK1	d	2	1	1.5
JUD1	e	0.5	1	3	JUENK2	c	0.5	1	3
JUD2	a	1	1.5	2.5	JUENK2	d	0.5	1	3
JUD2	e	1	2	3	JUENK2	e	0.5	1	3
JUD3	a	1.5	1.55	2.0	JUENK3	a	1	1.5	2.5
JUD3	c	0.5	1	3	JUENK3	b	0.5	1	3
JUD3	d	0.5	0.75	2.5	JUENK3	c	1	1	2
JUD4	a	0.5	1.5	4	JUENK3	d	1	1	2
JUD4	b	1.5	2	2.3	JUENK3	e	0.5	1	3
JUD4	c	1	1.5	2.5	JUENK4	b	0.5	1	3
JUD4	d	2	2.5	2.3	JUENK4	c	1	1	2
JUD5	b	1	2	3	JUENK5	b	0.5	1	3
JUD5	c	0.5	1.5	4	JUENK5	c	0.5	1	3
JUD5	d	0.5	0.55	2.0	JUENK5	d	1	0.5	2
JUCHOP1	b	1	2	3	JUENK5	e	0.5	1.75	4.5
JUCHOP1	c	1	1.5	2.5	JUChala1	a	1	2	3
JUCHOP1	d	0.5	1	3	JUChala1	b	0.5	1	3
JUCHOP1	e	0.5	2	5	JUChala1	c	0.5	1	3
JUCHOP2	a	1	1.5	2.5	JUChala1	d	0.5	2	5

JUCHOP2	b	0.75	2	3.5	JUChala1	e	0.5	1.5	4
JUCHOP2	d	1	2	3	JUChala2	a	0.5	1	3
JUCHOP2	e	0.5	1.75	4.5	JUChala2	b	1	2	3
JUCHOP3	b	1	2	3	JUChala2	c	1	2	3
JUCHOP3	c	1	2	3	JUChala2	d	0.5	2	5
JUCHOP3	d	0.5	1	3	JUChala2	e	0.5	1.5	4
JUCHOP3	e	1	2	3	JUChala3	a	0.5	1	3
JUCHOP4	b	0.5	1	3	JUChala3	b	1	2	3
JUCHOP5	c	1	1.5	2.5	JUChala3	c	0.5	1	3
JUCHOP5	d	0.5	1.5	4	JUChal3	d	0.5	1.5	4
JUG1	a	1	1.5	2.5	JUChala3	e	0.5	1	3
JUG1	b	1	1.5	2.5	JUChala4	a	1	2	3
JUG1	c	1	2	3	JUchala4	b	0.5	2	5
JUG1	d	0.5	2	5	JUChala4	c	0.5	2	5
JUG1	e	1	2	3	JUChala4	d	1	2.5	3.5
JUG2	a	0.5	1	3	JUChala5	a	0.5	1	3
JUG2	b	0.5	1	3	JUChala5	b	1.5	2	2.3
JUG2	c	1	1.5	2.5	JUChala5	e	0.5	2	5
JUG2	d	0.5	1	3	JUW1	b	1	1.5	2.5
JUG2	e	0.5	1	3	JUW1	c	0.5	1	3
JUG3	a	0.5	1	3	JUW1	d	0.5	1	3

JUG3	b	0.5	1	3	JUW1	e	0.5	1	3
JUG3	c	1	2	3	JUW2	b	1	1.5	2.5
JUG3	d	1	2	3	JUW2	d	0.5	1	3
JUG3	e	1	2	3	JUW3	a	0.5	1	3
JUG4	a	2	3	2.5	JUW3	b	0.5	2	5
JUG4	d	0.5	0.75	2.5	JUW3	d	1	2	3
JUG5	d	1	1.5	2.5	JUW4	a	1	1.5	2.5
JUALGAE1	c	0.5	1	3	JUW4	b	0.5	1	3
JUALGAE2	a	1	1.5	2.5	JUW4	c	0.5	1	3
JUALGAE2	e	1	1.5	2.5	JUW4	e	0.5	2	5
JUALGAE3	a	0.5	1	3	JUW5	a	0.5	1	3
JUALGAE3	c	0.5	0.55	2.1	JUW5	b	0.5	1.5	4
JUALGAE3	e	1	2	3	JUW5	c	1	1.5	2.5
JUALGAE4	a	0.5	2	5	JUW5	d	0.5	1.5	4
JUALGAE4	b	0.5	0.75	2.5	JUBORE1	a	0.5	1	3
JUALGAE4	c	1	2	3	JUBORE1	e	0.5	1	3
JUALGAE4	d	1	1.5	2.5	JUBORE2	a	1	2	3
JUALGAE4	e	0.5	1	3	JUBORE2	b	1	1	2
JUALGAE5	a	0.5	2	5	JUBORE3	a	1	1.5	2.5

JUALGAE5	b	0.5	0.75	2.5	JUBORE3	b	1	1.25	2.25
JUALGAE5	d	1	2	3	JUBORE3	c	1	1.5	2.5
JUBore1	b	1	1	2	JUBORE3	d	1	1.5	2.5
					JUBORE3	e	1	1.5	2.5
					JUBORE4	a	1	1.5	2.5
					JUBORE4	b	1	1.5	2.5
					JUBORE4	c	1	1.5	2.5
					JUBORE4	d	1	2	3
					JUBORE4	e	1	1.5	2.5
					JUBORE5	a	1	1	2
					JUBORE5	b	1	1.5	2.5
					JUBORE5	c	1	1.5	2.5
					JUBORE5	d	1	1.5	2.5
					JUBORE5	e	0.5	2	5

Annex 3 Biochemical Identification Tests of the isolates (lab. Test results)

Sample code	isolate code	HCN produc. test	NH_3 produc. Test: brown ppt.	H_2S prod. and, motility test	Chitinase produc. Test	Catalase test	Urase test, pink colour	KOH test
JUD1	a	+ve	-	Non-mo	+ve	-ve	+ve	G-ve
	c	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
	d	+ve	-	Mo	+ve	+ve	+ve	G-ve
	e	-ve	+	Mo	+ve	+ve	+ve	G+ve
JUD2	a	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
	e	+ve	-	Mo	+ve	+ve	+ve	G-ve
JUD3	a	-ve	+	Mo	+ve	+ve	+ve	G+ve
	c	+ve	-	Mo	+ve	+ve	+ve	G-ve
	d	-ve	+	Mo	+ve	-ve	+ve	G-ve
JUD4	a	+ve	-	Non-mo	-ve	+ve	+ve	G+ve
	b	+ve	-	Mo	-ve	+ve	+ve	G-ve
	c	+ve	-	Mo	-ve	+ve	+ve	G-ve
	d	+ve	-	Mo	-ve	+ve	+ve	G-ve
JUD5	b	-ve	+	Mo	+ve	+ve	+ve	G-ve
	c	+ve	-	Mo	+ve	+ve	+ve	G-ve
	d	+ve	-	Mo	+ve	+ve	+ve	G-ve
JUChopa1	b	+ve	-	Mo	-ve	+ve	+ve	G-ve
	c	+ve	-	Mo	-ve	+ve	+ve	G-ve
	d	+ve	-	Mo	-ve	+ve	+ve	G-ve
	e	+ve	-	Mo	+ve	+ve	+ve	G-ve
JUChopa2	a	+ve	-	Mo	+ve	+ve	+ve	G-ve
	b	+ve	-	Mo	+ve	+ve	+ve	G-ve
	d	+ve	-	Mo	+ve	+ve	+ve	G-ve
	e	+ve	-	Mo	-ve	+ve	+ve	G-ve
JUChopa3	b	+ve	-	No-mo	+ve	+ve	+ve	G-ve
	c	-ve	-	Non-mo	+ve	+ve	+ve	G-ve
	d	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
	e	+ve	-	Mo	+ve	+ve	+ve	G-ve
JUChopa4	b	+ve	-	Mo	-ve	+ve	+ve	G-ve
JUChopa5	c	+ve	-	Mo	-ve	+ve	+ve	G-ve
	d	+ve	-	Mo	+ve	+ve	+ve	G-ve
JUG1	a	+ve	-	Mo	+ve	+ve	+ve	G-ve
	b	+ve	-	Mo	-ve	+ve	+ve	G-ve

	c	+ve	-	Mo	+ve	-ve	+ve	G-ve
	d	+ve	-	Mo	+ve	+ve	+ve	G-ve
	e	-ve	+	Mo	+ve	+ve	+ve	G-ve
JUG2	a	+ve	-	Mo	+ve	+ve	+ve	G-ve
	b	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
	c	-ve	+	Mo	-ve	+ve	+ve	G-ve
	d	-ve	+	Mo	-ve	+ve	+ve	G-ve
	e	+ve	-	Mo	+ve	+ve	+ve	G-ve
JUG3	a	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
	b	+ve	-	Mo	+ve	+ve	+ve	G-ve
	c	+ve	-	Mo	-ve	+ve	+ve	G-ve
	d	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
	e	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
JUG4	a	+ve	-	Mo	+ve	+ve	+ve	G-ve
	b	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
	d	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
JUG5	d	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
JUAlg1	c	+ve	-	Mo	-ve	+ve	+ve	G-ve
JUAlg2	a	+ve	-	Non-mo	-ve	-ve	+ve	G-ve
	e	+ve	-	Mo	+ve	+ve	+ve	G-ve
JUAlg3	a	+ve	-	Mo	-ve	-ve	+ve	G-ve
	c	+ve	-	Mo	+ve	+ve	+ve	G-ve
	e	+ve	-	Mo	-ve	+ve	+ve	G-ve
JUAlg4	a	+ve	-	Mo	+ve	+ve	+ve	G-ve
	b	-ve	+	Mo	+ve	+ve	+ve	G-ve
	c	+ve	-	Mo	+ve	+ve	+ve	G+ve
	d	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
	e	-ve	+	Mo	+ve	+ve	+ve	G-ve
JUAlg5	a	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
	b	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
	d	+ve	-	Mo	-ve	+ve	+ve	G-ve
JUChala1	a	+ve	-	Mo	+ve	+ve	+ve	G+ve
	b	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
	c	+ve	-	Mo	+ve	+ve	+ve	G-ve
	d	+ve	-	Mo	+ve	+ve	+ve	G-ve
	e	-ve	+	Mo	+ve	+ve	+ve	G-ve
JUChala2	a	+ve	-	Mo	+ve	-ve	+ve	G-ve

	b	+ve	-	Mo	+ve	+ve	+ve	G-ve
	c	+ve	-	Mo	+ve	+ve	+ve	G+ve
	d	+ve	-	Mo	+ve	+ve	+ve	G-ve
	e	+ve	-	Mo	+ve	+ve	+ve	G-ve
JUChala3	a	+ve	-	Mo	+ve	-ve	+ve	G-ve
	b	+ve	-	Mo	+ve	+ve	+ve	G-ve
	c	+ve	-	Mo	+ve	+ve	+ve	G-ve
	d	+ve	-	Mo	+ve	+ve	+ve	G-ve
	e	+ve	-	Mo	+ve	-ve	+ve	G-ve
JUChala4	a	+ve	-	Mo	+ve	+ve	+ve	G-ve
	b	+ve	-	Mo	+ve	+ve	+ve	G-ve
	c	-ve	+	Mo	+ve	+ve	+ve	G-ve
	d	+ve	-	Mo	-ve	+ve	+ve	G-ve
JUChala5	a	+ve	-	Mo	+ve	+ve	+ve	G-ve
	b	+ve	-	Mo	+ve	+ve	+ve	G-ve
	e	+ve	-	Mo	+ve	+ve	+ve	G-ve
JUEnk1	b	+ve	-	Mo	+ve	+ve	+ve	G-ve
	c	+ve	-	Mo	+ve	+ve	+ve	G-ve
	d	+ve	-	Mo	+ve	+ve	+ve	G+ve
JUEnk2	c	+ve	-	Mo	+ve	+ve	+ve	G-ve
	d	+ve	-	Mo	+ve	+ve	+ve	G+ve
	e	-ve	+	Mo	-ve	+ve	+ve	G-ve
JUEnk3	a	+ve	-	Mo	+ve	+ve	+ve	G-ve
	b	+ve	-	Mo	+ve	+ve	+ve	G-ve
	c	+ve	-	Mo	+ve	+ve	+ve	G-ve
	d	-ve	+	Mo	-ve	+ve	+ve	G-ve
	e	+ve	-	Mo	+ve	+ve	+ve	G-ve
JUEnk4	b	+ve	-	Mo	+ve	+ve	+ve	G-ve
	c	+ve	-	Non-mo	+ve	+ve	+ve	G+ve
JUEnk5	b	-ve	+	Mo	-ve	+ve	+ve	G-ve
	c	+ve	-	Mo	+ve	+ve	+ve	G+ve
	d	+ve	-	Mo	+ve	+ve	+ve	G-ve
	e	-ve	+	Mo	+ve	+ve	+ve	G-ve
JUW1	b	+ve	-	Black	+ve	+ve	+ve	G-ve
	c	+ve	-	Black	+ve	-ve	+ve	G-ve
	d	+ve	-	Black	-ve	+ve	+ve	G-ve
	e	-ve	+	Black	-ve	+ve	+ve	G-ve
JUW2	b	+ve	-	Black	+ve	+ve	+ve	G-ve
	d	+ve	-	Black	+ve	+ve	+ve	G-ve

JUW3	a	+ve	-	Black	+ve	+ve	+ve	G-ve
	b	+ve	-	Black	+ve	+ve	+ve	G-ve
	d	+ve	-	Black	+ve	+ve	+ve	G-ve
JUW4	a	+ve	-	Non-mo	+ve	-ve	+ve	G-ve
	b	+ve	-	Non-mo	+ve	+ve	+ve	G+ve
	c	+ve	-	Black	+ve	+ve	+ve	G-ve
	e	+ve	-	Black	-ve	+ve	+ve	G-ve
JUW5	a	+ve	-	Black	+ve	+ve	+ve	G-ve
	b	+ve	-	Black	-ve	+ve	+ve	G-ve
	c	+ve	-	Non-mo	+ve	+ve	+ve	G-ve
	d	+ve	-	Black	-ve	+ve	+ve	G-ve
JUBore1	a	-ve	+	Mo	-ve	+ve	+ve	G+ve
	e	-ve	+	Mo	-ve	+ve	+ve	G+ve
JUBore2	a	-ve	+	Mo	-ve	+ve	+ve	G-ve
	b	-ve	+	Mo	+ve	+ve	+ve	G-ve
JUBore3	a	+ve	-	Mo	-ve	+ve	+ve	G-ve
	b	+ve	-	Mo	+ve	+ve	+ve	G-ve
	c	-ve	+	Mo	+ve	+ve	+ve	G-ve
	d	+ve	-	Mo	+ve	+ve	+ve	G-ve
	e	-ve	+	Mo	+ve	+ve	+ve	G-ve
JUBore4	a	-ve	+	Mo	-ve	+ve	+ve	G-ve
	b	-ve	+	Mo	-ve	+ve	+ve	G-ve
	c	+ve	-	Mo	-ve	+ve	+ve	G-ve
	d	+ve	-	Mo	+ve	+ve	+ve	G-ve
	e	-ve	+	Mo	+ve	+ve	+ve	G-ve
JUBore5	a	+ve	-	Mo	+ve	+ve	+ve	G-ve
	b	-ve	+	Mo	+ve	+ve	+ve	G-ve
	c	-ve	+	Mo	-ve	+ve	+ve	G-ve
	d	+ve	-	Mo	-ve	+ve	+ve	G-ve
	e	-ve	+	Mo	-ve	+ve	+ve	G-ve

Keys : +ve =positive, -ve=Negative, mo=motile, Non-mo=None motile, G+ve= Gram-positive, G-ve= Gram-negative.

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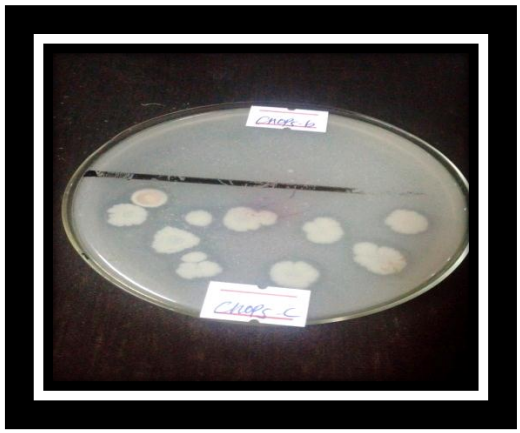

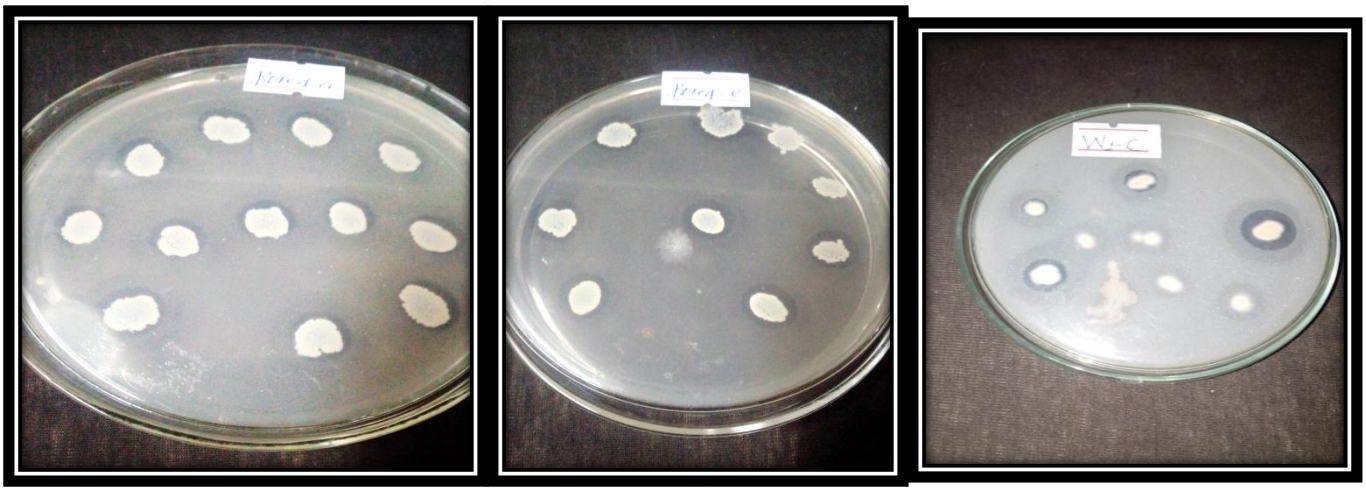

fig.3. phosphate solubilizing bacterial isolates

Fig. 4a,urease test result

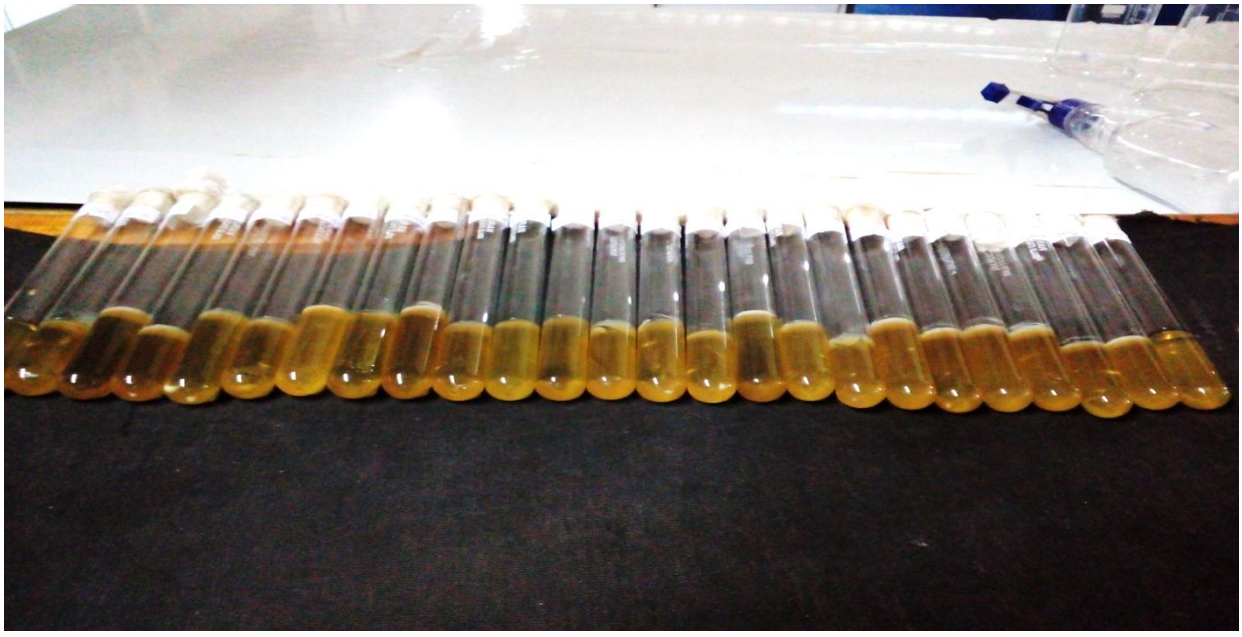

Fig.4b motility test

Fig. 5 Sample with plastic bags