PHENOTYPIC AND SYMBIOTIC CHARACTERIZATION OF RHIZOBIA NODULATING COMMON BEAN (*PHASEOLUS VULGARIS L.*) FROM EAST SHOA ZONE OROMIA, ETHIOPIA

By: Dugo Nura Beriso

A Thesis Submitted to Applied Biology Program

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirements for the Degree of Master of science in Applied Biology (Microbiology)

Office of Graduate Studies

Adama Science and Technology University

Adama,

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Advisors: Dr. Zerihun Belay (PHD)

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DECLARATION

I hereby declare that this MSc Specialty or equivalent thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: Dugo Nura

Signature: _____

This MSc Specialty or equivalent thesis has been submitted for examination with my approval as Thesis advisor.

Name: Zerihun Belay (PhD)

Signature: _____

Adama,

November, 2018

DEDICATION

I dedicate this work to my brother Dori Nura, who had a great dream for my success in education but passed away at a very early age without seeing any of those long journeys and to my mother Kule Hawas for her contribution for this work. Also to my lover Shega Tuji for her loving, treating and challenging me since 2014; she teaches me patience and love, I have much appreciation for her.

SCHOOL OF GRADUATE STUDIES

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY ADVISOR

APPROVAL SHEET

(Submission sheet-1)

This is to certify that the title entitled "**Phenotypic and Symbiotic Characterization of Rhizobia Nodulating Common Beans (*Phaseolus Vulgaris L.*) From East Shoa Zone Oromia, Ethiopia**", Submitted in the partial fulfillment of the requirements for the degree of **Master of Science in Biology** with specialization **Applied Microbiology** the Graduate Program of the **Department of Biology**, **Adama Science And Technology University** and has been carried out By: Dugo Nura under my supervision. Therefore I recommend that the student has fulfilled the requirements and hence hereby can submit the thesis to the department.

Zerihun Belay (PhD)	_____	_____
Name of advisor	Signature	Date

SCHOOL OF GRADUATE STUDIES

ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

EXAMINERS' APPROVAL SHEET

(Submission sheet-2)

We, the undersigned, members of board of the examiners of the final open defense by Dugo Nura have read and evaluated his thesis entitled: "**Phenotypic and Symbiotic Characterization of Rhizobia Nodulating Common Bean (*Phaseolus Vulgaris L.*) From East Shoa Zone Oromia, Ethiopia**", and examined the candidate. This is therefore to certify that the thesis has been accepted in fulfillment of the requirement for the degree of Master Sciences in Applied Biology (Microbiology).

Name of External Examiner	Signature	Date
Advisor/Supervisor/	Signature	Date
Name of Internal Examiner	Signature	Date
Chair Person	Signature	Date

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LISTS OF ABBREVIATIONS

ASTU	Adama Science and Technology University
BNF	Biological Nitrogen Fixation
BTB	Bromothymol Blue
CRD	Complete Random Design
E	Effective
EPS	Exopolysaccharide
GPS	Global Positioning System
HE	Highly Effective
LB	Luria Bertani Agar
LE	Lowly Effective
NDW	Nodule Dry Weight
NN	Nodule Number
PY	Peptone Glucose
SE	Symbiotic Efficiency
SNF	Symbiosis Nitrogen Fixation
TY	Tryptone Yeast Extracted Agar
YEMA	Yeast Extracted Manitol Agar
YEMB	Yeast Extracted Manitol Broth

ABSTRACT

This study was aimed to isolate rhizobia, nodulating common bean and characterize phenotypically and its symbiotic performance from East Shoa Zone Oromia, Ethiopia. The Common bean is a legume plant, important source of nutrients and also used as medicine. The bean is grown in the warm and low land areas of the country including East Shoa. It plays a major role in soil fertility through biological nitrogen fixation. Common bean is relatively permissive host, nodulating effectively with different rhizobial species. Nitrogen fixation by rhizobia reduces on the use of artificial fertilizers; this saves money and prevents many problems by artificial fertilizers. Soil samples were collected from 11 representative Kebeles of 7 Woredas, where common bean has been grown for long time with no history of inoculation; samples were pooled from 5-15 cm depth and transferred to ASTU for nodule trapping. Phenotypic features such as substrate utilization, resistance to antibiotics, pH, temperature and concentration of NaCl was analyzed. All isolates were observed to be Gram-negative, rod shaped, non-absorber of Congo-red, colony size 1.5mm to 5mm; they were also found to be resistance to most of the tested antibiotics. Most of the isolates were found to change YEMA-BTB media to yellow showing acid producers and fast growers. More than 73% of the tested isolates were observed to be phosphorus solubilizer. Based on relative effectiveness isolates were found to be effective as (highly effective, lowly effective and effective) except two isolates.

***Keywords**: Common bean, Congored, Re-inoculation, Re-nodulation, Rhizobia, Symbiotic effectiveness.*

1. INTRODUCTION

Common bean *(Phaseolus vulgaris L.)* is species of the genus *Phaseolus* which are all native to America. The bean is an annual herbaceous plant, climber or erect, warm climate, reproduced by seed (Romaro *et al*., 2013), diploid, pinnately compound trifoliate leaves. It is the third most important legume crop grown worldwide, next to soya bean (*Glycine max* L.) and peanut (*Arachis hypogea* L.) and contains protein, dietary fiber, complex carbohydrates, minerals, and vitamins for millions of people in both developing and developed nations, and is one of the basic foods of the indigenous populations of South America and Eastern and Southern Africa (Darkwa *et al*., 2016). It contains 65% of total protein uptake, 32% of energy, and micronutrients such as: iron, zinc, thiamin and folic acid; and has high (55 µg/g) concentration of iron with respect to; crops such as wheat, rice and maize (Petry *et al.*, 2015).

Lin *et al* (2014) reported that common bean is consumed as dry seeds, green pods and green shelled seeds. The common bean has medicinal importance for treating difficult urination (diuretic), hypoglycemic action, kidney, heart, rheumatic and diabetes and used as anti-bacterial, antiviral and mutagenic, fungicidal (Romero *et al*,. 2013). Most legume species such as common bean (*Phaseolus vulgaris L*.), fix atmospheric nitrogen by symbiotic rhizobia in root nodules, that favor growth under low soil nitrogen conditions. Besides, nitrogen fixation by legumes is major input of nitrogen into natural and agricultural ecosystems (Andrews and Andrews, 2017), to play role in soil fertility by enhancing soil organic matter, and prevention of nutrient leaching (Darkwa *et al*., 2016).

Common bean is relatively permissive host, nodulating effectively with rhizobial species; *Rhizobium etli, Rhizobium tropici, Rhizobium leguminosarum* biovar, *Phaseoli, Rhizobium giardinii, Rhizobium gallicum* and *Bradyrhizobium*, nodulate this promiscuous legume (Bayou, 2015). Other plants use N-fixed when the bacteria die and release nitrogen to the environment, or when the bacteria live in symbiosis with the plant (Flynn and Idowu, 2015). Utilizing crop rotation with legumes could save millions or billions of dollars currently being spent on synthetic nitrogen forms used extensively in monoculture agriculture, namely continuous corn production (Fox *et al.*, 2007).

Sustainable soil fertility management is needed to food production and environmental stability. Nitrogen fixation by rhizobia plays a great role in agriculture. Crop yields are improved in nodulated plants; legumes can grow well in less fertilized soils where there is not enough fixed nitrogen to support other types of plants (Burdass, 2002).

In Ethiopia it is reported that more than 337,000 ha were assigned for the production of 455,000 tons of common beans annually. Common bean is grown in the warm and low land areas of the country which include East Shoa. A Common bean contributes to food and cash commodity, which serving as a source of income and employment to a large supply chain and the stems are also used as fodder for livestock (Kedir, 2017). This study was initiated with the aim to isolate rhizobia, nodulating common bean and characterize phenotypically and its symbiotic performance from East shoa zone Oromia, Ethiopia.

1.1. Statement of the Problem

Fixed nitrogen is the limiting factor for plant growth in all environments where there is a suitable climate and availability of water to support life. Common bean cultivation is common in many parts of East Shoa. However, the potential productivity level of the crop was yet to be achieved. The farmers of the area are using artificial fertilizer, which so costy, environmentally unfriend and has impact on human healthy. Research was carried out to find ways of improving the amounts available to plants. This includes not only enhancing the efficiency of rhizobia as nitrogen fixers in legumes, but also to bring about nitrogen fixation in other crops.

1.2. Objectives of the Study

1.2.1. General Objective

- ❖ To evaluate phenotypic and symbiotic diversity of rhizobia, nodulating common bean (*Phaseolus vulgaris L.*) From East Shoa Zone Oromia, Ethiopia

1.2.2. Specific Objectives

- ✧ To isolate and authenticate common bean-nodulating bacteria from nodules
- ✧ To examine phenotypic characteristics of the isolates
- ✧ To evaluate symbiotic effectiveness of the isolates

2. LITERATURE REVIEWS

2.1. Rhizobium

Rhizobia are soil bacteria that colonize the roots of leguminous plants, to fix atmospheric nitrogen (Hunter *et al*., 2007). In the soil these bacteria are free-living and motile, feeding on the remains of dead organisms. Free living rhizobia cannot fix nitrogen and they have a different shape from the bacteria found in root nodules (Burdass, 2002). Nitrogenase is the enzyme for conversion of nitrogen to ammonium, exists in three forms that include molybdenum nitrogenase, vanadium nitrogenase, and iron nitrogenase. The colonies were entire, opaque with regular margin, milky white, translucent, circular in shape, shiny, raised (convex), sticky consistency, musky odour of the colony and 2-4mm in diameter. The isolated bacterium was aerobic, non spore forming, pink coloured gram negative rods and motile.

2.2. Common Bean (Phaseolus Vulgaris L.)

2.2.1. Origin of the Beans

The bean (*Phaseolus vulgaris* L.), is originated in the western area of Mexico and Guatemala (Romero *et al*., 2013). Botanical explorations in Mexico showed that the wild varieties of *Phaseolus vulgaris* L. grow along the Sierra Madre Occidental, in a transition band ecological located between 500 and 1,800 (m.s.l), although the higher frequency of these varieties occurs at 1,200 meters approximately. Its origin is based on the fact that there is a large genetic diversity, both in *P. vulgaris.* The wild and cultivated Common beans are distributed from Mexico to the Southern ends of the southern Andes. Among the cultivated types these great variation in habit of growth and other morphological traits, characteristics of seed, adaptation and performance potential (Romero *et al*., 2013)

2.2.2. Common Bean in Ethiopia

Common beans (*Phaseolus vulgaris* L.) are grown in Ethiopia primarily as a crop for small-scale production with little inputs. They are grown in Ethiopia in areas with temperature of less than 30^0C and greater than 10^0C, with rainfall of 350-700 mm. It can be grown on light sandy soils to heavy clay soils. Common bean production in the country mainly grown as cash

crop and corns by farmers is about 163,688ha. Even though common bean has high economic importance, the yield of bean obtained in Ethiopia is low (800-900Kg/ha) when compared to 2500 kg/ha in Egypt, which is the highest yield in the world (CSA, 2006). Katungi *et al* (2009) reported that bean is produced in almost all of the regional states which, Oromia and Southern National Nationality Peoples Region (SNNPR) contribute to about 75% of production.

The white beans dominate in the Oromia region (Northeast rift valley), where more than 95% of farmers grow it and account for about 50% of total common bean production. The coloured bean type dominates SNNPR, south of Lake Danbal. Coloured food types are preferred in SNNPR because of their popularity in the local diet and relatively lower production costs compared to white beans. The varieties within the coloured bean type include the reds, white and black, but the reds are the most important. About 80-90 % of the area allocated to common bean in SNNPR is designated for red varieties while the white varieties occupy 10-20 % of the area. Among the white canning type, the most preferred canning type seed are of oval shaped, with a sparkling white color and of upright growth habit to avoid damage by soil and of early maturity (Katungi *et al.*, 2009).

2.3. Biological Nitrogen Fixation

The biological nitrogen fixation (BNF) is a natural process of rhizobial inoculants production. The rhizobial inoculant is a cheap and non polluting source of bacteria that can provide high rates of nitrogen to the legume, and the use of improved inoculants is a strategy to increase BNF rates in agro-ecosystems. Nitrogen is vital nutrient for plant growth and required for high crop yielding; it is the major component of chlorophyll, DNA and RNA, building material of proteins. There is about 78% nitrogen in atmosphere, but it is unusable freely by plants. Nitrogen-fixing bacteria fix atmospheric nitrogen using nitrogenase enzyme (Nadal and Hooda, 2013). The reduction of nitrogen gas to ammonia (NH_3) requires molecules of ATP and a complex set of enzymes to break the nitrogen bonds so that it can combine with hydrogen.

$$N_2 + 3H_2 \xrightarrow{\text{energy}} 2NH_3$$

Rhizobia enter the roots of most legume species via root hair infection. Here, rhizobia enter root hairs, and host cell wall material grows around the developing "infection", forming an infection thread, which grows through the cortex of the root, branching repeatedly (Andrews and Andrews 2017). About half (1/2) of the 23 million metric tons of nitrogen eaten as human food sources gained from BNF by microorganisms of which rhizobia can contribute to 50-70% of the world's annual biological fixation of atmospheric nitrogen (10^8 and $1.8x10^8$ Mt per year). Common bean fix nitrogen to the tune of 25 to 210 kg N ha^{-1}.

Common bean is poor nitrogen fixing legumes, to inoculation in experiment, promiscuity, high rate of nitrogen-fertilizer used, existence of native bacteria and lack of available phosphorus in the soil. In Ethiopia, there is a need for inoculation of common bean with rhizobia to increase the yield of common beans. The inoculated *Phaseolus vulgaris* L fixed 12-20Kg/ha, of nitrogen than uninoculated one. Symbiotic properties of the effective isolates showed different response depending on soil factors such as cations, cation exchange capacity, and available phosphorus (Antenah, 2007).

2.4. Factors Affecting Nitrogen Fixation and Legume Production

Biological nitrogen fixation is very important to sustainable agricultural system in tropical soils, which are frequently deficient in nitrogen. Environmental factors influence all aspects of nodulation and symbiotic nitrogen fixation. Nitrogen-fixing organisms (diazatrophs) are prokaryotes. Symbiotic nitrogen fixation takes place in a symbiosome, an organelle inside the root nodules (Raymond *et al.*, 2004).

2.4.1. Extreme Temperature

Temperature is one of the major factors affecting rhizobial growth, survival in the soil and the symbiotic process itself (Niste *et al.*, 2013). High temperature may affect symbiotic relationships, nitrogen content, formation of roots, binding of rhizobia to root hairs, nodule development and function, and dry matter production (Boboye *et al.*, 2011). Low temperature affects nodulation, as the process of nodule initiation is completely inhibited under low temperature (Sadowsky, 2005). High temperature also alters the pattern of cell surface

components (EPS and LPS) secreted by rhizobia that affects the process of nodulation and Nitrogen fixation (Yadav *et al.,* 2010).

2.4.2. Soil Acidity

The soil acidity is the major limiting factors for the production of haricot bean in Ethiopia (Ayalew, 2011). Therefore, these acid tolerant isolates may play a great role in improving the yield and production of haricot bean and also having the paramount agronomic and ecological importance in relation to development of sustainable agricultural system. Soil acidity disrupts the symbiotic association between *Phaseolus* and *Rhizobium*. All stages of the legume-*Rhizobium* symbiosis, including strain survival in the soil, root hair infection, nodule initiation, and nitrogen fixation are affected by low pH of the soil. Since *Rhizobium* strain are unable to survive in acid soil below pH 5 this cause poor nodulation. Soil acidity causes toxicity of Al and Mn on the host, rhizobia and symbiosis. In Eastern Africa P availability is reduced by increasing soil acidity due to high P fixation. Soil acidity for nitrogen fixation can be managed by selecting of acid-tolerant legume cultivars and compatible rhizobia (Antenah, 2007).

2.4.3. Soil Salinity

About 10% of the world's such as tropical and Mediterranean regions are classified as endangered area because of salinity. Among legume crops, there is variability in salt tolerance. For instance, *Pisum sativum* is less salt tolerant than legumes such as *Phaseolus vulgaris, Vicia faba* and *Glycine max*. Strains of fast growing acid-producing rhizobia such as *R. etli are* more salt tolerant than slow growing alkali producing strains (Anteneh, 2007).

High salt concentration decrease nodulation and amounts of nitrogen fixed; the amount of nitrogen fixed per unit weight of nodules decline with salt stress. So, the success of *Rhizobium*-legume symbiosis under salt stress requires a good selection for both salt tolerant rhizobial strains and host plants (Anteneh, 2007). Rhizobia utilize the mechanism of osmotic adaptation, responding to high salt stress by changing their cellular morphology. Some rhizobia can survive in extremely high levels of salt both in culture and in soil. Salinity resulted in decreased plant growth, less N_2 fixation, decreased nodule numbers and reduced percentage of tissue nitrogen (Garg and Singla, 2009).

3. MATERIALS AND METHODS

3.1. Study Area

The rhizobia isolation, characterization and pot experiment were carried out at ASTU, Biology Department laboratory. The soil sample collection was carried out in the different districts of East Shoa Zone with the help of GPS. The study site was selected based on agroclimatic conditions and known common bean cultivation. East Shoa Zone extends between $7^0$33'50"N-$9^0$08'56"N and from $38^0$24'10"E-$40^0$05' 34"E. The temperature in East Shoa Zone varies from less than 10^0c along high altitudes to above 30^0c in Rift Valley lowland areas and, the mean temperature is 20^0C. Since the large portion of the zone is located along the Rift valley system, rainfall varies from 600mm-1000mm with mean annual rainfall of 816 mm (Nigatu, 2013). The Appendix 1: Shows map of East Shoa Zone.

3.2. Soil Sampling

Soil samples were collected randomly from 11 kebele's of East Shoa Zone where common bean has been grown for long time with no history of inoculation. Three Kg of soil samples from each district were pooled from 5-15 cm depth. The sample was taken to the laboratory for nodulation test and analysis. The corresponding GPS data including altitude and province name was also recorded (Table 1).

3.3. Nodule Trapping

Each representative soil sample was mixed and sieved using 2mm sieve. The soil from each sample was filled into 3 kg capacity plastic pots, which surface sterilized by swabbing with 95% alcohol. Seeds of common bean (variety, **Ser-119** taken from Melkassa Agricultural Research Center) were surface sterilized in 95% ethanol for 30s and then socked in NaOCl (0.25%) for 3 min and washed with distilled and sterilized water (Vincent, 1970); And was planted on soil samples. The seedlings were maintained for 5 weeks and harvested to obtain nodules. The plants were uprooted and cut at crown and soil was washed off from the root and nodules. The fresh and undamaged nodules were picked and preserved for isolation of the rhizobial isolates (Somasegaran and Hoben, 1994).

Table 1: Samples Location, Altitude, pH and type of the crop on the Fields

Number	Name of sites		Location			Field history		
	(Woredas)	(Kebeles)	Altitude (m.a.s.l)	Latitude(N)	Longitude(E)	Previous	Current	PH of the soil
1	Fantalle	Ilala	926	$08^0$55'18.7"	$39^0$49'17.3"	Maize	Common bean	7.79
2		Nukusa	1002	$08^0$53'14.6"	$39^0$48'46.7"	Maize	>>	8.32
3	Boset	Sifa Bate	1210	$08^0$31'37.6"	$39^0$34'49.6"	Teff	>>	7.99
4		Buta Donkore	1473	$08^0$41'59"	$39^0$26'33"	>>	>>	8.07
5		Rukecha Bokore	1555	$08^0$37'14.3"	$39^0$28'07.2"	>>	>>	7.60
6	Liben	Gadulla	1655	$08^0$30'07.5"	$38^0$54'19.4"	>>	>>	8.40
7		Oda Jidda	1695	$08^0$30'12.1"	$38^0$54'13.5"	Pea	>>	8.37
8	Ada'a	Dire	1945	$08^0$41'41"	$38^0$53'55"	Barley	>>	7.29
9	Dugda	Woyyo Gabrel	1624	$08^0$03'50.8"	$38^0$44'56.4"	Maize	>>	7.61
10	Bora	Tuchi Dako	1644	$08^0$16'45.4"	$38^0$54'43.8"	Teff	>>	7.70
11	Adami/T/J Kombolcha	Abosa (Batu 03)	1643	$08^0$01'03"	$38^0$43'21.1"	Maize	>>	8.35

3.4. Isolation of Rhizobia

The nodules were soaked and rinsed in distilled water before surface sterilization. They were dipped in 95 % ethanol for 5sec. and surface sterilized with 3%NaOCl for 4 min. Then rinsed in sterile water and crushed in normal saline solution (0.85% NaCl). A loop-full of the crushed nodule was then streaked across the surface of petri dish containing YEMA and incubated at 28^0C for 3-5 days (Vincent, 1970).

All colonies were checked on YEMA containing 25 mg ml^{-1} Congo red to evaluate their ability to absorb the dye. The isolates were also inoculated on YMA containing 25 mg ml^{-1} bromothymol blue (BTB) to determine their ability to produce acid or base and change the medium (Lupwayi and Haque, 1994). The isolates were also characterized by colony appearance, EPS production and the size of the colony, on YEMA plates incubated at 28^0C for 3-5 days (Ahmed *et al*., 1984).

YEMA medium is composed of 10g Mannitol, 0.5g K_2HPO_4, 0.2g $MgSO_2.7H_2O$, 0.1g NaCl, 1g Yeast extract, 0.025ml Congo red, 15g Agar and 1000ml Distilled water (Somasegaran and Hoben, 1994). Each isolate was designated as ASTUR to remember a University where experiment has been done and the last R is for rhizobia with different number.

3.5. Characterization of the Isolates

3.5.1. Colony Characteristic

Colony was characterized based on its color (watery and white), size (mm), shape (circular), texture (elastic or buttery) extracellular polysaccharide (EPS) production (low and high), on YEMA plates incubated at 28^0C for 3-5 days (Ahmed *et al*., 1984). Phosphate solublizing ability was checked by inoculating each of them on Picovaskaya Agar medium containing (g/l): Glucose (10), Tricalcium phosphate (5), Ammonium Sulphate (0.5), Yeast extract (0.5), Magnesium Sulphate (0.1), Sodium Chloride (0.2), Manganese Sulphate (0.002), and Agar (15). The pH of the medium was adjusted to 7. The medium was supplemented with 2.5 g/lt of Ca_{3}-$(PO4)_2$ (TCP) as P source. The growth and clear zone formation around the colonies showed phosphate solubilization (Somasgaran and Hobben, 1994)

3.5.2. Physiological Characteristics

Salt tolerance was measured, weather the bacterial cells can grow and reproduce in NaCl concentration. Growth in different salt concentrations was investigated on YEMA medium supplemented with NaCl at a concentration of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 11%. The ability of bacterial strains to grow at high and low temperatures was monitored using YEMA medium incubated at 5, 10, 15, 37, 40 and 45^{0}C on YEMA medium. Tolerance to extreme pH was tested on TY agar medium set at 4, 5, 9, 10, and 11 pH values (Singh *et al.*, 2011).

3.6. Intrinsic Antibiotic Resistance

Resistance of the isolates to different concentrations of antibiotics was determined by preparing the fresh solutions (0.22µm) antibiotics in YMA medium. Seven antibiotics, Chloramphenicol, Erythromycin, Ampicillin, Vancomycin, Norfloxacin, Ciprofloxacin and tetracycline were used at 5 and 10 %(µg/ml) concentrations (Amarger *et al.*, 1997).

3.7. Substrate Utilization

All the isolates were tested for their ability to utilize the various carbon sources in carbohydrate free basal medium. Carbon sources including starch, glucose, Lactose, sucrose, dextrose white, benzophenone, citrate and mannitol were used in the study. The carbohydrate free basal medium was prepared by dissolving the $MgSO_4.7H_2O$ 0.2 g; NaCl 0.2 g; K_2HPO_4 0.5 g; KH_2PO_4 0.5 g; Yeast extract 0.05 g and agar 15.0 g in 1,000 ml of distilled water. The pH of the medium was adjusted to 7. The isolates were streaked on the Petri plates in three replicates. The presence or absence of growth was recorded after 5 days. Ability of isolates to utilize the following N sources, Urea, Triethanolamine, Aniline, Ammonium Acetate, Glycine and L(+) Asparagine-1-hydrate, were determined as described by Amarger *et al*. (1997).

3.8. Symbiotic Effectiveness Test

Symbiotic effectiveness was evaluated in pot experiment conducted under greenhouse condition. Seeds of common bean (**Ser-119**) were surface sterilized using NaOCl and then rinsed in sterile water prior to sowing. Seeds were sown in each 3kg plastic pot filled with washed, sieved and sterilized sand. Each strain of rhizobia grown on YEM liquid medium to

logarithmic phase (10^{9} cells ml^{-1}) of the culture was inoculated. Two controls, without inoculation and N-addition and uninoculated with 0.05% (W/V) KNO_3 per week were included. Plants were supplied with distilled water every 2 days and fertilized once a week with N-free nutrient solution (Broughton and Dilworth, 1970 cited in Lupwayi and Haque, 1994).

The pots were arranged in CRD; after 6 weeks, the plants were uprooted and their effectiveness was evaluated on the basis of nodule dry weight, nodule number and shoot dry weights after drying at 70^{0}C for 2 days in oven. Strain effectiveness was calculated according to the equation proposed by Date *et al.* (1993) cited in Purcino *et al.* (2000) [100× inoculated plant DM/ N-fertilized plant DM) with Nitrogen fixing effectiveness classified as ineffective, <35%; lowly-effective, 35-50%; effective, 50-80%; and highly effective, >80%.

3.9. Data Analysis

Phenotypic features including carbon source and nitrogen source, resistance to antibiotics, growth in a range of pH, temperature and concentration of NaCl were analyzed by Tables, graphs and figures.

4. RESULTS

4.1. Cultural and growth characteristics

Twenty two isolates of root nodule bacteria were isolated from East Shoa Zone Oromia, Ethiopia, but soil from Ada'a woreda of dire Kebele and Boset woreda of Rukecha Kebele failed to nodulate. All isolates were gram negative (pink after Gram-staining) and rod shaped bacteria and no absorption of congored grown on YEM-CR medium.

Five isolates grew on peptone- glucose medium with bromocresol purple; 15 isolates grow on Luria Bertani (LB) and 73% were able to solubilize tricalcium phosphate in sperber solid medium. Isolates $ASTU_{6,}$ $ASTU_{9,}$ $ASTU_{10,}$ $ASTU_{13,}$ $ASTU_{20}$ and $ASTU_{21}$ produced large halo zones greater or equal than 1mm as shown in (Table 2). All isolates were reinoculated into the host on sterilized river sand to evaluate their ability to form nodules and all of the tested isolates renodulated the host plant; and hence were authenticated as root nodule bacteria.

Most of the isolates except ($ASTU_{3,}$ $ASTU_{5,}$ $ASTU_{7,}$ $ASTU_{15,}$ $ASTU_{19}$ and $ASTU_{20}$ from Adami Tulu G/Kombolcha, Boset, Fantalle and Liben) woreda respectively produced acid on YEMA-BTB medium and did change the YEMA-BTB medium in to yellow, showing acid producers and fast growers and 27% of the isolates did not change YEMA-BTB to yellow showing alkaline producers and slow growing isolates (Table 2). Based on colony size the isolates were observed to be 1.5-5mm colony size (Table 3).

Table 2: Growth on some media and Phosphate solubilization of the isolates

Isolates	PG-BCP	YEMA-BTB	LB	PO_4
$ASTUR_1$	-	Yellow	-	0.2mm
$ASTUR_2$	-	Yellow	+	0.3mm
$ASTUR_3$	+	Blue	+	-
$ASTUR_4$	-	Yellow	-	0.6mm
$ASTUR_5$	-	Blue	-	-
$ASTUR_6$	-	Yellow	+	1.2mm
$ASTUR_7$	+	Blue	+	0.8mm
$ASTUR_8$	-	Yellow	-	0.4mm
$ASTUR_9$	-	Yellow	+	1.4mm
$ASTUR_{10}$	-	Yellow	+	1.2cm
$ASTUR_{11}$	-	Yellow	-	0.2mm
$ASTUR_{12}$	-	Yellow	-	-
$ASTUR_{13}$	-	Yellow	+	1mm
$ASTUR_{14}$	-	Yellow	+	0.6mm
$ASTUR_{15}$	-	Yellow	+	0.7mm
$ASTUR_{16}$	+	Blue	+	0.4mm
$ASTUR_{17}$	+	Yellow	+	-
$ASTUR_{18}$	-	Yellow	+	0.2mm
$ASTUR_{19}$	+	Blue	+	1.4mm
$ASTUR_{20}$	-	Blue	+	-
$ASTUR_{21}$	-	Yellow	+	1mm
$ASTUR_{22}$	-	Yellow	-	-

Keys: + Presence (positive), - Absence (negative), LB- Luria Bertani, PG-BCP- peptone glucose with bromocresol purple.

4.1.1. Cultural Characteristics

All the colonies were circular in shape, different in color (watery and white), texture (buttery and elastic) and produced EPS (low and high) on YEMA media. About 73 % formed colonies size greater than 2.5mm; whereas more than 27% formed colony size less than 2.5mm. 73% of isolates produce high EPS and 27% produce low EPS. 14 isolates showed buttery whereas the rest displayed elastic colonies. Isolates $ASTU_3$, $ASTU_5$, $ASTU_7$, $ASTU_{16,}$ $ASTU_{19}$ and $ASTU_{20}$ showed rough growth as (Table: 3).

Table 3: Colony characteristics of the Isolates

Isolates	Color	Shape	Texture	EPS production	Colony size
$ASTUR_1$	Watery	Circular	Buttery	High	2.5mm
$ASTUR_2$	White	Circular	Buttery	High	3mm
$ASTUR_3$	White	Circular	Buttery	Low	1.5mm
$ASTUR_4$	White	Circular	Elastic	High	2.5mm
$ASTUR_5$	Watery	Circular	Elastic	Low	2mm
$ASTUR_6$	White	Circular	Elastic	High	3mm
$ASTUR_7$	Watery	Circular	Buttery	Low	1.5mm
$ASTUR_8$	Watery	Circular	Buttery	High	3mm
$ASTUR_9$	Watery	Circular	Buttery	High	2.4mm
$ASTUR_{10}$	White	Circular	Elastic	High	3mm
$ASTUR_{11}$	White	Circular	Elastic	High	3mm
$ASTUR_{12}$	Watery	Circular	Buttery	High	3mm
$ASTUR_{13}$	White	Circular	Elastic	High	3mm
$ASTUR_{14}$	White	Circular	Elastic	High	3mm
$ASTUR_{15}$	Watery	Circular	Buttery	High	3mm
$ASTUR_{16}$	White	Circular	Buttery	Low	2mm
$ASTUR_{17}$	Watery	Circular	Buttery	High	3mm
$ASTUR_{18}$	Watery	Circular	Buttery	High	5mm
$ASTUR_{19}$	Watery	Circular	Elastic	Low	2mm
$ASTUR_{20}$	Watery	Circular	Buttery	Low	2mm
$ASTUR_{21}$	Watery	Circular	Buttery	High	4mm
$ASTUR_{22}$	White	Circular	Buttery	High	3mm

4.1.2. Physiological Characteristics

Salt tolerance

All the isolates tolerated 1% NaCl and 73% were tolerated 2% NaCl. About 64% isolates tolerated 3% NaCl, whereas 55% tolerated 4% NaCl and about 50% of the isolates tolerated up to 5%NaCl. Isolates of $ASTUR_{13,}$ $ASTUR_{16}$ and $ASTUR_{17}$ isolated from Fantalle, $ASTUR_{11}$ from Dugda, $ASTUR_6$ from Boset and $ASTUR_9$ isolated from Bora were observed to be sensitive to %NaCl greater than one; two isolates $ASTUR_{21}$ and $ASTUR_3$ isolated from Liben and Adami Tullu respectively were sensitive to above 2%NaCl concentrations. In contrast two isolates, $ASTUR_1$ and $ASTUR_5$ isolated from Adami Tullu and Boset Woreda respectively were found to be tolerant to up to 8% NaCl; whereas $ASTUR_{20}$ isolated from Liben woreda was tolerant to 9% NaCl (Table 4).

Temperature tolerance

All isolates were able to grow at 15-37^{0}c and only 23% survived to a temperature of 40^{0}c; 18% grew at 5^{0}c and 73% grew at 10^{0}c. Isolates $ASTUR_9$, $ASTUR_{13,}$ $ASTUR_{18,}$ $ASTUR_{19}$ and $ASTUR_{20}$ were found to grow at a temperature of 40^{0}c, whereas isolates $ASTUR_{2,}$ $ASTUR_{13,}$ $ASTUR_{19}$ and $ASTUR_{20}$ showed growth at temperatures of 5^{0}c. Isolates ($ASTUR_{13,}$ $ASTUR_{19}$ and $ASTUR_{20}$) showed growth in both low and high temperatures of 5^0 and 40^{0}c respectively; $ASTUR_9$ and $ASTUR_{14}$ were tolerant to temperature of 10^{0}c to 40^{0}c respectively (Table 4).

PH tolerance

Isolates $ASTUR_{13}$, $ASTUR_{14}$ and $ASTUR_8$ were able to grow at pH-4 and 55% could tolerate pH-5. Whereas, 91% of isolates were able to grow at pH-6 and all the isolates were grown at pH-7 and 8; about 95% of the isolates were able to grow on pH-9 and half of the isolates were able to grow at pH-10, but only 5 isolates were able to grown at pH-11. Isolate $ASTUR_8$ isolated from Boset woreda was tolerant to both acidic and alkaline of media of pH 4-9 and isolates $ASTUR_{13}$ and $ASTUR_{14}$ isolated from Fantalle woreda were tolerate to pH 4-11. Two isolates from Adami Tulu ($ASTUR_1$, $ASTUR_3$), one from Boset ($ASTUR_5$), one from Bora ($ASTUR_{10}$) and one from Fantalle ($ASTUR_{15}$) were tolerant at pH 5-10 (Table 4).

Table 4: Physiological (PH, NaCl and Temperature) Tolerance Test

Isolates	ASTUR$_1$	ASTUR$_2$	ASTUR$_3$	ASTUR$_4$	ASTUR$_5$	ASTUR$_6$	ASTUR$_7$	ASTUR8	ASTUR$_9$	ASTUR$_{10}$	ASTUR$_{11}$	ASTUR$_{12}$	ASTUR$_{13}$	ASTUR$_{14}$	ASTUR$_{15}$	ASTUR$_{16}$	ASTUR$_{17}$	ASTUR$_{18}$	ASTUR$_{19}$	ASTUR$_{20}$	ASTUR$_{21}$	ASTUR$_{22}$	% of tolerance
Temperature tolerance																							
5^0	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	18
10^0	+	+	+	-	-	-	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	73
15^0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
37^0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
40^0	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	+	+	-	-	23
45^0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
PH tolerance																							
4	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	14
5	+	+	+	-	+	-	-	+	-	+	-	-	+	+	+	-	+	+	+	-	-	+	55
9	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	95
10	-	-	+	-	-	+	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	50
11	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+	+	-	23
NaCl % tolerance																							
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
2	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	+	+	73
3	+	+	-	+	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	-	+	64
4	+	+	-	-	+	+	+	-	-	+	+	-	-	+	-	-	-	+	+	+	-	+	55
5	+	+	-	-	+	+	+	-	-	+	+	-	-	+	-	-	-	+	-	+	-	+	50
6	+	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-	-	+	-	+	-	+	36
7	+	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	+	-	+	-	-	27
8	+	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	23
9	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	14
10	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	9
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0

Keys: (+) Presence of growth, (-) Absence of growth

4.2. Substrate Utilization Test

All isolates were grown on mannitol and glucose and 82% of isolates were grown on starch, 50% of isolates were grown on Dextrin white and lactose, 45% on Benzophenone and 41% on sucrose; but no one of the isolates grew on citrate. About 95% of the tested isolates were able to use ammonium acetate, 91% L (+) Asparagines-1-hydrate, 86% Urea, 73% Glycine, 50% Triethanolamine, 41% Aniline, (Table 5).

Table 5: Substrate Utilization Tests

Substrates	**Isolates**	
Carbon sources	**No**	**% of utilization**
Mannitol	22	100
Starch	18	82
Glucose	22	100
Sucrose	9	41
Lactose	11	50
Benzophenone	10	45
Citrate	0	0
Dextrin white	11	50
Nitrogen source	**No**	**% of utilization**
L(+) Asparagines-1-hydrate	20	91
Glycine	16	73
Ammonium Acetate	21	95
Aniline(alanine)	9	41
Triethanolamine	11	50
Urea	19	86

4.3. Intrinsic Antibiotic Resistance

IAR test was carried out to show *Rhizobia* were sensitive or resistant to the following antibiotics namely: Vancomycin, Chloramphenicol, Ampicillin, Tetracycline, Norfloxacin, Erythromycin and Ciprofloxacin. Accordingly, 91% of the isolates were resistant to vancomycin and Chloramphenicol at both 5&10 % (μgml^{-1}) concentrations. Isolates like: $ASTUR_2$, $ASTUR_8$, $ASTUR_{11}$, $ASTUR_{13}$, $ASTUR_{15}$, $ASTUR_{17}$, $ASTUR_{18}$ and $ASTUR_{19}$ showed resistance to all antibiotics, whereas $ASTUR_1$, $ASTUR_7$, $ASTUR_{10}$, $ASTUR_{12}$ and $ASTUR_{21}$ were resistant strains, which were susceptible to two antibiotics (Table 6).

Isolate $ASTUR_5$ was found to be sensitive to all antibiotics except chloramphenicol. Isolates $ASTUR_{20}$ and $ASTUR_{16}$ were found to be sensitive to tetracycline and $ASTUR_9$ was found to be sensitive only to Ciprofloxacin; $ASTUR_4$ was observed to be sensitive to all antibiotics except for tetracycline and chloramphenicol. Ciprofloxacin, Norfloxacin and Tetracycline were the most potent antibiotics that allow the growth of a few isolates. About 50% of the isolates were unable to grow in the presence of ciprofloxacin (Table 6). The data showed the following pattern was the Antibiotic resistance: Chloramphenicol and vancomycin> Erythromycin>Ampicillin > Tetracycline and Norfloxacin> Ciprofloxacin (Table 6).

Table 6: Antibiotics Susceptibility Test

	% of Resistance of the Isolates	
Antibiotics	**5% µgml^{-1}**	**10 %µgml^{-1}**
Ciprofloxacin	50	50
Tetracycline	64	64
Ampicillin	77	77
Vancomycine	91	91
Erythromycin	82	86
Chloramphenicol	91	91
Norfloxacin	64	64

4.4. Evaluation of Symbiotic Effectiveness

The ineffectiveness and effectiveness of the twenty-two isolates were studied on sand pots under green house conditions. The minimum nodule number record was 16 for isolate of $ASTUR_3$ and maximum nodule number record was 109 for isolate of $ASTUR_{16}$ as presented in (Table 7). The highest NDW was 0.121 gm/pl and the least NDW was 0.039gm/pl for isolates $ASTUR_{16}$ and $ASTUR_{11}$, respectively. Regarding to relative effectiveness based on shoot dry mass of the isolates over positive control, 27% of the isolates were found to be highly effective, more than 36% isolates were found to be effective, about 27% isolates were found to be lowly effective and only two of the tested isolates were ineffective. HE isolates were; $ASTUR_3$, $ASTUR_4$, $ASTUR_8$, $ASTUR_{11}$, $ASTUR_{12}$ and $ASTUR_{22}$ with SDW greater than 0.725g/pl. Isolates $ASTUR_1$, $ASTUR_2$, $ASTUR_5$, $ASTUR_{10}$, $ASTUR_{13}$, $ASTUR_{14}$, $ASTUR_{15}$ and $ASTUR_{19}$ were found to be effective; isolates $ASTUR_6$, $ASTUR_7$, $ASTUR_9$, $ASTUR_{16}$,

$ASTUR_{21,}$ and $ASTUR_{20}$ were lowly effective whereas $ASTUR_{17}$ and $ASTUR_{18}$ were ineffective as indicated in (Table 7).

Table 7: Symbiotic effectiveness test

ISOLATES	NN	NDW	SDW	SE%	Effectiveness
Control(-)	0	0	0.192	21.94	
Control(+)	0	0	0.875	100.00	
$ASTUR_1$	25	0.071	0.479	54.74	E
$ASTUR_2$	75	0.059	0.557	63.65	E
$ASTUR_3$	16	0.069	0.725	82.85	HE
$ASTUR_4$	90	0.045	0.914	104.45	HE
$ASTUR_5$	85	0.091	0.519	59.31	E
$ASTUR_6$	100	0.102	0.397	45.37	LE
$ASTUR_7$	56	0.091	0.328	37.48	LE
$ASTUR_8$	61	0.089	0.993	113.48	HE
$ASTUR_9$	82	0.100	0.399	45.60	LE
$ASTUR_{10}$	94	0.078	0.576	65.82	E
$ASTUR_{11}$	23	0.039	0.889	101.6	HE
$ASTUR_{12}$	69	0.079	0.725	82.85	HE
$ASTUR_{13}$	24	0.065	0.659	75.31	E
$ASTUR_{14}$	89	0.091	0.439	50.17	E
$ASTUR_{15}$	93	0.103	0.558	63.77	E
$ASTUR_{16}$	109	0.121	0.389	44.45	LE
$ASTUR_{17}$	61	0.109	0.256	29.25	I
$ASTUR_{18}$	73	0.070	0.299	34.17	I
$ASTUR_{19}$	33	0.069	0.681	77.82	E
$ASTUR_{20}$	19	0.044	0.309	35.31	LE
$ASTUR_{21}$	95	0.079	0.357	40.80	LE
$ASTUR_{22}$	101	0.111	0.969	110.74	HE

Keys: - Control (+) – positive control, - Control (-) – Negative control, E – Effective, HE – highly effective, LE – lowly effective, I – ineffective,

5. DISCUSSION

5.1. Cultural and growth characteristics

Twenty two isolates of root nodule bacteria were isolated from East Shoa Zone Oromia, Ethiopia. All isolates were Gram-negative (pink after Gram-staining); rod shaped bacteria and failed to absorb congored. All the isolates re-nodulated the host and were authenticated as root nodule bacteria; Alemayehu (2006) also observed that all collected rhizobia from common bean nodules found to be authenticated as root nodule bacteria. Fifteen isolates were grown on Luria Bertani (LB) but 7 isolates were unable to grow on LB. Anteneh (2007) revealed that 79 % of tested isolates were able to grow on LB-medium and the rest were unable to grow on the LB Media. Alemayehu (2006) found that two isolates were able to grow on LB media and the rest failed to grow. Similarly, Andrade *et al* (2002) isolated some isolates that were not able to grow on LB media, which are the characteristics of all rhizobia nodulating common bean except *R.tropici*.

Most of the isolates except $ASTUR_{3,}$ $ASTUR_{5,}$ $ASTUR_{7,}$ $ASTUR_{16,}$ $ASTUR_{19}$ and $ASTUR_{20}$ produced acid on YEMA-BTB medium and did change the YEMA-BTB medium in to yellow and 27% of the isolates showed slow growing. Based on colony size the isolates were observed to 1.5-5mm colony size. All the isolates were in the range of colony size between 1.5-4mm, except isolate from Fantalle Nukusa got higher size of 5mm; 1.5 - 4.5mm were reported by Bayou (2015). According to Jordan (1984), most of the isolates were categorized in to fast growing rhizobia based on their acid production and large growth with production of high exopolysaccharid and colony size greater than 2mm; Bayou (2015) reported almost all isolates he isolated were categorized under fast growing rhizobia. Smooth and gummy colonies of bean rhizobia could be *R. leguminosarum* or *R. etli* whereas rough growth is a characteristic of *R. gallicum* (Silva *et al.,* 2003). In this study Isolates $ASTUR_{3,}$ $ASTUR_{5,}$ $ASTUR_{7,}$ $ASTUR_{16,}$ $ASTUR_{19}$ and $ASTUR_{20}$ were formed colony size of 1.5mm, 2mm, 1.5mm, 2mm, 2mm and 2mm respectively; which were alkaline producers and categorized as slow growing *Rhizobium* known as *Bradyrhizobium* according to (Jordan, 1984) and may be *Rhizobium gallicum* as Silva et *al (*2003).

15 isolates were able to solubilize tricalcium phosphate in sperber solid medium. Isolates $ASTUR_{13,}$ $ASTUR_{21,}$ $ASTUR_{9,}$ $ASTUR_{10,}$ $ASTUR_{6}$ and $ASTUR_{19}$ isolated from Fantalle of Ilala, Liben of Oda Jida, Bora of Tuchi Dako, Boset of Sifa Bate and Liben of Liben Gadulla woreda produced large halo zones greater than 1mm. Anteneh (2007) reported isolates from Boset woreda were grouped under better inorganic phosphorus solubilizer rhizobia. Bayou (2015) observed that able to solubilize tricalcium phosphate.

All the isolates were tolerant to 1% NaCl; about 73% were tolerated to 2%NaCl, about 64% isolates tolerated to 3% NaCl, about 55% to 4% NaCl and about 50% of the isolates tolerated up to 5%NaCl; Hungaria *et al.,* (2000) report that *R. Phaseoli* is the most halotolerant rhizobia with high salt concentrations of (4%-5%). Priefer *et al* (2001) reported isolates of high salt concentration (2-10) % tolerant, Anteneh (2007) also isolated, isolates of highly salt tolerant up to 10% NaCl concentration. Maximum and minimum temperature for *the growth* of the isolates was recorded at 40°C and 5°C, respectively. All isolates were able to grow between 15-37^{0}C and only 23% survived a temperature of 40^{0}C; Hungria *et al.,* (2000) also showed common bean rhizobia tolerated 40^{0}C; Kucuk *et al.,* (2006) isolated common bean rhizobia surviving an incubation temperature of 42^{0}C. Mulugeta *et al* (2013) observed that all isolates were able to grow at 37-40°C.

Three isolates were able to grow at pH-4; Alemayehu (2006) reported that one of his isolate from southern Ethiopia tolerated pH-4; some fast growing strains isolated by Jordan (1984) were also able to grow at pH-4. Anteneh (2007) reported that, some of the isolates were able to grow at pH-4.5; Amarger *et al*. (1997) revealed *R. tropici* were able to grow at pH 4. About 55% of the isolates in this study were tolerant to pH-5; Musiyiwa *et al* (2005) demonstrated that *R. etli* and *R. gallicum* isolates were able to grow at pH 5. About 95% of the isolates were able to grow at pH-9 and half of the isolates were able to grow at pH-10, 5 isolates grew at pH-11, Mulugeta *et al* (2013) isolated 9 isolates were grown at pH-11. All isolates grew on mannitol and glucose and 82% of isolates were grown on starch; the same result was reported by De Oliveira *et al.,* (2007) who reported that *Rhizobium* strains have capability to use starch and found positive for utilization of glucose as carbon source. Jordon (1984) observed that dextrin was rarely utilized by *Rhizobium*, but 50% of the isolates in this study were able to utilize dextrin as a carbon source. None of the isolates grew on citrate; however, Datta *et al*.,

(2015) found that citrate utilization as a carbon source was positive in *Rhizobium Phaseoli* and *Rhizobium trifolii.* About 91% of L (+) Asparagines-1-hydrate was utilized; Anteneh (2007) reported all isolates utilize L- Asparagine. 73% of Glycine was utilized; Alemayehu (2006) showed that Glycine was found to be utilized by 87% of tested isolates.

About 91% of the tested isolates were resistant to vancomycin and Chloramphenicol at both 5and 10 (μgml^{-1}). Isolates like: $ASTUR_2$, $ASTUR_8$, $ASTUR_{11}$, $ASTUR_{13}$, $ASTUR_{15}$, $ASTUR_{17}$, $ASTUR_{18}$ and $ASTUR_{19}$ were observed to be tolerant to all antibiotics, whereas $ASTUR_1$, $ASTUR_7$, $ASTUR_{10}$, $ASTUR_{12}$ and $ASTUR_{21}$ were only susceptible on two of tested antibiotics. Isolate $ASTUR_5$ was found to be sensitive to all antibiotics except chloramphenicol. Isolates $ASTUR_{20}$ and $ASTUR_{16}$ were sensitive only for tetracycline and $ASTUR_9$ was found to be sensitive only for Ciprofloxacin; $ASTUR_4$ was observed to be sensitive to all antibiotics except for tetracycline and chloramphenicol. Ciprofloxacin, Norfloxacin and Tetracycline were found to be the most potent antibiotics that allow the growth of a few isolates. About 50% of the isolates were unable to grow with the presence of ciprofloxacin and about 9% of the tested isolate were susceptible to chloramphenicol and vancomycine. Sharma *et al.*, (2010) reported that most of the *Rhizobium* isolates were sensitive to chloramphenicol but in this study most of the isolates were sensitive to Ciprofloxacin.

5.2. Evaluation of Symbiotic Effectiveness on Sand Culture

The minimum and maximum nodule number recorded was 16 and 109 respectively. More than 27% of the tested isolates were found to be highly effective (80-100%). More than 36% of the tested isolates were found to be effective (50-80%), about 27% isolates were found to be lowly effective (35-50%) and only two (about 9%) of the tested isolates were ineffective (< 35%). Antenah (2007) reported that 89% of the tested were effective and very effective in terms of symbiotic effectiveness. In this study isolates $ASTUR_3$, $ASTUR_4$, $ASTUR_8$, $ASTUR_{11}$, $ASTUR_{12}$ and $ASTUR_{22}$ with SDW > 0.725g/pl were obtained as highly effectives (HE); Which is similar to the result of Anteneh (2007) who reported that all isolates from East Shoa were found to be effective. This shows the presence of effective bean rhizobia in East Shoa soils with the possibility of selecting interesting strains able to nodulate the host abundantly and effectively.

6. CONCLUSION

Based on different tests twenty two isolates were isolated from East Shoa Zone Oromia, Ethiopia; from which, isolates like $ASTUR_{1,}$ $ASTUR_{2,}$ $ASTUR_{3,}$ $ASTUR_{4,}$ $ASTUR_{5,}$ $ASTUR_{8}$ $ASTUR_{9,}$ $ASTUR_{10,}$ $ASTUR_{11,}$ $ASTUR_{13,}$ $ASTUR_{14,}$ and $ASTUR_{20}$ were observed phosphate solubilizer, antibiotic resistant, halotolerant, extreme temperature tolerant, tolerant to acid and alkaline and highly effective or effective. They were halotolerant in the range of concentration of NaCl, 1% up to 11%; they also tolerated to extreme temperature from 5^0c to 40^0c in minimum growth and all the tested isolates found to tolerate 15^0c -37^0c. Rhizobia also tolerated different pH from acidic to alkaline condition in the range of 4- 11pH. They utilized broader range of carbon and nitrogen source. They were resistance to most of antibiotic and could survive in the presence of most antibiotics; some of isolates might be sensitive to ciprofloxacin and tetracycline. More than 60% rhizobia isolated from East Shoa Zone, proved to be effective and highly effective. This organism will greatly enhance agricultural production thereby reducing the environmental threat of synthetic nitrogen fertilizers. These characteristics are very important in the screening of elite strains as inoculants for plant production under extreme environmental conditions provided that their performance validate under different stressed soil environments in field trials.

7. RECOMMENDATION

Isolates those were able to grow at high pH and high salt concentration may favour the establishment of the rhizobia and may represent an advantage of these over other inoculate use for common bean. Nevertheless, rhizobial strains that are tolerance to high salinity, pH and temperature levels in laboratory may not be effective in nitrogen fixation and survival these adverse condition under external environment. Thus, intensive study is needed under field condition or field trial of the elite isolates is needed. Molecular characterization of the screened isolates is needed with *Rhizobium* isolates from common bean to confirm whether they represent different species. Soils from other locations of East Shoa Zone and other Rift Valley area not covered in this study should be investigated in order to provide further information about the species of rhizobia that effectively nodulate common bean and developing a multi-inoculum for common bean in Ethiopia. It is also recommended that isolating rhizobia species that can do cross nodulation.

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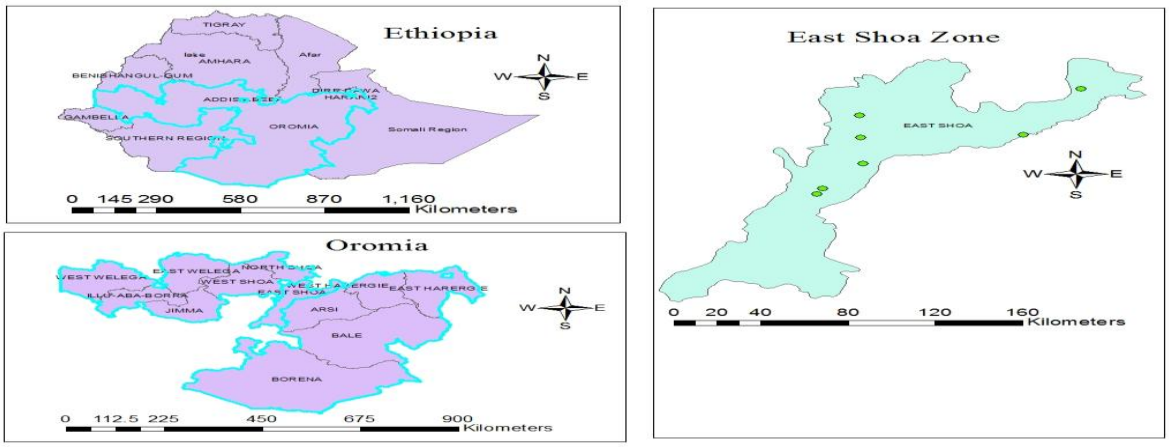
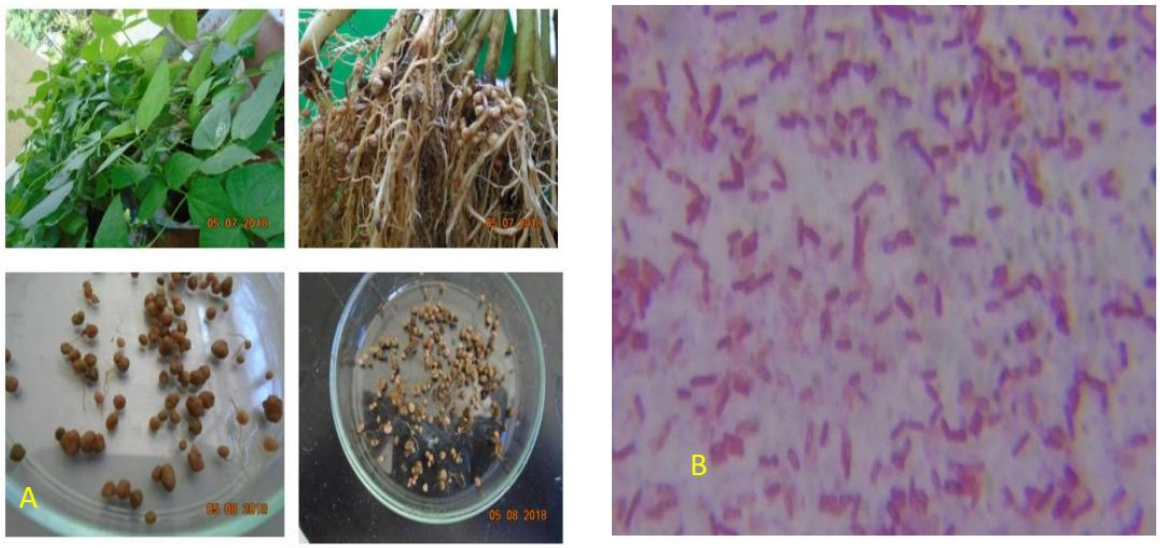
APPENDIXES



Appendix 1: Map of the Study Area



Appendix 2: Nodule trapped from common bean(A) and rhizobia gram-staining(B)