Ethiop. J. Biol. Sci. **18(1): 37–53, 2019**
 ISSN: 1819-8678

RESEARCH ARTICLE

PHENOTYPIC AND SYMBIOTIC CHARACTERISTICS OF NATIVE RHIZOBIA NODULATING COMMON BEAN (*PHASEOLUS VULGARIS* L.) IN SOILS OF EAST SHOA, ETHIOPIA

Dugo Nura[1] and Zerihun Belay[1,*]

ABSTRACT: The common bean (*Phaseolus vulgaris* L.) is a cultivated legume, important source of protein, vitamins and micronutrients. Common bean is a relatively permissive host, nodulated by different genera and species of fast-growing and slow-growing rhizobia. This study was aimed to evaluate symbiotic and phenotypic diversity of common bean nodulating rhizobia in various areas of East Shoa Zone, Oromia, Ethiopia. Soil samples were collected from 11 representative kebeles and transported to Adama Science and Technology University (ASTU) for nodule trapping and isolation of rhizobia, and evaluating for their symbiotic effectiveness in pot experiments under greenhouse condition. The isolates were tested for their physiological characteristics such as substrate utilization, inherent antibiotic resistance, and tolerance to different pH, temperature and concentrations of NaCl. Thus, twenty-two isolates were collected, and most of them changed YEMA-BTB media to yellow colour showing that they are acid producers and fast growers. More than 73% of the isolates showed the ability to solubilize tricalcium phosphate on Picovaskaya agar medium with solubilization index ranging from 1.04 to 2.4. Twenty-seven percent of the isolates were highly effective, and more than 36% of the isolates were effective. Our results demonstrated the presence of compatible indigenous rhizobia, some of which could have high potential of symbiotic nitrogen fixation, and can be evaluated under field condition for inoculant production.

Key words/phrases: Highly effective, Nodule trapping, Phosphate solubilisation, Physiological characters.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) 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 (Darkwa *et al*., 2016). The crop contains

[1]School of Applied Natural Science, Department of Applied Biology, Adama Science and Technology University, Adama, Ethiopia. E-mail: zebelay2009@yahoo.com
* Author to whom all correspondence should be addressed.

65% of total protein, 32% of energy and high concentration of iron (55 μg/g) compared to crops such as wheat, rice and maize (Petry *et al*., 2015).

Most legume species including common bean (*Phaseolus vulgaris* L.), fix atmospheric nitrogen by symbiotic rhizobia that favour growth under low soil nitrogen conditions. Nitrogen fixation by legumes is a major input of nitrogen into natural and agricultural ecosystems (Andrews and Andrews, 2017). It is a relatively permissive host, nodulated by different species of rhizobia; *Rhizobium etli*, *R. tropici*, *R. leguminosarum* symbiovar (sv.) *phaseoli*, *R. giardinii*, *R. gallicum* and *Bradyrhizobium* spp. (Amarger *et al*., 1997).

Common beans are considered to be poor nitrogen fixers among other leguminous crop (Hardarson and Danso, 1993). It was concluded that rates of nitrogen fixation in bean were low, at 25 to 71 kg N_2 fixed ha^{-1} for mid-to long-season cultivars (Graham, 1981). However, recently Farid (2015) reported that nitrogen fixing capacity of common beans ranged from 2.7 to 69.7 kg N_2 fixed ha^{-1}, which represents a range of 5.2 to 78.5% nitrogen derived from the atmosphere (%Ndfa). Heilig (2015) also examined 79 navy and black commercial cultivars and advanced breeding lines under organic production and found a similar range for nitrogen fixing capacity (16 to 94 kg N_2 ha^{-1}) and for %Ndfa (9.8 to 71.7%).

In Ethiopia, some studies have been made on the genetic diversity (Desta Beyene *et al*., 2004), phenotypic and symbiotic effectiveness (Alemayehu Workalemahu and Fassil Assefa, 2007a;b; Anteneh Argaw, 2007) of indigenous common bean rhizobia from some part of central, southern and eastern parts of Ethiopia. However, there is still a need for more information about the taxonomic and symbiotic diversity of rhizobia nodulating common bean from different agro-ecological zones, particularly in the warm and lowland areas of the country. Hence, this study was initiated with the aim of isolating rhizobia nodulating common bean and to characterize the phenotypic and symbiotic performance of the isolates collected from some bean growing area of East Showa Zone, Oromia, Ethiopia.

MATERIALS AND METHODS

Study area

The rhizobia isolation, characterization and pot experiment were carried out at Adama Science Technology University (ASTU), Department of Applied Biology. The soil sample collection was carried out in the different districts of East Shoa Zone with the help of GPS and previous history for common

bean cultivation. East Shoa Zone is located between 7°33′50″N–9°08′56″N and from 38°24′10″E–40°05′34″E, with altitudes ranging from 926–1,945 m asl. The temperature in the study area varies from less than 10°C along high altitudes to above 30°C in Rift Valley lowland areas and the mean temperature is 20°C. The rainfall in the area is ranged from 600 mm–1,000 mm with mean annual rainfall of 816 mm (Nigatu Alemayehu, 2013) (Fig. 1).

Soil sampling

Soil samples were collected randomly during March 2017 from sampling sites of East Shoa zone where common bean has been grown for long time. Three kg of soil samples from each district were pooled from 5–15 cm depth. The samples were taken to the laboratory for nodulation test and analysis. The corresponding GPS data, including altitude and local names were also recorded (Table 1).

Table 1. Sample location, altitude, soil pH and history of the crop in the fields.

Sample site		Geographical location			Field history		
(Woreda)	**(Kebele)**	**Altitude (m.a.s.l)**	**Latitude (N)**	**Longitude (E)**	**Previous**	**Current**	**Soil pH**
Fantalle	Ilala	926	08°55′18.7″	39°49′17.3″	Maize	Common bean	7.79
	Nukusa	1002	08°53′14.6″	39°48′46.7″	Maize	Common bean	8.32
Boset	Sifa Bate	1210	08°31′37.6″	39°34′49.6″	Teff	Common bean	7.99
	Buta Donkore	1473	08°41’59″	39°26′33″	Teff	Common bean	8.07
	Rukecha Bokore	1555	08°37′14.3″	39°28′07.2″	Teff	Common bean	7.60
Liben	Gadulla	1655	08°30′07.5″	38°54′19.4″		Common bean	8.40
	Oda Jidda	1695	08°30’12.1″	38°54′13.5″	Pea	Common bean	8.37
Ada’a	Dire	1945	08°41′41″	38°53′55″	Barley	Common bean	7.29
Dugda	Woyyo Gabrel	1624	08°03′50.8″	38°44′56.4″	Maize	Common bean	7.61
Bora	Tuchi Dako	1644	08°16′45.4″	38°54′43.8″	Teff	Common bean	7.70
Adami/T/J Kombolcha	Abosa (Batu 03)	1643	08°01′03″	38°43′21.1″	Maize	Common bean	8.35

Nodule trapping

Each representative soil sample was mixed and sieved using 2 mm sieve, and filled into 3 kg capacity plastic pot, surface sterilized by swabbing with 95% alcohol. Seeds of common bean (variety, Ser-119) obtained from Melkassa Agricultural Research Centre (MARC) was surface sterilized with 95% ethanol for 30 seconds and then soaked in NaOCl (0.25%) for 3 min and washed with distilled and sterilized water (Vincent, 1970). The seeds were planted on each pot to trap the native rhizobia from each location. The seedlings were maintained for 5 weeks under greenhouse conditions. The plants were then uprooted and cut at crown and soil was washed off from

the root and nodules. The fresh and undamaged nodules were picked and preserved in closed container over silica gel until isolation (Somasegaran and Hoben, 1994).

Isolation of rhizobia

The preserved nodules were soaked and rinsed in distilled water and surface sterilized briefly (5 sec) with 95% ethanol and 3% NaOCl for 4 min and rinsed five times in sterile water and crushed in normal saline solution (0.85% NaCl). A loop-full of the crushed nodule was then streaked on YEMA medium containing 10 g Mannitol, 0.5 g K_2HPO_4, 0.2 g $MgSO_2.7H_2O$, 0.1 g NaCl, 1 g yeast extract, 15 g agar and 1000 ml distilled water (Somasegaran and Hoben, 1994) and incubated at 28°C for 3–5 days. The isolates were preserved in culture slants at 4°C for further characterization.

Designation of isolates

All isolates were named as ASTUR; Adama Science and Technology University Rhizobia with different numbers to differentiate the isolates.

Growth and colony characters

All isolates 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 YEMA containing 25 mg ml^{-1} bromothymol blue (BTB) to determine their ability to produce acid or base and change the medium (Jordan, 1984). The isolates were characterized based on their colour (watery and white), size (mm), shape (circular) and extracellular polysaccharide (EPS) production (low and high) after having grown on YEMA plates at 28°C for 3–5 days (Ahmed *et al*., 1984).

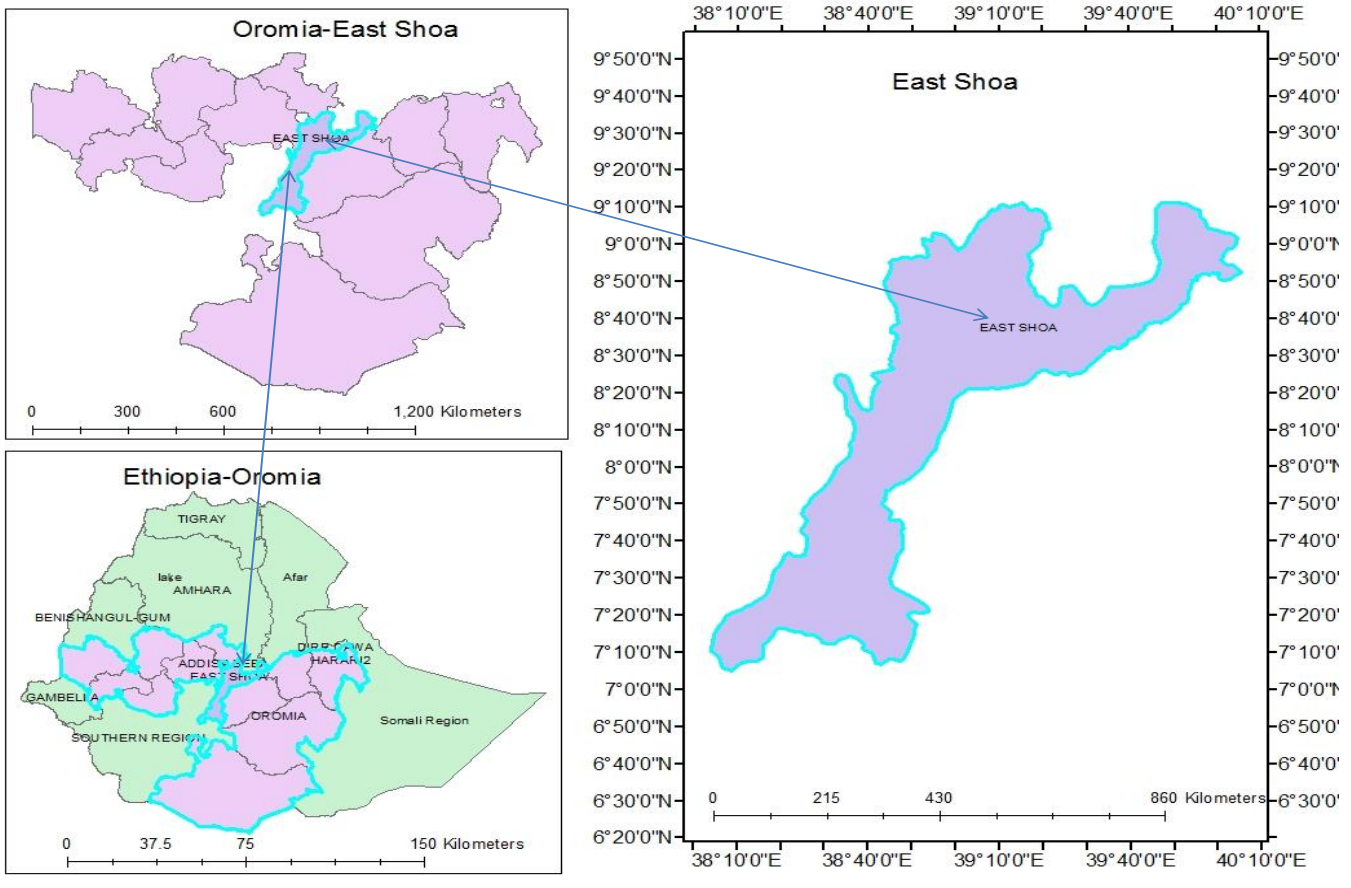

Fig.1. Map of the study area.

Phosphate solubilization

The ability of rhizobia isolates to solubilize tricalcium phosphate [$Ca_3(PO_4)_2$] was estimated using Picovaskaya Agar medium. Rhizobium isolate was inoculated to separate Petri plates of Picovaskaya Agar medium containing (g/l): Glucose 10 g; tricalcium phosphate (TCP) as P source 5 g; ammonium sulphate 0.5 g; yeast extract 0.5 g; magnesium sulphate 0.1 g; sodium chloride 0.2 g; manganese sulphate 0.002 g; and agar 15 g. Rhizobia isolate that has potential to solubilize phosphorus was identified by production of a clear halo zone around the colonies. Phosphate solubilization index was determined according to Premono *et al*. (1996). Solubilization Index (SI) = Ratio of the total diameter (colony + halozone) to colony diameter on PA.

Physiological characteristics

Salt tolerance was checked with wither the bacterial isolates could grow and reproduce in NaCl concentration. Growth of the isolates on salt was investigated by cultivating them on YEMA medium supplemented with NaCl at a concentration of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10%. 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°C on YEMA medium. Tolerance to extreme pH was tested on TY agar medium set at 4, 5, 9 and 10 pH values (Singh *et al*., 2011).

Intrinsic antibiotic resistance

Resistance of the isolates to different concentrations of antibiotics (µg/ml) was determined using YEMA to which filter sterilized (0.22 µm) antibiotics was added. Seven antibiotics, chloramphenicol, erythromycin, ampicillin, vancomycin, norfloxacin, ciprofloxacin and tetracycline were used at 5 and 10% (µg/ml) concentrations (Amarger *et al*., 1997).

Carbon source utilization

All isolates were tested for their ability to utilize various carbon sources on carbohydrate-free basal medium containing: $MgSO_4.7H_2O$ 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 g in 1000 ml of distilled water (Somasegaran and Hoben (1994). The pH of the medium was adjusted to 7. The carbon sources were; starch, glucose, lactose, sucrose, dextrose, benzophenone, citrate and mannitol. The presence or absence of growth was recorded after 5 days.

Nitrogen source utilization

The ability of isolates to utilize different N sources: urea, triethanolamine, aniline, ammonium acetate, glycine and L (+) asparagine-1-hydrate, was determined as described by Amarger *et al*. (1997). The different nitrogen sources were added at a concentration of 0.5 g/litre to a basal medium containing (per litre): K_2HPO_4 1 g; KH_2PO_4 1 g; $FeCl_3.6H_2O$ 0.01 g; $MgSO_4.7H_2O$ 0.2 g; $CaCl_2$ 0.1 g; Mannitol 1 g; and agar 15 g. All of the substrates were filter sterilized before they were added to the basal medium. Cultures were incubated at 28°C, and the growth was recorded after 4 or 5 days.

Authentication and symbiotic effectiveness test

All isolates were evaluated for authentication and symbiotic effectiveness in pot experiment conducted under greenhouse condition (Somesegaran and Hoben, 1994). Seeds of common bean (Ser-119) were surface sterilized as before prior to sowing. Three uniform seeds were sown in each surface disinfected 3 kg capacity plastic pot filled with sieved, acid-washed sand. Each isolate was grown on YEM liquid medium to logarithmic phase (10^9 cells ml^{-1}) and inoculated on the base of each seedling. Two controls were included, negative control with no inoculation and positive control with addition of chemical fertilizer 0.05% (W/V) KNO_3 per week. All pots were supplied with distilled water every 2 days and fertilized once a week with N-free nutrient solution (Broughton and Dilworth, 1970). The pots were arranged in Complete Random Design (CRD); the plants were uprooted after 6 weeks and their effectiveness was evaluated on the basis of nodule number, nodule dry weight and shoot dry weights after drying at 70°C for 2 days in oven. Symbiotic effectiveness of isolates was calculated according to the equation proposed by Date *et al*. (1993) cited in Purcino *et al*. (2000) [100 x 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%.

Data analysis

The data were recorded, organized, and summarized in a Microsoft excel sheet. Phenotypic and features including carbon source and nitrogen source, resistance to antibiotics, growth in a range of pH, temperature and concentration of NaCl and symbiotic effectiveness were analyzed by percent and presented in tables and figures.

RESULTS

Cultural and growth characteristics

In this study, 22 isolates of rhizobia were isolated from root nodule of common bean (*Phaseolus vulgaris*) grown on soils collected from different sites of East Shoa Zone Oromia, Ethiopia, except soils from Dire of Ada'a woreda and Rukecha of Boset Woreda. Sixteen isolates changed the YEMA-BTB medium in to yellow colour indicating that they were acid producers; whereas the remaining six isolates changed the medium to blue. Five isolates grew on peptone-glucose medium with bromocresol purple; 15 isolates grew on Luria Bertani (LB) and almost 73% were able to solubilize tricalcium phosphate on Picovaskaya agar medium. The solubilization index (SI) of the isolates ranged from 1.04 to 2.4. Among these, isolate $ASTU_6$, $ASTU_{13}$, $ASTU_{19}$ and $ASTU_{21}$ produced SI equal or greater than 2 as shown in Table 2. The result of cultural characteristics of the isolates is presented in Table 2. All isolates showed circular colony shape, with white mucoid (45.4%) and watery colonies (54.5). Most of the isolates (73%) produced high EPSs. Sixty eight percent of the isolates formed colony size greater than or equal to 2.5 mm while 32% formed colony size less than 2.5 mm (Table 2).

Table 2. Growth characteristics of rhizobia nodulating common bean (*Phaseolus vulgaris*).

Isolates	Colour	EPS production	Colony size (mm)	YEMA-BTB	PSI
$ASTUR_1$	Watery	High	2.5	Yellow	1.2
$ASTUR_2$	White	High	3	Yellow	1.3
$ASTUR_3$	White	Low	1.5	Blue	-
$ASTUR_4$	White	High	2.5	Yellow	1.6
$ASTUR_5$	Watery	Low	2	Blue	-
$ASTUR_6$	White	High	3	Yellow	2.2
$ASTUR_7$	Watery	Low	1.5	Blue	1.8
$ASTUR_8$	Watery	High	3	Yellow	1.4
$ASTUR_9$	Watery	High	2.4	Yellow	1.58
$ASTUR_{10}$	White	High	3	Yellow	1.4
$ASTUR_{11}$	White	High	3	Yellow	1.2
$ASTUR_{12}$	Watery	High	3	Yellow	-
$ASTUR_{13}$	White	High	3	Yellow	2
$ASTUR_{14}$	White	High	3	Yellow	1.6
$ASTUR_{15}$	Watery	High	3	Yellow	1.2
$ASTUR_{16}$	White	Low	2	Blue	1.4
$ASTUR_{17}$	Watery	High	3	Yellow	-
$ASTUR_{18}$	Watery	High	5	Yellow	1.04
$ASTUR_{19}$	Watery	Low	2	Blue	2.4
$ASTUR_{20}$	Watery	Low	2	Blue	-
$ASTUR_{21}$	Watery	High	4	Yellow	2
$ASTUR_{22}$	White	High	3	Yellow	-

Keys: ASTUR: Adama Science and Technology University rhizobia, EPS: extracellular polysaccharide, YEMA-BTB: yeast extract mannitol agar-bromothymol blue, PSI: phosphate solubilization index

Physiological characteristics

Salt tolerance

The isolates exhibited variations in their NaCl tolerance (Table 3). All isolates tolerated 1% NaCl and 73% tolerated 2% NaCl; whereas nearly 50% of the isolates were able to grow on 5% NaCl. Isolate $ASTUR_{13}$, $ASTUR_{16}$ and $ASTUR_{17}$ from Fantalle, $ASTUR_{11}$ from Dugda, $ASTUR_{6}$ from Boset and $ASTUR_{9}$ from Bora were observed to be sensitive to percent of NaCl greater than one; two isolates $ASTUR_{21}$ and $ASTUR_{3}$ from Liben and Adami Tullu, respectively, were found to be sensitive to above 2% NaCl concentrations. However, isolates $ASTUR_{1}$ (Adami Tullu) and $ASTUR_{5}$ (Boset) and $ASTUR_{20}$ from Liben Woreda were tolerant to 8% and 9% NaCl, respectively; whereas $ASTUR_{10}$ from Bora and $ASTUR_{14}$ from Fentale Woreda were extraordinarily tolerant to 9% NaCl concentration (Table 3).

Temperature tolerance

All isolates were able to grow at 15–37°C and only 23% survived to the highest temperature of 40°C; and 18% grew at the lowest temperature of 5°C (Table 3). Isolates $ASTUR_{9}$, $ASTUR_{13}$, $ASTUR_{18}$, $ASTUR_{19}$ and $ASTUR_{20}$ showed growth at a temperature of 40°C, whereas isolates $ASTUR_{13}$, $ASTUR_{19}$ and $ASTUR_{20}$ showed growth at both low and high temperatures of 5°C and 40°C, respectively (Table 3).

pH tolerance

Almost 50% of the isolates were able to grow between pH 5–pH 10 (Table 3). Isolates $ASTUR_{8}$, $ASTUR_{13}$, and $ASTUR_{14}$ were able to grow at pH 4 but only 11 isolates were able to grown at pH 10. Remarkably, isolate $ASTUR_{13}$ and $ASTUR_{14}$ from Fantalle Woreda showed tolerance to pH 4–10 and $ASTUR_{8}$ from Boset Woreda was tolerant to pH 4–9 (Table 3).

Substrate utilization test

All isolates were able to assimilate mannitol and glucose whereas 82% and 50% of the isolates catabolized starch and lactose, respectively (Table 4). However, none of the isolates grew on citrate. Likewise, almost all isolates utilized ammonium acetate, asparagine, and urea; and more than half of the isolates utilized glycine and triethanolamine.

Table 3. Temperature, pH and NaCl tolerance of common bean rhizobia isolated from different locations of East Showa Zone, Oromia, Ethiopia.

Isolates	ASTUR1	ASTUR2	ASTUR3	ASTUR4	ASTUR5	ASTUR6	ASTUR7	ASTUR8	ASTUR9	ASTUR10	ASTUR11	ASTUR12	ASTUR13	ASTUR14	ASTUR15	ASTUR16	ASTUR17	ASTUR18	ASTUR19	ASTUR20	ASTUR21	ASTUR22	% of tolerance
Temperature tolerance																							
50	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	18
100	+	+	+	-	-	-	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	73
150	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
370	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
400	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	+	+	+	-	-	23
450	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
pH tolerance																							
4	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	14
5	+	+	+	-	+	-	-	+	-	+	-	-	+	+	+	-	+	+	+	-	-	+	55
9	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	95
10	-	-	+	-	-	+	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	50
NaCl % tolerance																							
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
2	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	+	+	73
3	+	+	-	+	+	+	+	-	-	+	+	-	-	+	+	-	-	+	+	+	-	+	64
4	+	+	-	-	+	+	+	-	-	+	+	-	-	+	-	-	-	+	+	+	-	+	55
5	+	+	-	-	+	+	+	-	-	+	+	-	-	+	-	-	-	+	-	+	-	+	50
6	+	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-	-	+	-	+	-	+	36
7	+	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	+	-	+	-	-	27
8	+	-	-	-	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	23
9	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	14
10	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	9

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

Intrinsic antibiotic resistance

IAR test was carried out to show resistance/sensitivity of the isolates to the following antibiotics: vancomycin, chloramphenicol, ampicillin, tetracycline, norfloxacin, erythromycin and ciprofloxacin. The data showed that majority of the isolates were resistant to vancomycin and chloramphenicol at all tested concentrations (Table 4). Isolates $ASTUR_2$, $ASTUR_8$, $ASTUR_{11}$, $ASTUR_{13}$, $ASTUR_{15}$, $ASTUR_{17}$, $ASTUR_{18}$ and $ASTUR_{19}$ showed resistance to all antibiotics (data not shown). About 50% of the isolates were unable to grow in the presence of ciprofloxacin. The data indicated the following pattern of antibiotic resistance: chloramphenicol and vancomycin>erythromycin>ampicillin>tetracycline and norfloxacin>ciprofloxacin.

Table 4. Carbon and nitrogen source utilization and antibiotic resistance of the isolates grown on YEMA and incubated at 30°C for 5–7 days.

Carbon source	**No of isolates**	**Proportion (%)**
Mannitol	22	100
Starch	18	82
Glucose	22	100
Sucrose	9	41
Lactose	11	50
Benzophenone	10	45
Citrate	0	0
Dextrin	11	50
Nitrogen source		
Asparagine	20	91
Glycine	16	73
Ammonium Acetate	21	95
Alanine	9	41
Triethanolamine	11	50
Urea	19	86
Antibiotics	**% of resistant isolates**	
	5% µg ml^{-1}	**10 % µg ml^{-1}**
Ciprofloxacin	50	50
Tetracycline	64	64
Ampicillin	77	77
Vancomycin	91	91
Erythromycin	82	86
Chloramphenicol	91	91
Norfloxacin	64	64

Evaluation of symbiotic effectiveness

All isolates were authenticated as root nodule bacteria for they re-nodulated the host under greenhouse condition. They induced nodules on the host ranging from 16/plant for isolate of $ASTUR_3$ and 109/plant for isolate of

$ASTUR_{16}$; and with nodule dry weight of (0.039 gm/plant) and (0.121 gm/plant) obtained from isolates $ASTUR_{11}$ and $ASTUR_{16}$, respectively (Table 5). The inoculated plants produced shoot dry mass between 0.256 g/plant and 0.993 g/plant. The isolates showed variation in relative symbiotic effectiveness (SE) in which 27% of the isolates were highly effective, and 36% of the isolates were effective, about 27% isolates were lowly effective (Table 5). The highly effective isolates were $ASTUR_3$, $ASTUR_4$, $ASTUR_8$, $ASTUR_{11}$, $ASTUR_{12}$ and $ASTUR_{22}$ indicating that 63% of the isolates have the potential to be further tested as inoculants under field conditions.

Table 5. Symbiotic characteristics of common bean rhizobia isolates on sand experiment.

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, NN-nodule number, NDW-nodule dry weight, SDW-shoot dry weight

DISCUSSION

Cultural and growth characteristics

Twenty two root nodule bacteria were isolated from the nodules induced by growing the host plant on soil samples collected from common bean growing sites in East Shoa Zone, Oromia, Ethiopia. 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 colour. The colony size of all isolates lied between 1.5–5 mm and more than 73% of the isolates exhibited high production of EPS and colony size > 2 mm. According to Jordan (1984), isolates producing acid reaction and large production of EPS and colony diameter greater than 2 mm are categorized as fast growing rhizobia whereas isolates producing colony diameter ≤ 2 mm and showing alkaline reaction were categorized as slow growing. The large production of EPS by the isolates could be an indicative of endurance to high temperatures, metal toxicity, low soil pH and salinity (Karthik *et al*., 2017). This result is consistent with other studies in Ethiopia (Bayou Bunkura, 2015; Endalkachew Wolde-meskel *et al*., 2018) showing that different species of rhizobia can be recovered from nodules of the same plant (Silva *et al*., 2003; Aregu Amsalu *et al*., 2012).

In this study, 68% of the isolates showed the ability to solubilize tricalcium phosphate on Picovaskaya Agar Medium with solubilization index ranging from 1.04 to 2.4, of which four isolates recorded SI greater than or equal to 2. These results are high compared to those described by Bayou Bunkura (2015) who reported that 34% of the isolates were able to solubilize tricalcium phosphate from haricot bean nodulating rhizobia at Hawassa and Zeway, Ethiopia. Contrary to this, Alemayehu Workalemahu and Fassil Assefa (2007a) reported that no isolate was found to exhibit the ability to solubilize phosphate in their study of common bean (*Phaseolus vulgaris*)-nodulating rhizobia in some parts of southern Ethiopia. These results show that rhizobia can act as a phosphate-solubilizer along with nitrogen fixation making these strains efficient to be utilized for the production of inoculants, which can improve the availability of major growth limiting nutrients like phosphorus for the common bean production system in Ethiopia.

The physiological characteristics of the isolates showed they were diverse in their ability to grow at different pH values, NaCl concentrations and temperatures (Table 3). These characteristics are important to determine the ability of isolates to grow under different environmental stress conditions. In this study, all, half and only five of the isolates were able to tolerate 1%, 5% and 8% of NaCl concentration, respectively. In previous studies, several isolates of common bean rhizobia were observed tolerant to different concentration of NaCl including 2–7% from Ethiopia (Endalkachew Wolde-meskel *et al*., 2018), 2–10% from Mediterranean soils (Priefer *et al*., 2001) and up to 10% (Jordan, 1984; Zahran, 1999) of salt concentration. However, common bean rhizobia isolated from southern Ethiopia was found salt

sensitive and 89% of the isolates failed to grow above 0.5% NaCl (Alemayehu Workalemahu and Fassil Assefa, 2007a).

All the isolates were able grow on a wide range of pH values. Half of the tested isolates were able to grow at high value up to pH 10. Isolates $ASTUR_{14}$ and $ASTUR_{20}$ were able to withstand high salt concentration up to 9% and alkaline pH of up to 10. Alemayehu Workalemahu and Fassil Assefa (2007a) reported an isolate tolerant to extreme pH, salt, and temperature from southern Ethiopia. These findings showed that high salt concentration and alkaline pH tolerant isolates can be screened from low land area of Ethiopian Rift Valley.

With regard to temperature profile, all isolates in this study were able to grow between 15–37°C and only a few of them survived a temperature of 40°C. Elsewhere, several isolates of common bean rhizobia have been reported to grow at temperature of 35–45°C (Endalkachew Wolde-meskel *et al*., 2018), 20–40°C (Bayou Bunkura, 2015), 40°C (Hungria *et al*., 2000), 42°C (Küçük *et al*., 2006) and 50°C (Anteneh Argaw, 2007). Despite geographical differences, common bean rhizobia show big variation and wide range of temperature tolerance.

All isolates grew on mannitol and glucose and 82% of isolates were grown on starch; similar result was reported by De Oliveira *et al*. (2007) who suggested that Rhizobium strains have capability to use starch and were positive for utilization of glucose as carbon source. Although dextrin is rarely utilized by Rhizobium (Jordan, 1984), 50% of the isolates in this study were able to utilize dextrin as a carbon source. None of the isolates grew on citrate. Many authors including Zerihun Belay and Fassil Assefa (2011) worked on faba bean and other rhizobia that failed to utilize citrate (Küçük *et al*., 2006; Endalkachew Wolde-meskel *et al*., 2018). However, Datta *et al*. (2015) observed that citrate utilization as a carbon source was positive in *Rhizobium phaseoli* and *Rhizobium trifolii*.

Majority of the isolates showed growth on a wide range of nitrogen sources except for alanine which was utilized only by 41%. Bayou Bunkura (2015) also reported that 36% of the tested isolates utilized alanine. Most (73%) of the isolates utilized glycine compared to less number of isolates (39%) (Endalkachew Wolde-meskel *et al*., 2018) and 10% (Bayou Bunkura, 2015) reported to utilize glycine.

All isolates exhibited variations in their intrinsic antibiotic resistance to different types of antibiotics and concentrations. More than 50% of the tested isolates were tolerant to all antibiotic at 10% µg ml^{-1} concentration. The antibiotic resistance pattern of these isolates was generally similar to other common bean rhizobia reported by Endalkachew Wolde-meskel *et al*. (2018) and Alemayehu Workalemahu and Fassil Assefa (2007a).

Evaluation of symbiotic effectiveness

The preliminary screening of symbiotic effectiveness of the isolates under controlled greenhouse conditions showed that more than 63% of rhizobia showed desirable characteristics of good performance of nitrogen fixation indicated by Purcino *et al*. (2000). About 27% and 36% of the tested isolates were found to be highly effective and effective nitrogen fixers, respectively. This indicated that there is a great variation among rhizobial isolates nodulating common bean in eastern Shoa Zone of Oromia and can be possible to select superior rhizobia in terms of symbiotic effectiveness. Similarly, Anteneh Argaw (2007) reported that 89% of the isolates collected from Eastern Ethiopia were effective and highly effective nitrogen fixers. Alemayehu Workalemahu and Fassil Assfea (2007b) reported that five of the sixteen isolates (31%) from southern Ethiopia showed very good symbiotic performance on sand culture under greenhouse conditions.

CONCLUSION

The present study showed the presence of compatible indigenous rhizobia that are superior to the inorganic fertilizer and can be exploited to enhance common bean inoculation programmes in the area. The superior performance of isolate $ASTUR_{14}$ that tolerated high salt concentration, high pH and showed effective symbiotic characteristics demonstrate the presence of inoculum candidate from native common bean rhizobia. Further testing of inoculation efficiency under various environmental conditions is necessary.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Office of Post Graduate Studies, Adama Science and Technology University. We are grateful to Melkassa Agricultural Centre for providing us seeds of common bean variety ‘Ser-19’.

REFERENCES

Ahmed, M.H., Rafique Uddin, M. and Mclaughlin, W. (1984). Characterization of indigenous rhizobia from wild legumes. *FEMS Microbiol. Lett*. **24**: 197–203.

Alemayehu Workalemahu and Fassil Assefa (2007a). Phenotypic characteristics of

common bean (*Phaseolus vulgaris*)-nodulating rhizobia from some areas of Southern Ethiopia. *Ethiop. J. Biol. Res.* **6**: 99–118.

Alemayehu Workalemahu and Fassil Assefa (2007b). Symbiotic characteristics of common bean (*Phaseolus vulgaris*)-nodulating rhizobia from some areas of Southern Ethiopia. *Ethiop. J. Nat. Res.* **9**(2): 289–304

Amarger, N., Macheret, V. and Aguerre, G. (1997). *Rhizobium gallicum* sp. nov. and *Rhizobium giardinii* sp. nov., from *Phaseolus vulgaris* nodules. *Int. J. Syst. Bacteriol.* **47**: 996–1006.

Andrews, M. and Andrews, ME. (2017). Specificity in legume-rhizobia symbioses. *Int. J. Mol. Sci.* **18**(4): 705.

Anteneh Argaw (2007). **Symbiotic and Phenotypic Characterization of Rhizobia Nodulating Common Bean (*Phaseolus vulgaris* L.) from Eastern Ethiopia**. M.Sc. Thesis, Addis Ababa University, Addis Ababa.

Aregu Amsalu, Räsänen, L.A., Fassil Assefa, Asfaw Hailemariam and Lindström, K. (2012). Phylogeny and genetic diversity of native rhizobia nodulating common bean (*Phaseolus vulgaris* L.) in Ethiopia. *Syst. Appl. Microbiol*. **35**: 120–131.

Bayou Bunkura (2015). Soil population and phenotypic characterization of soybean (*Glycine max* L.) and haricot bean (*Phaseolus vulgaris* L.) nodulating rhizobia at Hawassa and Ziway. *SJAS* **5**: 30–38.

Broughton, W.J. and Dilworth, M.J. (1970). Control of leghaemoglobin synthesis in snake beans. *Biochem. J.* **12**: 1075–1080.

Darkwa, K., Daniel Ambachew, Hussein Mohammed, Asrat Asfaw, and Blair, M.B. (2016). Evaluation of common bean (*Phaseolus vulgaris* L.) genotypes for drought stress adaptation in Ethiopia. *Crop J.* **4**: 367–376.

Datta, A., Singh, R.K. and Kumar, S. (2015). Isolation, characterization and growth of *Rhizobium* strains under optimum conditions for effective biofertilizers production. *Int. J. Pharm. Sci. Rev. Res*. **32**(1): 199–208.

De Oliveira, A.N., de Oliveira, L.A., Andrads, J.S. and Chagas, J.A.F. (2007). Rhizobia amylase production using various starchy substances as carbon substrates. *Braz. J. Microbiol.* **38**: 208–216.

Desta Beyene, Serawit Kassa, Franklin, A.M., Amha Assefa, Tadesse Gebrmedhin and Van Berkum, P. (2004). Ethiopian soils harbour natural population of rhizobia that form symbiosis with common bean (*P. vulgaris*). *Arch. Microbiol.* **181**: 129–132.

Endalkachew Wolde-meskel, Tulu Degefu, Brehanu Gebo, Asnake Fikre, Tilahun Amede, and Ojiewo, C. (2018). Phenotypic characteristics and preliminary symbiotic effectiveness of rhizobia associated with haricot bean growing in diverse locations of southern Ethiopia. *Ethiop. J. Crop Sci*. **6**(2): 119–139.

Farid, M. (2015). **Symbiotic Nitrogen Fixation in Common Bean**. Ph.D. dissertation, University of Guelph, Guelph.

Graham, P.H. (1981). Some problems of nodulation and symbiotic nitrogen fixation in *Phaseolus vulgaris* L.: A review. *Field Crops Res*. **4**: 93.

Hardarson, G. and Danso, S.K.A. (1993). Methods for measuring biological nitrogen fixation in grain legumes. *Plant Soil* **152**: 19–23.

Heilig, J.A. (2015). **QTL Mapping of Symbiotic Nitrogen Fixation in Dry Bean; Dry bean performance under organic productions systems.** Ph.D. dissertation, Michigan State University, East Lansing, MI.

Hungria, M., Vargas, M.T., Campo, R.J., Chueire, L.O. and Andrade, D.S. (2000). The Brazilian experience with the soybean (*Glycine max*) and common bean

(*Phaseolus vulgaris*) symbioses. In: **Nitrogen Fixation: From Molecules to Crop Productivity**, pp. 515. Kluwer Academic Publishers, Dordrecht.

Jordan, D.C. (1984). Family III. Rhizobiaceae. In: **Bergey's Manual of Systematic Bacteriology**, pp. 234–254 (Krieg, N.R. and Holt, J.G., eds.). The Williams and Wilkins, Baltimore.

Karthik, C., Oves, M., Sathya, K., Sri Ramkumar, V. and Arulselvi, P.I. (2017). Isolation and characterization of multi-potential *Rhizobium* strain ND2 and its plant growth-promoting activities under Cr (VI) stress. *Arch. Agron. Soil Sci.* **63**: 1058–1069.

Küçük, C., Kivanc, M. and Kinaçi, E. (2006). Characterization of *Rhizobium* spp. isolated from bean. *Turk J. Biol*. **30**: 127–132.

Nigatu Alemayehu (2013). Zonal diagnosis and intervention plan for East Shoa, Oromia.

Petry, N., Boy, E., Wirth, J.P. and Hurrell, R.F. (2015). Review: The potential of the common bean (*Phaseolus vulgaris*) as a vehicle for iron biofortification. *Nutrients* **7**: 1144–1173.

Premono, M.E., Moawad, A.M. and Vlek, P.L. (1996). Effect of phosphate-solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. I *J. Crop Sci.* **11**: 13–23.

Priefer, U., Aurag, J., Boesten, B., Bouhmouch, I., Defez, R., Filali-Maltouf, A., Miklis, M., Moawad, H., Mouhsine, B., Prell, J., Schluter, A. and Senatore, B. (2001). Characterization of *Phaseolus symbionts* isolated from Mediterranean soils and analysis of genetic factors related to pH tolerance. *J. Biotechnol*. **91**: 223–236.

Purcino, H.M.A., Festin, P.M. and Elkan, G.H. (2000). Identification of effective strains of *Bradyrhizobium. Archis Pintoi. Trop.* **77**: 226–232.

Silva, C., Vinuesa, P., Eguiarte, L.E., Martinez-Romero, E. and Souza, V. (2003). *Rhizobium etli* and *Rhizobium gallicum* nodulate common bean (*Phaseolus vulgaris*) in a traditionally managed milpa plot in Mexico: Population genetics and biogeographic implications. *Appl. Environ. Microbiol*. **69**(2): 884–893.

Singh, A.K., Bhatt, R.P., Pant, S., Bedi, M.K. and Naglot, A. (2011). Characterization of Rhizobium isolated from root nodules of *Trifolium alexandrinum*. *J. Agric. Technol.* **7**(6): 1705–1723.

Somasegaran, P. and Hoben, H. J. (1994). **Handbook for Rhizobia**. Springer-Verlag.

Vincent, J.M. (1970). **A Manual for the Practical Study of Root Nodule Bacteria**. IBP Handbook, 15, Blackwell, Oxford.

Zahran, H.H. (1999). Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiol. Mol. Biol. Rev*. **63**(4): 968–989.

Zerihun Belay and Fassil Assefa (2011). Symbiotic and phenotypic diversity of *Rhizobium leguminosarum* bv. *viciae* from Northern Gondar, Ethiopia. *Afr. J. Biotechnol*. **10**(21): 4372–4379.