African Journal of Biotechnology Vol. 10(21), pp. 4372-4379, 23 May, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.431
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Symbiotic and phenotypic diversity of *Rhizobium leguminosarum* bv. *viciae* from Northern Gondar, Ethiopia

Zerihun Belay* and Fassil Assefa

Faculty of Life Science, Microbial, Cellular and Molecular Program Unit, Addis Ababa University, Ethiopia

Accepted 19 November, 2010

Rhizobia that nodulate cool season legumes are widely spread in the Mediterranean and highland agro ecosystems. Faba bean is one of these important crops that represent the major protein source of human nutrition, cash crop for farmers and component of the farming systems. This study is aimed at identifying rhizobia isolates capable of guarantying efficient nitrogen derived from biological nitrogen fixation. Due to this, a total of twenty-one isolates of *Rhizobium* were isolated from as many sampling sites of northern Gondar using plant infection method. The isolates were characterized morphologically and physiologically and tested on sand and soils to evaluate their symbiotic effectiveness. Studies on symbiotic effectiveness on sand culture indicated that, the strains showed shoot dry matter ranging from 0.4 (AUFR127) to 2.3 g/plant (AUFR124), with negative control (0.2 g/plant) and positive control of 2.4 g/plant. Eighty percent of the isolates were found to be effective and have very effective nitrogen fixers based on their shoot dry matter accumulation (50 to 100%) in relation to the nitrogen fertilized control plants. Culturally, almost all of them displayed large colonies with diameters of 2 to 4.5 mm, generation time of 1.9 to 4.3 h and showed characteristics of fast growing rhizobia. With a few exceptions, isolates grew at temperatures of 15 and 35°C and were found to be sensitive to salt, except AUFR118 that grew up to 5% NaCl. The isolates also grew on a wide range of moderate acidity and alkalinity (pH5.5-9).With the exception of gluconate, citrate and tartarate, almost all isolates grew on all carbohydrates. The pattern of intrinsic antibiotic resistance showed that, almost all of them were tolerant to chloramphenicol, nalidixic acid and erythromycin. Isolates AUFR118, AUFR128 and AUFR132 were found to be resistant to almost all tested antibiotics. These isolates were also included in the effective and very effective symbiotic groups. The trial of the 5 selected effective isolates on two soil types showed that, they performed well without any significant difference on their respective soils. Interestingly, the isolates were found to nodulate the host on the highly acidic soil (pH 4.8) that failed to be nodulated by the indigenous rhizobia. Generally, the present work shows the physiological and symbiotic diversity of the isolates in the traditional agricultural areas of the region and the potential of these rhizobia to be used as effective commercial inoculants in areas where the indigenous rhizobia fail to do so. This will help to reclaim acidic soils for faba bean production.

Key words: Faba bean, Northern Gondar, *Rhizobium,* phenotypic diversity, symbiotic effectiveness.

INTRODUCTION

Faba bean (*Vicia faba* L) is one of the cool season legumes widely cultivated in temperate, subtropical regions and tropical regions with higher elevations (Hawtin and Hebblethwaite, 1983). Ethiopia and Afghanistan are considered as the centre of secondary diversity of the crop (Bond et al., 1976). In Ethiopia, it is grown between the altitudes of 1800 and 3200 m (Westphal, 1974; Bond

*Corresponding author. E-mail: zebelay2009@gmail.com. Cell: 0911728023.

et al., 1985). It is an important food source, it contains high protein content and appreciable amount of minerals and vitamins (Senayit and Asrat, 1994), and is an important export commodity of the country (Asfaw et al., 1994).

Faba bean is a legume capable of fixing nitrogen with root nodule bacteria known as *Rhizobium leguminosarum* bv. *viciae*. It is the most efficient nitrogen fixer of all cool season pulse crops (McVicar et al., 2005). The amounts of N_2-fixed by faba bean is estimated to be between 240 and 325 kg ha^{-1} (Somasegaran and Hoben, 1994; Maidl et al., 1996), with percentage efficiency (66% Ndfa; Nitrogen derived from the air) (Jensen, 1986) and fulfills 80% of its nitrogen requirements (Zapta et al., 1987). For this reason, it is incorporated in the traditional low-input mixed agriculture providing a cheaper and more effective agronomic practice by ensuring an adequate supply of N for legume-based crop and pasture production.

The ability of faba bean to fix the desired amount of nitrogen depends on many factors, such as the effectiveness of the rhizobium strain, the genetic variation of the host plant and other environmental and agronomic factors (Nutman, 1976). In order to obtain a very effective faba bean *R. leguminosarum* bv. *viciae* system, it is necessary to search for effective indigenous rhizobia with the host under laboratory and greenhouse conditions.

In Ethiopia, attempts have been made to conduct research on rhizobiology of cool season legumes such as faba bean and field bean for the last two decades (Asfaw and Angaw, 2006). However, most of the works were limited to inoculation trials with and without the application of nitrogen/phosphorus fertilizers on different soil types (Asgelil, 2000; Amanuel et al., 2000; Ayneabeba et al., 2001). However, there is still a dearth of information about the taxonomic and symbiotic diversity of rhizobia nodulating faba bean from different agro ecological zones of the country. The objective of this study is to evaluate the status and symbiotic effectiveness of faba bean rhizobia from North Gondar and to assess the performance of selected isolates on two soil types with contrasting physical and chemical characters.

MATERIALS AND METHODS

The *Rhizobium* isolation, identifications, pot experiments and total nitrogen analysis were carried out at the Department of Biology, Addis Ababa University. Soil chemical analysis was done at the National Soil Laboratory (EARO).

Soil sites and sampling

Twenty one sampling sites from North Gondar, Amhara Regional State were selected for the study (Figure 1). According to FAO (1990) classification, they are found in agro ecological zones between 1500 m sub moist and 3500 m cold in altitude. The study sites are characterized by mean annual temperature of 10 to 20°C and average annual rainfall of 900 mm. Soils are characterized by vertisol with mildly acidic and near neutral pH (Table 1). Composite soil samples from 20 to 30 cm depth of each representative site were collected (October, 2004) in alcohol (70%) sterilized plastic bags. They were taken and preserved in cold rooms at the Department of Biology, Addis Ababa University.

Isolation of rhizoba

Rhizobia were isolated from the soil samples by using 'plant infection' method (Vincent, 1970). Each soil sample representative was thoroughly mixed, sieved using 2 mm mesh-size sieve and filled into 3 kg capacity surface-sterilized (70% alcohol) plastic pots. Undamaged and selected seeds of faba bean of "Degaga" cultivar were used to induce nodulation. Five seeds were surface- sterilezed, according to Vincent (1970) and were planted on each pot, which were later thinned down to three after germination. The pots were watered twice a week at full capacity and arranged in a complete random block design in greenhouse with a photo period of 12 h for 45 days. The rhizobia were isolated from surface-sterilized nodules, as before by streaking on Yeast-extract mannitol agar medium (YEMA) and incubated at 28°C for 5 to 7 days. YEMA contains g l^{-1}: Mannitol, 10; K_2HPO_4, 0.5; $MgSO_47H_2O$, 0.2; NaCl, 0.1; yeast extract, 0.5; agar, 15.

Purified isolates were then preserved on YEMA slants containing 0.3% (w/v) $CaCO_3$ and stored at 4°C (Vincent, 1970). Rhizobia were routinely cultured at room temperature in YEMB (without agar) on rotary shaker operating at 100 cycles per minute to standardize the inoculum size of (10^8 cells ml^{-1}) and in YEMA at 28°C for seven days, for every experiment unless stated otherwise.

Colony morphology and growth rate

Colony morphology was characterized on YEMA according to (Ahmed et al., 1984) and acid/base reaction was evaluated on YEMA containing 25 µg ml^{-1} bromothymol blue (BTB) (Lupwayi and Haque, 1994). Growth rate of the isolates was determined as described by Somasegaran and Hoben (1994).

Tolerance to acidity, alkalinity, salinity and temperature

All experiments on tolerance to acidity, alkalinity, temperature and growth on carbohydrates and intrinsic antibiotic resistance were performed according to Lupwayi and Haque (1994). Tolerance to acidity and alkalinity of each isolate was evaluated on CIAT-Modified Keyser medium (CIAT-KM) (CIAT, 1988) with pH previously adjusted to 4.0, 4.5, 5.0, 5.5, 8.0 and 9.0 with sterile HCl or NaOH. For salt tolerance, the isolates were transferred to YEMA plates supplemented with NaCl at concentrations of 0.1, 0.3, 0.8, 1, 2, 3, 4, 5% (w/v). The ability of bacterial strains to grow at high and low temperatures was monitored at incubation temperatures of 5, 10, 15, 35, 40 and 45°C.

Utilization of carbohydrates

Carbon utilization of strains was determined following the method of Somasegaran and Hoben (1994) on seventeen carbohydrates prepared as 10% (w/v) solution in water. The carbohydrate free YEMA medium was modified by reducing the yeast extract to 0.05 gl^{-1} liter.

Intrinsic antibiotic resistance

This intrinsic resistance of isolates was determined by inoculating (10^8cells ml^{-1}) on solid YEMA medium containing seven filter sterilized (0, 22 µm Millipore filters) antibiotics at concentrations of 2.5, 5, and 10 µm ml^{-1} of water according to Josey et al. (1979).

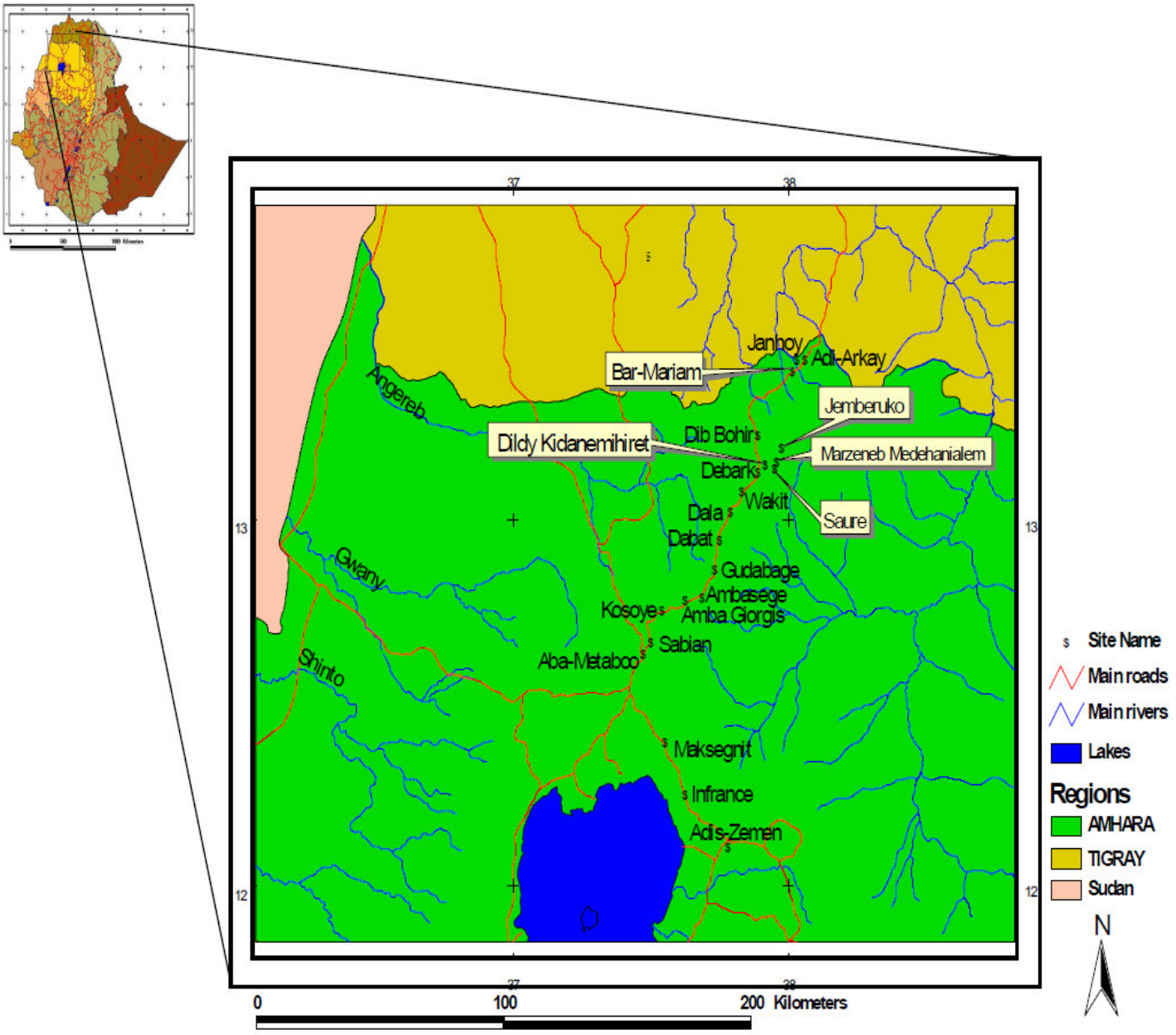

Figure 1. Location map of the sampling sites.

Authentication and preliminary evaluation of nitrogen-fixing effectiveness (sand pot experiment)

Authentication of isolates as root nodule bacteria and prescreening of their relative symbiotic effectiveness were undertaken on sand pot experiment. Each isolate was grown on YMB, for three days and adjusted to a concentration of (10^9 cells ml^{-1}). About 3 kg of carefully washed, sieved and sterilized river sand was filled into surface disinfected plastic pots (70% alcohol). Seeds of "Degaga" faba bean cultivar were surface-sterilized as before and planted on 3 kg capacity alcohol-swabbed plastic pots containing acid washed river sand. Four seeds were planted per pot individually treated with 1 ml of inoculum and thinned to three per pot after 5 days of emergence (DAE). They were once fertilized with 0.05% KNO_3 as a starter nitrogen source and grown under greenhouse conditions (12 h photoperiod; 29/18°C day/ night temperature). They received quarter strength of N-free nutrient solution (Broughton and Dilworth, 1971) and distilled water once a week and every two days , respectively. Control pots were included for an unfertilized and an uninoculated negative control (TO) and uninoculated but nitrogen fertilized (0.05% KNO3/week) positive control (TN).

Plants were harvested 45 DAE (day after emergence), evaluating nodule number, nodule dry mass and shoot dry mass. The experiment was statistically laid out with three replications using randomized black design (Somasegaran and Hoben, 1994). Relative effectiveness of isolates was calculated according to the

Table 1. Sample location, altitude, soil pH and isolation types from the soil field.

S/N	Isolation types	Origin of isolation	Altitude (m)	pH of soil	Colony diameter (mm)	Growth rate (h)	Colony texture
1	AUFR112	Infranze	ND*	6.7	2.5	2.1	LT
2	AUFR113	Maksgnit	1929	7.2	<1	2.6	SD
3	AUFR114	Aba Matabo	2264	5.8	3.5	3.1	LT
4	AUFR115	Sabian	2419	6.7	3.5	3.2	LT
5	AUFR116	Janhoy	2693	6.6	3.0	4.2	LT
6	AUFR117	Kosaye	2898	6.3	4.0	4.1	LW
7	AUFR118	Ambasege	2694	6.8	5.0	2.9	LW
8	AUFR119	Godabage	2767	7.4	4.5	3.0	LW
9	AUFR120	Dabat	2634	6.7	4.5	1.9	LW
10	AUFR121	Dala	2672	6.7	3.5	2.9	LT
11	AUFR122	Wakit	2758	6.5	3.0	4.1	LW
12	AUFR123	Debark	2795	6.3	3.0	2.9	LT
13	AUFR124	Jemberko	3196	6.9	3.5	3.2	LT
14	AUFR125	Merzeneb medhanlem	3074	6.1	3.0	3.3	LT
15	AUFR126	Saurae	3042	7.3	4.0	3.8	LW
16	AUFR127	Dildykdanemihret	2923	6.6	4.0	3.0	LT
17	AUFR128	Dib-bahir	2163	6.1	2.5	3.3	LT
18	AUFR129	Bar-Mariyam	1586	7.5	2.5	3.1	LT
19	AUFR130	Adi-Arkay	1529	6.8	2.0	3.2	LT
20	AUFR132	Amba-Georgis	2837	7.3	2.5	4.3	LW
21	AUFR134	Adis-Zemen	1923	7.5	2.5	2.5	LT

*ND, Not done; LT, large translucent; LW, large watery; SD: small dry.

equation proposed by Date et al. (1993) in Purchino et al. (2000) (100 × inoculated plant DM/ N-fertilized plant DM) with Nitrogen fixing effectiveness classified as ineffective <35%; lowly-effective, 35 to 50%; effective, 50 to 80%; and highly effective, >80%.

Symbiotic effectiveness under soil pot experiment

Two soil samples with contrasting texture and pH were selected from Amba Girogis (North Gondar) and Holetta (Central Shoa). The soil physical and chemical characters are not shown. Fine soil samples from each site were sieved with 2 mm mesh-size and filled in 3 kg capacity surface sterilized (70% alcohol) plastic pots. They were once treated with fertilizer recommended by Somasegaran and Hoben (1994). The composition of the fertilizers in mg/pt: 468 KH_2PO_4, 404.2 KCl, 533 $MgS0_4.7H_2O$, 49.5 $ZnS0_4.7H_2O$, 1.95 (NH_4) and 6 MO_2 $24.H_2O$. 219 mg pot^{-1} and urea was added as nitrogen source for N-Control pots.

Before planting, the soils were watered to approximately 75% field capacity. Selected seeds of "Degaga" faba bean were surface sterilized as described before (Vincent, 1970) and allowed to germinate on water agar for three days at 25°C. Five superior strains from previously screened sand culture were selected and prepared by growing in YEMB nutrients broth media to approximately 10^9 cells ml^{-1}. One ml of each culture suspension was flooded on each seedling for one hour. Four inoculated seedling were planted on each pot which were later thinned down to three, and watered twice a week. The experiment was set up in a randomized complete block design with three blocks or replications in a greenhouse with a 12/12 h photoperiod for 45 days. The whole plants were carefully up rooted and their nodule number, nodule dry weight and shoot dry mass and total nitrogen were determined.

Plant total nitrogen analysis

Total nitrogen was quantitatively determined by the modified "Wet" Kjeldahl method after Sahelemedihin and Taye (2000).

Data analysis

Comparison between treatments was analyzed using one-way ANOVA (Tukey's HSD tests) (SPSS.10).

RESULTS AND DISCUSSION

Twenty-one isolates were recovered from nodules of faba bean grown on soils from various sites of Northern Gondar (Table 1). All isolates displayed fast growth with doubling times between 1.9 and 4.3 h and large colonies with mucoid and watery textures upon 5 days of incubation on YEMA medium. They changed the YEMA-BTB medium into yellow color indicating that they produced acid by preferentially utilizing the sugar component of the medium for their growth (Tan and Broughton, 1981). All these features are characteristics of fast growing rhizobia, including the cross-inoculation group of *R. leguminosarum b*v. *viciae* that are nodulates member of the tribe *Viceae* (Faba bean, Field pea, Lentil and Grass pea) (Vincent, 1977; Jordan, 1984; Somasegaran and Hoben, 1994).

Table 2. *In vitro* ecological competence and symbiotic effectiveness of isolates collected from faba bean of North Gondar, Ethiopia.

Isolate	Carbohydrate utilization %	Temperature tolerance (°C)	Salt tolerance (%)	pH tolerance	Antibiotic tolerance*	Symbiotic effectiveness*
AUFR114	100	15 - 35	0.3	5.5 - 9	Ery, Nal, Chl	VE
AUFR115	85	15 - 35	0.1	5.5 - 9	Str, Rif, Ery, Nal, Chl	VE
AUFR116	85	15 - 35	0.1	5.5 - 9	Str, Rif, Ery, Nal, Chl	E
AUFR117	85	15 - 35	0.3	5.5 - 9	Rif, Ery, Nal, Chl	E
AUFR118	100	5 - 35	5.0	5.5 - 9	Amp, Str, Kan, Nal, Chl	E
AUFR119	90	15 - 35	0.1	5.5 - 9	Rif, Kan, Ery, Nal, Chl	E
AUFR120	85	15 - 35	0.1	5.5 - 9	Ery, Nal, Chl	E
AUFR121	85	15 - 35	0.3	5.5 - 9	Rif, Ery, Chl	E
AUFR122	90	15 - 40	0.5	7.0 - 9	Str, Chl. Ery, Nal, Chl	E
AUFR123	80	15 - 35	0.1	5.5 - 9	Str, Rif, Ery, Nal, Chl	E
AUFR124	85	15 - 35	0.1	5.5 - 9	Rif, Kan, Ery, Nal, Chl	VE
AUFR125	85	15 - 35	0.3	5.5 - 9	Str, Rif, Ery, Nal, Chl	E
AUFR126	100	5 - 35	0.5	5.5 - 9	Amp, Str, Rif, Kan, Ery, Nal, Chl	E
AUFR128	100	15 - 40	2.0	5.0 - 9	Amp, Str, Rif, Kan, Ery, Nal, Chl	VE
AUFR130	85	15 - 35	0.1	5.5 - 9	Ery, Nal, Chl	E
AUFR132	85	15 - 35	0.1	5.5 - 9	Str, Rif, Kan, Ery Nal, Chl	VE
AUFR134	85	15 - 35	0.1	5.5 - 9	Str, Ery, Nal, Chl	E

*Amp, Ampicillin; Chl, chloramphenicol; Ery, erythromycin; Kan, kanamycin; Nal, nalidixic acid; Rif, rifampicin; VE, very effective; E, effective.

The isolates also grew on a wide range of moderate acidity and alkalinity (pH 5.5 to 9) (Table 2). This is concurrent to the finding that *R. leguminosarum* bv. *viciae* strains are generally sensitive to low pH and grow well on near neutral and basic pH (Graham and Parker, 1964; Jordan, 1984). The isolates were found to utilize 80 to 100% of the tested monosaccharide and disaccharides, while many failed to metabolize gluconate and tartarate. No isolate did grow on citrate. This finding corroborates with previous reports of carbohydrate metabolism of *R. leguminosarum* bv. *viciae* (Graham and Parker, 1964; Jordan, 1984; Stowers, 1985; Lindstrom and Lhetomaki, 1988).

Almost all rhizobia/ isolates grew between 15 and 35°C (Table 2), with a few isolates grew at incubation temperatures of 5 and10°C (AUFR118 and AUFR126) and 40°C (AUFR 122 and AUFR128). This pattern of temperature tolerance is within the range of T max; 32.5 to 34.5°C reported for *R. leguminosarum* bv. *viciae* HAMBI499, HAMBI 1125 and MPI 6001 isolated from faba bean and field pea from USA, UK and the Netherlands (Lindstrom and Lehtomaki, 1988).

With regard to salt tolerance, all isolates grew on the medium containing 0.1% of NaCl (Table 2). However, only isolates AURF128 and AUFR118 were found to be tolerant to NaCl concentrations of 2 and 5%, respectively. This finding is contrary to previous reports that, fast growing *Rhizobium* in general, grew well at NaCl concentration between 3 and 5% (Abdul-wahab and Zahran, 1979; Zerhari et al., 2000).

All isolates exhibited variations in their intrinsic antibiotic resistance (IAR) to different concentrations and types of antibiotics. Ninety five percent of the tested isolates were resistant to erythromycin, chloramphenicol and nalidixic acid at all tested concentrations. AUFR128 was found to be tolerant to all antibiotics followed by AUFR126 that were able to grow on all, except higher concentrations of streptomycin (10 µg ml, $^{-1}$) and nalidixic acid (10 µg ml, $^{-1}$) (data not shown). The pattern of antibiotic resistance of the isolates was generally similar to the findings on other isolates of *R. leguminosarum* bv. *viciae* with respect to streptomycin and erythromycin (Josey et al., 1979; Brockman and Bezdicek, 1989).

Preliminary screening of symbiotic effectiveness in sand culture

All isolates, except AUFR113 were authenticated as root nodule bacteria by forming nodules on the test host plants (Table 3). The nodulating isolates showed differences in nodule number 67 and 168/plant) and dry weight (37 and 111 mg/plant) and shoot dry weight ranging from 0.4 g/plant (AUFR127) to 2.3 g/plant (AUFR 124). The data showed that there is a 6 fold difference between the isolates accumulating the lowest and highest shoots dry mass in the host which is a very good indicator of effectiveness in nitrogen fixation of legumes (Sorwill and Myaton, 1986; Somasegaran and Hoben 1994; Date, 1993 in Purchino, 2000; Peoples et al, 2002).

Table 3. Nodulation and relative effectiveness of nitrogen fixation of *R. leguminosarum* bv. *viciae* isolates of North Gondar tested on 'Degaga' variety of faba bean on sand culture.

Treatment	Nodule number/pl	Nodule dry mass (mg/pl)	Shoot dry mass (g/pl)	Efficiency N-fixation (%)
AUFR112	133 [a]	108[a]	1.0 [b]	42
AUFR113	-	-	-	-
AUFR114	148 [a]	91.1[ab]	2.1 [a]	88
AUFR115	136 [a]	78.8[a]	2.1[a]	88
AUFR116	134 [a]	55.2[ab]	1.7 [a]	71
AUFR117	151 [a]	88.4[ab]	1.5 [a]	63
AUFR118	123[a]	111.1 [a]	1.8 [a]	75
AUFR119	125 [a]	83 [a]	1.8 [a]	75
AUFR120	137[a]	81.8 [a]	1.9 [a]	79
AUFR121	91[ab]	54.5[ab]	1.7[ab]	71
AUFR122	154 [a]	55.2[ab]	1.8 [a]	75
AUFR123	119 [a]	77.7[ab]	1.6 [a]	67
AUFR124	168 [a]	78.8 [a]	2.3 [a]	96
AUFR125	128[ab]	69.1[ab]	1.4 [a]	58
AUFR126	156[a]	79.2[ab]	1.8 [a]	75
AUFR127	67[b]	37.0[b]	0.4[b]	17
AUFR128	128 [a]	99.5[ab]	2.1 [a]	88
AUFR129	78[ab]	42.8[b]	0.70[b]	29
AUFR130	91[ab]	53.7[ab]	1.4 [b]	58
AUFR132	137 [a]	79.9[ab]	2.2[a]	92
AUFR134	88[b]	74.9[ab]	1.3[ab]	54
N-	-	-	0.2 [b]	8
N+	-	-	2.4[a]	100

-, Not found. Means in columns followed by the same letters are not significantly different at $p < 0.05$ (Tukey's HSD test).

Based on the percentage differences of shoot dry weight of inoculated and nitrogen-fertilized plants a measure of effectiveness (Date, 1993 in Purcinho, 2002), more than 23% of the isolates were found to be highly effective (80 to 100%) and 57% were effective (50 to 80%) nitrogen fixers (Table 3). The highest scores of 88 to 96% effectiveness were displayed by isolates AUFR114, AUFR115, AUFR124, AUFR128 and AUFR132 (Table 3). The data show that, more than 80% of the sampling sites in North Gondar harbor effective and very effective rhizobia in the soil. This is contrary to the previous finding of Desta and Angaw (1987) where only 11% of the isolates from Central Shewa were effective. Such variability in symbiotic effectiveness of faba bean *Rhizobium* was also found to be widespread in Ethiopia (Van Berkum et al., 1995) and in the USA (Brockman and Bezdicek, 1989).

Symbiotic effectiveness of selected isolates on soil culture

Based on the total shoot dry mass production, five elite isolates were selected as inoculants to determine their *In situ* symbiotic effectiveness on faba bean on soils from Ambaghiorgis (North Gondar) (pH 7.3) and Holetta (West Shoa) (pH 4.8) (Table 4). All isolates did not show significant differences on nodule number, nodule dry mass and shoot dry weight on their respective soils. The treated plants also accumulated 81 to 100% of their shoot dry weight compared to their respective N-fertilized controls on the tested soil types, indicating that there were no significant inter-isolate differences in nitrogen fixation on tested soils. However, the data showed a significantly higher increase in nodule number (171 to 239%), slightly higher nodule dry weight (4 to 30%) and shoot dry matter contents (4 to 30%) of the treated plants on Ambaghorgis soil than the Holetta soil. The high nitrogen and phosphorus contents and the high pH (7.3) in Ambagiorgis soil may have induced more favorable environment than Holetta soil (4.8) for effective legume-*Rhizobium* symbiosis.

The uninoculated, N+ and N- treatments of the Holetta soil failed to produce nodules whereas sufficient, but reduced number of nodules were produced with treatments of Ambagiorgis (Table 4). This indicates that, the low pH, low nitrogen and phosphorus status of the Holetta soil severely affected the survival of the indigenous rhizobia, their nodulation and nitrogen fixation. A

Table 4. Comparative effectiveness of selected strains of *R. leguminosarum* bv. *viciae* from North Gondar on the growth and nitrogen fixation of faba bean on soil culture.

Treatment	Holeta				Ambagiorgis			
	Nodule number/plant	Nodule dry mass (mg/pl)	Shoot dry mass (g/pl)	Total nitrogen (%)	Nodule number /plant	Nodule dry mass (mg/pl)	Shoot dry mass (g/pl)	Total nitrogen (%)
AUFR114	51[ab]	54.0 [a]	2.4 [a]	2.3 [a]	122[a]	26[a]	2.8 [a]	3.9 [a]
AUFR115	41[ab]	28.3[ab]	2.3 [a]	2.8 [a]	67[a]	27[a]	2.6 [a]	3.5 [a]
AUFR124	78[a]	49.3 [a]	2.5[a]	2.7 [a]	84[a]	31.6[a]	2.6 [a]	3.9 [a]
AUFR128	86[a]	46 [a]	2.3 [a]	2.9 [a]	104[a]	34[a]	3.0[a]	3.3 [a]
AUFR132	73[a]	45.3 [a]	2.2 [a]	1.9 [a]	99[a]	43.6[a]	2.7[a]	2.6[a]
N +	-	-	2.7 [a]	3.2 [a]	31[b]	14.0[a]	2.9 [a]	3.5 [a]
N -	-	-	1.5[b]	1.9 [a]	79[a]	19.33[a]	2.4 [a]	3.3 [a]

-, Not found. *Means in columns followed by the same letters are not significantly different at $p < 0.05$ (Tukey's HSD test).

strong correlation between soil pH, survival and persistence of rhizobia and nodule activity (Coventry and Evans, 1989; Brockwell et al., 1991) and failure of nodulation of faba bean at lower pH <5.5 (Evans et al., 1980) and reduced growth at <pH 5.4 (Schubert et al., 1990) and <pH 6 (Tang and Thomson, 1996) was well recorded. In Ethiopia, Desta and Angaw (1988) also reported limited nodulation on faba bean at Bekoji, south eastern Ethiopia with a pH of 5.1.

All isolates were also found to increase shoot dry weight in the range of 20 to 25% higher than their respective sand cultures. This increase in shoot dry weight in soil culture may be attributed to the high nitrogen content of the soil and other rhizosphere effects on plant growth (Kang and Mills, 2004). An increase in shoot dry weight between sand and soil culture experiments on faba bean production areas in Ethiopia was also reported (Ayeneabeba et al., 2001; Assefa et al., 2010).

The fact that the inoculated selected isolates increased shoot dry matter on Holetta soil indicates that, they fix nitrogen on acidic soil although they were originally isolated from a mildly acidic or near neutral pH. This shows that it is possible to reverse failed nodulation and extend the growth of legumes on acidic soils by primarily selecting acid tolerant rhizobia. Selection and application of acid tolerant strains retrieve production of faba bean (Carter et al., 1994) and medics (Howieson et al., 1988; Dilworth et al., 2001) on marginalized acidic soils of Western Australia. A work on Assosa (pH 5.8), Kulumsa (pH 6.0) and Bekoji soils (pH 5.1) in the central part of Ethiopia also showed an increase of 13 to 24% of shoot dry matter by inoculating effective rhizobia and fertilizer treatment (Amanuel et al., 2000).

Although correlation of tolerance to different environmental factors and symbiotic effectiveness was not undertaken in the present work, the ability of AUFR128, AUFR132 and AUFR118 to grow at higher temperatures and all carbohydrates tolerance to a wide range of pH and to most of the tested antibiotics and displaying the highest shoot dry mass (75 to 92%) are remarkable features for future screening of elite strains as inoculants for pulse production under extreme environmental conditions provided that their performance corroborate in field trials under different stressed soil environments. From this study, it can be concluded that the selection of highly performed strains are worthy of further investigation from different faba bean growing regions of Ethiopia. Given that Gondar region is one of the important regions of faba bean production, it may well be that more effective strains of *R. leguminosarum* bv. *viciae* can be isolated from other parts of Ethiopia.

ACKNOWLEDGEMENTS

We acknowledge SIDA/SAREC for funding this work under the project of "Microbial Inputs into Coffee Agro forestry and the Education Bureau of Oromia Regional State for sponsoring the MSc. training at the Biology Department of the Addis Ababa University.

REFERENCES

Abdel-Wahab SH, Zahran HH (1979). Salt tolerance of *Rhizobium* species in broth culture. *Z.* Allg. Mikrobiol. 19: 681-685.

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

Amanuel G, Kuhne RF,Tunner DG, Vlek PLG (2000). Biological nitrogen fixation in faba bean (*Vicia faba*) in the Ethiopian highlands as affected by P fertilization and inoculation. Biol. Fertil Soils, 32: 353-359.

Asefa Keneni, Fassil Assefa, Prabu PC (2010). Isolation of phosphate solubilizing bacteria from the rhizosphere of faba bean of Ethiopia and their abilities on solubilizing insoluble phosphates. J. Agric. Sci. Tech. 12: 79-89

Asegilil Debabe (2000).Effects of fertilizer on the yield and nodulation pattern on faba bean on a nitosol of Adet northwestern Ethiopia. Ethiopian Soil Sci. Soc. 2: 237-244.

Asfaw Hailemariam, Angaw Tsigie (2006). Symbiotic nitrogen fixation research on food legumes in Ethiopia. In: Food and forage legumes of Ethiopia, (Kemal Ali, Gemechu Keneni, Seid Ahmed, Rajendra Malhotra, Surendra Beniwal, Khaled Makkouk and M.H. Halila, eds). Progress and prospects. Proceedings of a Workshop on Food and Forage Legumes. 22-26 Sept, Addis Ababa, Ethiopia. ICARDA, Aleppo, Syria. pp. 172-176, 51.

Asfaw Telaye, Geletu Bejiga, Alem Berhe (1994). Role of cool-seasons food legumes and their production constructions in Ethiopian agriculture. In: Cool Season Food Legumes of Ethiopia, (Asefaw Tilaye, Geletu Bejiga, Saxena, M.C. and Solh, M.B., eds). The International Center for Agricultural Research in the Dry Areas (ICARDA). Addis Ababa, Ethiopia. pp. 1-18.

Ayneabeba Adamu, Fassil Assefa, Asfaw Hailemariam, Endashaw Bekele (2001). Studies of Rhizobium inoculation and fertilizer treatment on growth and production of faba bean (*Vicia faba*) in some yield depleted and yield sustained regions of Semien Showa. *SINET:* Ethiop. J. Sci. 24: 197-211.

Bond DA, Laws DA, Hawtin GC, Saxena MC, Stephens JH (1985). Faba bean (Vicia faba L.). In: Grain Legume Crops, (Summerfield, RJ and Roberts EH eds). William Collins and Co. Ltd., London. pp. 199-265.

Bond DA (1976). Field beans *Vicia faba.* In: Evolution of Crop Plants, (Simmonds NW ed). Long Man, London. pp.179-182.

Brockman FJ, Bezdicek DF (1989). Diversity within serogroups of *R. leguminosarum* bv. *viciae* in the Palouse region of eastern Washington as indicated by plasmid profiles, intrinsic antibiotic resistance, and topography. Appl. Environ. Microbiol. 55: 109-115.

Brockwell J, Pilka A, Holliday RA (1991). Soil pH is a major determinant of the numbers of naturally occurring *Rhizobium meliloti* in non-cultivated soils in central New South Wales. Aust. J. Exp. Agric. 31: 211-219.

Broughton WJ, Dilworth MJ (1971). Control of leghemoglobin synthesis in snakebean. Biochem. J. 125: 1075-1080.

Carter JM, Gardner WK, Gibson AH (1994). Improved growth and yield of faba beans (*Vicia faba* cv.Fiord) by inoculation with strains of *Rhizobium leguminosarum* bv. *viciae* in acid soils in South-West Victoria. Aust. J. Agric. Res. 45: 613-623.

CIAT (1988). Legume- Rhizobium symbiosis: Methods Manual for Evaluation, Selection and Agronomic Management. Cali: CIAT.

Coventry DR, Evans J (1989). Symbiotic nitrogen fixation and soil acidity In: Soil Acidity and Plant Growth, (Robson AD ed). Academic Press, Sydney, pp.103-137.

Desta Beyene, Angaw Tsigie (1987). Studies on nodulations and rhizobial strains of faba bean. In: Results of Research on Faba bean in Ethiopia, IAR/ICARDA/IFAD, Nile Valley Project During the 1986 Crop Season, IAR, Addis Ababa.

Desta Beyene, Angaw Tsigie (1988). Selection of effective Rhizobium strains of faba bean. In: Faba bean Research in Ethiopia, Under IAR/ICARDA/IFAD Nile Valley Project on Faba bean, 1985-1987, Crop Season. IAR, Addis Ababa.

Dilworth MJ, Howieson JG, Reeve WG, Tiwari RT, Glenn AR (2001). Acid tolerance in legume root nodule bacteria and selecting for it. Aust. J. Exp. Agric. 41: 453-446.

Evans LS, Lewin KF, Vella FA (1980). Effects .of nutrient pH on symbiotic nitrogen fixation by *Rhizobium leguminosarum* and *Pisum sativum*. Plant and soil, 56: 71-80.

FAO (1990). Guideline for Soil Description. Rome, p. 193.

Graham PH, Parker CA (1964). Diagnostic features in the characterization of the root- nodule bacteria of legumes. Plant Soil, 20: 169-231.

Hawtin GC, Hebblewate PD (1983). Back ground and history of faba bean production. In: The Faba bean (*Vicia faba* L.), (Hebblewat, P.D., ed.). Butter Worth's, Landon. pp. 1-22.

Howiesson JG, Ewing MA, D' Antuono (1988). Selection for acid tolerance in *Rhizobium meliloti.* Plant and Soil 105: 179-188.

Jesnen Erik S (1986). Symbiotic nitrogen fixation and nitrate uptake by the pea. Thesis(PhD) Royal Veternary and Agricultral University, Copenhagen.

Jordan DC (1984). Family III. Rhizobiaceae. In: Bergey's Manual of Systematic Bacteriology, (Krieg, N.R. and Holt, J.G. eds). The Williams and Wilkins, Baltimore. 1: 234-254.

Josey DP, Beynon JI, Johnson AWB, Bohlool BB (1979). Strain identification in *Rhizobium* using intrinsic antibiotic resistance. J. Appl. Bacteriol. 46: 343-350.

Kang S, Mills AL (2004). Soil bacterial community structure changes following disturbance of the overlying plant community. Soil Sci. 169: 55-65.

Lindstrom K, Lehtomaki S (1988). Metabolic properties, maximum growth temperature and phage sensitivity of *Rhizobium* sp (*galega*) compared with other fast-growing rhizobia. FEMS Microbiol. Lett. 50: 277-287.

Lupwayi NZ, Hague I (1994). Legume-Rhizobium Technology Manual. Environmental Science Division, International Livestock Center for Africa Addis Ababa, Ethiopia, p. 97.

Maidl FX, Haunz FX, Panse A, Fischbeck G (1996). Transfer of grain legume nitrogen within a crop rotation containing winter wheat and winter barley. J. Agron. Crop Sci. 176: 47-57.

McVicar R, Panchuk K, Brenzil C, Hartley S, Pearse P (2005). Faba bean in Sasktchewan. Saskatchewan Agriculture, Food and Rural Revitalization. A. Vandenberg, University of Saskatchewan

Nutman PS (1976). IBP field experiment on nitrogen fixation by nodulated legumes. In: Symbiotic Nitrogen Fixation in Plants, (Nutman, P. S., ed.). Cambridge University Press. pp. 211-237.

Peoples MB, Giller KE, Herridge DF, Vessey JK (2002). Limitations to biological nitrogen fixation as a renewable source of nitrogen for agriculture. In: Nitrogen Fixation Global Perspectives, pp. 356-360. (Finan TM, O'Brain MR, Lagzell DB, Vessey JK and Newton W eds). ABI Publishing, New York.

Purcino HMA, Festin PM, Elkan GH (2000). Identification of effective strains of *Bradyrhizobium*. Archis Pintoi. Trop. 77: 226-232.

Sahelemedihin Sertsu, Taye Bekele (2000). Procedures for soil and plant analysis. National Soil Research Center, Ethiopian Agricultural Research Organization, Addis Ababa, Ethiopia.

Schubert S, Mengel K, Schubert E (1990). Soil pH and calcium effect on nitrogen fixation and growth of broad bean. Agron. J. 82: 969-972.

Senayit Yethebarek, Asrat Wondimu (1994). Utilization of cool-season food legumes in Ethiopia. In: Cool-Season Food Legumes of Ethiopia, pp. 60-75, (Asfaw Telaye, Geletu Bejiga, Saxena MC and Solh MB eds). ICARDA, Addis Ababa, Ethiopia.

Somasegaran P, Hoben HJ (1994). Handbook for Rhizobia. Springer-Verlag.

Sorwil FK, Mytton LR (1986). The nitrogen fixing potential of Vicia faba rhizobia (*R. Leguminosarum*) from different agricultural locations. Plant Soil, 92: 249-254.

Stowers MD (1985). Carbon metabolism in Rhizobium species. Annu. Rev. Micrbiol. 39:89-108.

Tan IKP, Broughton WI (1981). Rhizobia in tropical legumes. XIII. Biochemical basis of acid and Alkali reactions. Soil Biol. Biochem. 13: 389-393.

Tang C, Thomson BD (1996). Effects of solution pH and bicarbonate on the growth and nodulation of a range of grain legume species. Plant Soil, 186: 321-330.

Van Berkum P, Desta Beyene, Fransisco TV, Keyes HH (1995).Variability among rhizobia strains originating from nodules of *Vicia faba*. Appl. Environ. Microbiol. 61: 2649-2653.

Vincent JM (1970). A Manual for the Practical Study of Root Nodule Bacteria. Blackwell, Oxford and Edinburgh, p. 164.

Vincent JM (1977). *Rhizohium:* general microbiology. In: A Treatise on Dinitrogen Fixation, (section III), (Hardy RWF and Silver WS eds). New York: John Wiley. pp. 277-366.

Westphal (1974). Pulse in Ethiopia their taxonomy and agricultural significance. Pub. Center for Agric. Pub-Doc., Wagenengen, p. 261.

Zapta F, Danso SKA, Hadarson G, Fried M (1987). Nitrogen fixation and translocation in field-grown faba bean. Agron J. 79: 505-509.

Zehari A, Aurag J, Khbaya B, Kharchat D, Filali-Maltouf A (2000). Phenotypic characteristics of rhizobia isolate nodulating *Acacia* species in the arid and Saharan regions of Moroco. Lett. Apppl. Microbiol. 30: 351-357.