academicJournals

Vol. 9(7), pp. 646-654, July, 2015
DOI: 10.5897/AJEST2015.1936
Article Number: DDA32E854520
ISSN 1996-0786

http://www.academicjournals.org/AJEST

African Journal of Environmental Science and Technology

Full Length Research Paper

Nutritional, eco-physiological and symbiotic characteristics of rhizobia nodulating faba bean (*Vicia faba* L.) collected from acidic soils of Ethiopia

Dereje Tsegaye[1]*, Fasil Assefa[2], Heluf Gebrekidan[3] and Gemechu Keneni[4]

[1]Arba Minch University, Soil Science Program, College of Agriculture and Environmental Sciences, P. O. Box 21, Arba Minch, Ethiopia.
[2]Addis Ababa University, Microbial, Cellular and Mollecular Biology Program, College of Life Science, P. O. Box 1176 Addis Ababa, Ethiopia.
[3]Haramaya University Soil Science Program, College of Agriculture and Environmental Sciences, P. O. Box 138, Dire Dawa, Ethiopia.
[4]Holeta Agricultural Research Center, P. O. Box 2003 Holleta, Ethiopia.

Received 28 May, 2015; Accepted 14 July, 2015

Characterization of root nodule bacteria is used for selecting and using them as inoculants to improve legume production. To this end, faba bean (*Vicia faba* L.) rhizobia were isolated from nodules collected from acidic soils of Central and Southern-Western parts of Ethiopia. A total of hundred rhizobial isolates were collected and characterized based upon their nutritional, ecophysiological and symbiotic characteristics. The isolates produced low to copious amount of extracellular polysaccharides (EPS) and attain colony sizes ranging from 2 to 7 mm with generation time ranging 0.75 to 3.9 h. Most of the isolates were grown at different pH levels ranging 4.5 to 7.0 and temperatures between 4 and 45°C. They were also capable of growing on many carbon sources and most of the nitrogen sources, and showed significant variations in resisting different types of antibiotics and heavy metals. Based on symbiotic efficiency (SE), 56% of the isolates were found to be very effective when applied with both Degaga and Dosha varieties. All taken together, two isolates, HUGAVf1 and HUCDVf5 were nutritionally versatile, showed a wide range of tolerance to the stress in many of the ecophysiological characters and very effective symbiotic performance should be utilized in future faba bean inoculants production.

Key words: *Rhizobium leguminosarum biovar. viceae*, antibiotic tolerance, carbon utilization, fast growing, heavy metals, temperature tolerance.

INTRODUCTION

It is estimated that 40.9% of highland areas of Ethiopia are affected by soil acidity (Schlede, 1989). However, soil acidity is one of the environmental factors that limit plant production because it is often associated with increased

*Corresponding author. E-mail: dassdere@yahoo.com. Tel: +251 911 33 84 40.

Aluminium (Al) and Manganese (Mn) toxicity and limit calcium and Phosphorus (P) up take by plants (Hungria and Vargas, 2000). Soil acidity hinders legume production more than any other crops as it affects the complex association of the legume host, the endosymbiont and the symbiosis (Graham, 1992). Faba bean (*Vicial faba* L.) is the leading grain legume crop grown as a food and cash crop throughout the mid altitudes and highlands between the altitudes 1800 and 3000 m above sea level in Ethiopia (ICARDA, 2006). The crop is integrated in the low input, traditional crop rotation and mixed agriculture system of the country to improve soil fertility (Gorfu, 1998). This is due to its ability to fix nitrogen in symbiosis with root nodule bacteria, *Rhizobium leguminosarum var. viceae* that also nodulate members of the tribe *Viceae*: field pea, lentil and grasspea (Jordan, 2005; Somasegaran and Hoben, 1994). It is one of the most efficient N fixer, and according to Somasegaran and Hoben (1994), it fixes nitrogen with fixation rate of 240-325 kg ha^{-1} $year^{-}1$. *R. leguminosarum* var *viceae*, is a fast growing and acid producing bacterium with generation time of 2-4 h, and colony size of 2-4 mm upon 3-5 days of incubation time (Jordan, 2005). However, recent studies both in Ethiopia and abroad showed isolates with relatively faster and slower growth and bigger colony size with much or little production of gum on the media. The isolates were also found to favor temperatures between 20 and 30°C, and tolerant to mild acidic and neutral pH (Abere et al., 2009; Zerihun and Fasil, 2011; Girmaye et al., 2014).

The rhizobial isolates were found to have diversity in their response to various nutritional and ecophysiological tests. The diversity of isolates might be an important factor to be considered in strain selection and presservation of culture for inocula production. The different environmental factors affect the rhizobia-legume symbiosis and there by nitrogen fixation. Legume productivity is limited significantly due to the sensitivity of the legume rhizobia symbiosis and nodule formation to pH, temperature, salinity and osmotic stress. Hence, strains that tolerated to these environmental constraints may be potential candidates for developing broad-host range inoculants.

Soil acidity for legume production can be alleviated through liming or selection of tolerant varieties and endosymbionts (Andrade et al., 2002). Montanez (2008) showed that pH mediated phosphorus fixation and P deficiency could be alleviated by inoculating phosphate solubilizing microorganisms to enhance legume production in acidic soils. Girmaye et al. (2014) revealed that acidic soil harbor phosphate solubilizing rhizobia and formed effective symbiotic association with faba bean plant. However; effective symbiosis on the host under acidic stress conditions depend up on the strain and the legume variety (Vijila and Jebaraj, 2008) indicating that isolating and characterizing of rhizobia from acidic environmental conditions is crucial for inocula production (Rengel, 2002).

The ability to utilizing a wide range of carbon and amino acid sources have significance for rhizobia strain classification and has also an ecological advantage in colonizing the soil and competing with other microorganism around the rhizosphere. Also, antibiotic resistance performances of the isolates to the different concentration can be useful for ecological investigations which have better survival ability and they can compete with other organism. The releasing of antibiotic by some group of microorganism may help to better colonize the rhizosphere. Heavy metal resistance patterns found among the indigenous rhizobial isolates are reflecting the stresses pressure predominant in their locations.

N and P elements are deficient in most of the faba bean growing acidic soils of Ethiopia (Tekalign et al., 1988; Tsegaye, 1992). Reports also indicated that the survival of host-strain interaction (Hubbell and Kidder 2003) and specificity of symbiotic association in many crops under stressed environments (Devi et al., 2010), which necessitated the search for elite rhizobia for potential development and application in legume production under such circumstances. In this study isolation and charcterization of rhizobia isolates was carried out from acidic soils of Central and Southern-Western Ethiopia.

MATERIALS AND METHODS

Sampling sites

Rhizobia were isolated directly from nodules collected from Gumer (45 sites), Holleta (35 sites), Jima Dedo (35 sites) and Chencha (35 sites) using the standard methods (Somasegaren and Hoben, 1994) (Figure 1). They were purified and preserved on YEMA (Yeast extract manitol agar) slants in screw cap tubes at 4°C. Some of the representative isolates are shown on Table 1

Designation of rhizobial Isolates

The pure isolates were designated as HU (Haramya University cultures), first letter of the name of the sampling sites and *Vf (*host plant *Vicia faba)* followed by the different serial numbers representing each isolate.

Characterization of the Isolates

A total of hundred isolates were characterized for their cultural, nutritional and ecophysiological features. For every experiment, the isolates were grown overnight on Yeast Extract Mannitol Broth (YEMB) (Oxoid), and standardized to inoculum concentration of 10^4 CFU/ml, and transferred to YEMA and incubated at 28±2°C for 5 to seven days (Somesegaran and Hoben, 1994). The tests were carried out in triplicates and result for growth tests were determined qualitatively, growth and no growth were represented as '+' and '-' respectively.

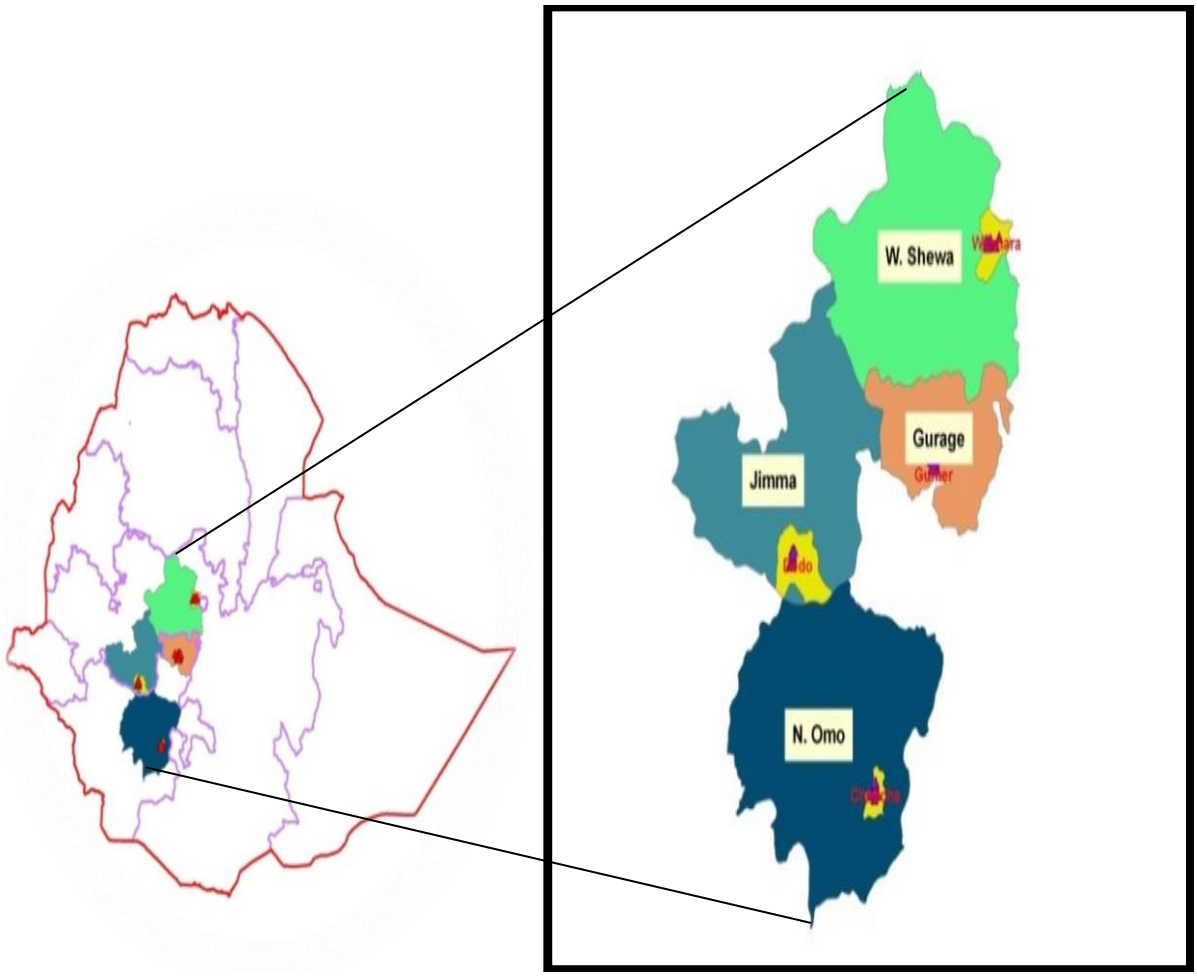

Figure 1. General location map of study area.

Cultural and growth characteristics

Individual colonies were characterized on YEMA medium based on their colony size (mm), capacity to produce extracellular polysaccharide gum (low, moderate and copious amount) and generation time (Maatallah, 2002).

Nutritional characteristics

The ablity of isolates to grow on different sources of carbon and nitrogen were tested using the methods of Somansegaran and Hoben (1994). The following filter sterilized carbon sources were added as 10% of the basal medium of YEMA without the original carbon source of the medium. The carbon sources were; glucose, lactose, tartarate, dextrin, citrate, sorbitol, D-fructose, rafinose, maltose, erythrose, malate, dulictol, galactose, glycerol, cellobiose, trehalose and starch (Difco). Similarly, filter sterilized nitrogen sources; alanine, arginine, tyrosine, L-lysine, L-valine, glutamine, L-Isoleucine, asparagien, glycine, L-phenylalanine, D-lystiene, and L-leucine were added to the basal medium at a concentration of 0.5 g l^{-1} from which ammonium sulfate was omitted and Manitol was added at a concentration of 1 g l^{-1}.

Eco-physiological characteristics

The ability of the isolates to grow at incubation temperatures of 4, 10, 20, 25, 35, 40 and 45°C and pH of 4.5, 5, 5.5 6, 6.5 and 7 was tested on TY (5 g Tryptone, 3 g yeast extract, 0.87 g $CaCl_2$ and 15 g agar) medium (Somansegaran and Hoben (1994). intrinsic resistance (IAR) to antibiotics and heavy metals was tested on solid YEMA medium containing the following filter-sterilized antibiotics or heavy metals at concentrations of µM: kanamycin (2.5; 10), streptomycin (2.5; 10), rifampicin (2.5; 10), ampicilin (2.5; 10), chloramphenicol (2.5; 10) spectinomycin (2.5; 10) nalidixic acid (2.5; 10) erythromycin (2.5; 10) and tetracycline (2.5; 10) ; and for heavy metals $ZnCl_2$ (50), $CdCl_2$ (20), $NiSO_4$ (100), $CoCl_2$ (25), $HgCl_2$ (10), $AlCl_3$ (250), Pb $(CH_3COO)_2$ (250), $CuCl_2$ (50) and $MnCl_2$ (500).

Symbiotic characteristics on sand culture

The experment was carried out at the National Soil Testing Center, Ethiopia, to select elite isolates of rhizobia forming effective symbiotic association with faba bean varieties following Somansegran and Hoben (1994). Prescreening of the relative

Table 1. Representative faba bean rhizobial test isolates with GPS data on site of isolation, and soil pH.

Isolate	Districts of origin	Locality	Latitude	Longitude	Altitude	Soil pH
HUGS*Vf3*	Gumer Sheleko		N 08°76'63.3''	E 39°63'61''	2894	4.8
HUGE*Vf5*	"	Essene	N 08°74'96.7''	E 39°70'03''	2895	4.8
HUGA*Vf1*	"	Abeke	N 08°75'38.9''	E 39°69'92''	2922	5.3
HUGZ*Vf1*	"	Zizencho	N 08°79'80.9''	E 39°33'05''	2893	5.5
HUGZ*Vf2*	"	Zizencho	N 08°79'61.1''	E 39°39'21''	2831	5.5
HUGD*Vf3*	"	Dember	N 08°85'03.2''	E 39°30'25''	2870	5.7
HUGD*Vf4*	"	Dember	N 08°84'95.0''	E 40°13'84''	3003	5.5
HUGI*Vf2*	"	Injefo	N 08°86'25.1''	E 40°24'38''	3020	5.3
HUGI*Vf4*	"	Injefo	N 08°87'34.1''	E 40°22'30''	2950	5.4
HUGJ*Vf1*	"	Jemboro	N 08°86'72.1''	E 38°48'59''	2817	5.1
HUGJ*Vf4*	"	Jemboro	N 08°75'04.2''	E 39°40'50''	2850	5.3
HUHR*Vf5*	Holleta	Rs. Center	N 10°00'19.8''	E 44°56'21''	2395	4.9
HUHR*Vf9*	"	Rs. Center	N 10°01'30.6''	E 44°47'90''	2415	4.7
HUHC*Vf1*	"	Choke	N 10°05'01.6''	E 44°82'03''	2457	5.1
HUHC*Vf5*	"	Choke	N 10°04'75.0''	E 44°82'34''	2435	5.4
HUHM*Vf1*	"	Menagesha	N 10°00'84.0''	E 45°22'28''	2536	5.0
HUHM*Vf2*	"	Menagesha	N 10°01'27.6''	E 45°37'00''	2578	5.4
HUHM*Vf4*	"	Menagesha	N 10°00;52.7''	E 45°26'10''	2500	5.1
HUHM*Vf5*	"	Menagesha	N 10°00'40.0''	E 45°30'33''	2520	4.9
HUHB*Vf5*	"	Bedi	N 10°05'76.2''	E 45°65'33''	2700	4.8
HUJS*Vf3*	J.Dedo	Sito	N 08°19'10.2''	E 26°43'97''	2314	5.6
HUJK*Vf1*	"	Keta	N 08°29''60.7''	E 26°63'25''	2233	5.2
HUJD*Vf2*	"	Debele	N 08°22'65.2''	E 26°24'09''	2540	5.7
HUJM*Vf5*	"	Metoso	N 08°21'0.26''	E 26°51;21''	2264	5.5
HUCM*Vf3*	Chencha	Mesho	N 06°92''3.88''	E 33°70'95''	2755	4.9
HUCO*Vf3*	"	Ote	N 06°97'27.6''	E 34°14'20''	3008	5.2
HUCD*Vf5*	"	Dorze Laka	N 06°98'61.7''	E 34°09'07''	2630	4.9

symbiotic effectiveness of the isolates was undertaken in a pot experiment using a sand culture. Each isolate was grown on Yeast extract manitol broth (YEMB) for three days and then adjusted to a concentration of 10^9 CFU ml^{-1}. About 3 kg of carefully washed, sieved and sterilized river sand was filled into surface disinfected (using 70% alcohol) plastic pots. Sterile paper towels were inserted aseptically at the base of the pot to prevent loss of nutrients and filled with acid treated sterile moisten sand. Seeds of two varieties of faba bean, *Degaga* and *Dosha,* were surface-sterilized as before. The seeds were planted in 3 kg capacity alcohol-swabbed plastic pots containing acid washed river sand as a media. Five seeds were planted pot^{-1}, individually treated with 1 ml of inoculum and thinned to three plants pot^{-1} 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. They received quarter strength of N-free nutrient solution and distilled water once a week and every two days, respectively. Two treatments, namely an unfertilized and an uninoculated check as a negative (TO) and uninoculated but nitrogen fertilizer (0.05% KNO_3/week) check as a positive (TN) controls. Plants were harvested 45 DAE and records were taken on root and shoot length, number of nodules $plant^{-1}$, nodule dry mass, shoot dry mass and symbiotic effectiveness. The experiment was laid out using a randomized block design with three replications in a factorial arrangement. Relative effectiveness of isolates were calculated according to the equation proposed by Purcino et al. (2000) as:

$$SE\ (\%) = \frac{DMI\ X\ 100}{DMN}$$

Where SE (%) = percent symbiotic efficiency, DMI = dry biomass produced by inoculated plants, and DMN = dry biomass produced by plants when N is applied.

The isolates were categorized into four efficiency groups using the method suggested by Purcino et al (2000) as: ineffective (< 35%), lowly-effective (35 to 50%), effective (50 to 80%) and highly effective (> 80%).

Data analysis

Separate analysis of variance (ANOVA) was computed to quantify the total variation among the isolates, the varieties and isolate by variety interaction effects using the following model of separate analysis of variance using SAS computer software (SAS Institute, 2002) as:

$$Y_{ijk} = \mu + B_i + I_j + V_k + (IV)_{ij} + E_{ijk}$$

Table 2. Cultural and growth characteristics of rhizobial isolates on YEMA medium with pH 7, incubated at 30°C for 5-7 days.

Cultural characteristics	Growth characteristics	Number of isolates by site				
		Gumer	Holleta	Jima Dedo	Chencha	Total
Colony size	> 5 mm	7	11	-	-	18
	3-5 mm	25	18	13	13	69
	≤ 3 mm	1	-	7	5	13
	Total	33	29	20	18	100
EPS[1]	L[2]	25	24	18	15	82
	M[3]	4	4	2	3	13
	C[4]	4	1	-	-	5
	Total	33	29	20	18	100
Growth rate	≤ 50 min)	2	0	2	0	4
	50 min < GT≤ 3 h	27	27	12	18	84
	3h[5] < GT[6]≤ 9 h	4	2	6	0	12
	Total	33	29	20	18	100

EPS[1]= Exopolysacchride, L[2]= low, M[3]= medium, C[4]= copious amount, h[5]= hours, GT[6]= generation time.

Where, Y_{ijk} = the total observation, μ = grand mean, B = the effect of block i, I = the effect of isolate j, V = the effect of variety k, IV = the interaction effect between isolate j and variety k, and e_{ijk} = random error. A mean separation 5% probability level was done using the Least Significant Difference (LSD) method following Gomez and Gomez (1984).

RESULTS AND DISCUSSION

In this study a total of 100 rhizobial isolates were collected from nodules of acidic soils (pH 4.7-5.7) of Gumer (33), Holetta (29), Jimma/Dedo (20), and Chencha (18) (data not shown). Most of the isolates showed convex or domed shaped (94%) colonies with elastic (85%) texture, almost similar with the colony shape (100% dome-shaped) and in contrary with colony texture (100% buttery) of faba bean rhizobia reported by Antneh (2012). They attained colonies diameters ranging from 2.0 to 7.0 mm, the majority of which (69%) fell within 2-4 mm, and 18% attained colony diameters greater than 5 mm after five to seven days of incubation (Table 2). The colony diameters obtained in this study were greater than the colony diameter of 1.5-5.00 mm of faba bean rhizobial isolates reported from acidic soils of East and West Wollega (Girmaye et al 2014) and other isolates from Vetch (*Vicia* spp.) in Turkey (Adiguzeli, 2010). Most of the rhizobial isolates (82%) produced low amount of EPS with a few exceptions (5%) of the isolates produce a copious amount of EPS. According to Yanmei et al (2007) rhizobial isolates producing more EPS have better capacity for P solublization.

All taken together, 84% of isolates were categorized into fast growing groups (50 min-3h), 4% and 12% of the isolates were within the categories of very fast growing (< 50 min) and slow growing groups (3-9 h) respectively, Except a few isolates, the majority of the isolates fell into fast growing groups according to Maatallah (2002). Girmaye et al (2014) reported that the mean generation times (MGT) of the rhizobial isolates collected from acidic soil (pH 4.6-6.3) were ranged between 1.07 and 6.24 h.

Nutritional characteristics

Carbon and amino acid utilization

The data on C and N nutritional properties showed that isolates were capable of utilizing 61-100% of the tested carbohydrates, and 75-100% of the amino acid sources (Table 3) indicating that they were more versatile in N utilization than C utilization. All isolates utilized glucose and glycerol and majority of the isolates (85%) were capable of utilized starch, fructose, sorbotil, lactose, maltose, rafinose, erythrose, celliobiose and sorbitol. Relatively, Citrate was the most recalcitrant substrate that was utilized by only 43% of the isolates). The most versatile isolates; HUGE*Vf5*, HUGA*Vf1*, HUGA*Vf4*, HUHA*Vf2*, HUHR*Vf1* and HUCT*Vf2*) (6%) were able to utilize all the carbon sources. Girmaye et al (2014) also indicated that more than 50% the faba bean isolates from acidic soils grew on basal growth medium containing all the twelve carbon sources tested.

The result, in general showed that there was no significant difference in carbohydrate utilization among isolates, except on citrate. Corroborating that fast growing rhizobia were able to utilize a large variety of carbon substrates (Stowers, 1985). All isolates were able to metabolize alanine, asparagien, L-Valine and glutamine, and the majority utilized arginine, tyrosine, asparagien, glycine, L- phenylalanine L-lysine, and L-

Table 3. Rhizobial isolates with superior nutritional and eco physiological characteristics.

Isolates type	pH tolerance	Temperature tolerance (°C)	Carbon utilization (%)	Amino acid utilization (%)	IAR[1] (%)	HMT[2] (%)
HUGS*Vf3*	5.0-7.0	20-35	78	83	89	56
HUGE*Vf5*	5.0-7.0	4-40	100	75	100	67
HUGA*Vf1*	4.5-7.0	10-40	100	100	94	78
HUGZ*Vf2*	5.0-7.0	20-40	88	92	100	44
HUGD*Vf3*	5.0-7.0	20-40	88	58	67	56
HUGD*Vf4*	4.5-7.0	20-35	66	100	89	44
HUGI*Vf2*	5.0-7.0	20-35	94	83	100	78
HUGI*Vf4*	5.0-7.0	20-35	78	100	83	44
HUGJ*Vf1*	5.0-7.0	20-35	78	100	100	44
HUGJ*Vf4*	5.0-7.0	20-35	88	100	83	56
HUHC*Vf1*	5.0-7.0	20-35	72	83	94	78
HUHC*Vf5*	5.5-7.0	4-40	94	92	89	56
HUHM*Vf1*	5.0-7.0	20-35	88	83	83	44
HUHM*Vf2*	5.0-7.0	20-35	61	100	78	56
HUHM*Vf4*	4.5-7.0	20-40	94	92	89	67
HUHM*Vf5*	5.0-7.0	20-35	78	83	78	56
HUJK*Vf1*	4.5-7.0	20-40	72	83	78	56
HUJD*Vf2*	5.0-7.0	4-45	94	92	100	89
HUJM*Vf5*	5.0-7.0	20-35	89	100	78	78
HUCM*Vf3*	5.0-7.0	20-35	89	100	78	56
HUCO*Vf3*	5.0-7.0	20-35	83	83	89	78
HUCD*Vf5*	5.5-7.0	10-40	94	100	89	78

IAR[1]= Intrinsic antibiotic resistance, HMT[2]= Heavy metal tolerance.

leucine, except that only 40% of them assimilate L-isoleucine. Unlike that of carbohydrates , forty percent of the isolates were able to utilize all amino acid tested similar to the findings of Girmaye et al. (2014) where 48% of the isolates were utilized all amino acids sources.

Ecophysiological characteristics

Temperature and pH tolerance

All rhizobial isolates were grown at 20-35^{o} emperature range, and pH 5.0-7.0 (Table 3) However, a few isolates were capable of growing on YEMA adjusted to a pH of 4.5 (12%) and incubated at temperatures of 4°C (11%) and 40°C (15%). Twelve (12) isolates namely, HUGE*Vf3*, HUGA*Vf1,* HUGZ*Vf3,* HUGD*Vf4,* HUHA*Vf1*, HUHC*Vf2*, HUHC*Vf4*, HUHM*Vf4*, HUJS*Vf1*, HUJS*Vf5*, HUJK*Vf1,* and HUJK*Vf1* were found to be relatively tolerant to a lower pH of 4.5. The temperature tolerance of the majority of the isolates in this study was similar with previous works on *Vicia faba* rhizobia (20-30°C) reported by Abere et al. (2009), Zerihun and Fasil (2011) and Girmaye et al. (2014).

The isolates showed wide differences in their pH tolerance (Table 3). Almost all of the isolates were found to grow at pH range of 5.5-7.0. Similar explanation was made by Girmaye et al. (2014); thirty two percent of the isolates tolerated the lower pH 4.

Intrinsic antibiotic resistance (IAR)

The isolates showed a wide range of tolerance to 78-94% of all the tested antibiotics were relatively tolerant to erythromycin and sensitive to rifampicine, naldixic acid and kanamycin. Six isolates (6%) HUGA*Vf5*, HUGI*Vf2*, HUGZ*Vf2*, HUGI*Vf1*, HUJD*Vf2* and HUJG$_S$*Vf5* were resistant to all antibiotics concentrations. Roughley et al. (1992) isolated highly antibiotic resistant rhizobia from Malaysian soils. In a similar study, faba bean rhizobia showed sensitivity to ampicillin and kanamycin than other types of antibiotics (Girmaye et al., 2014).

Heavy metals tolerance

The response of isolates to different heavy metals is shown on Table 4. The data showed that isolates were more sensitive to heavy metals than antibiotics, where they showed tolerance from 44%-89% of the tested heavy metals (Tables 2 and 3). The pattern of tolerance by the isolates to the different heavy metals was in the order of 74, 65, 63 and 49% to Zinc, Manganese, Copper and Aluminium, respectively, and sensitive to Lead, Nickel, Cobalt, Cyanide and Mercury to the tune of 24, 23, 22, 19, and 11%, respectively., Six Isolates (6%) HUGA*Vf4,* HUGF*Vf1,* HUGF*Vf4,* HUJS*Vf2,* HUJG*Vf4* and HUCT*Vf4* even did not grow on any heavy metals.

A comparable behavior was observed with different types and concentration of intrinsic antibiotic resistance

Table 4. Tolerance of isolates different types and concentration of antibiotic and heavy metals.

Sampling site	IAR[1] (80-100%)	HMT[2] (> 50%)	Both
Gumer	14 (42%)	7 (21%)	5 (15%)
Holleta	13 (45%)	10 (34%)	6 (20%)
Jima Dedo	6 (30%)	4 (20%)	3 (15 %)
Chencha	8 (41%)	3 (17%)	3 (17%)
Total	41%	24%	17%

IAR[1]= Intrinsic antibiotic resistance, HMT[2]= heavy metal tolerance.

and heavy metal tolerance of isolates on the sampling sites (Table 4.). Isolates collected from Gumer and Holleta areas were found to be inhibitorier, this might be related their adaptation at their soil of isolation sites.

Symbiotic effectiveness on sand culture

Nine rhizobial isolates were assessed for their symbiotic effectiveness using sterile and acid treated sand in a pot experiment under greenhouse condition (Table 5). Brockwell et al. (1998) indicated that, the ability to form nodules along with the subsequent capacity fixing nitrogen is widely used as means of evaluating the inherent links between rhizobia and respective hosts. On the basis of host plant specificity for infection and nodulation these species were generally assumed to be *Rhizobium leguminasorium bv viciae* (van Berkum et al., 1995).

Rhizobial inoculations significantly ($P \leq 0.05$) increased root and shoot length, number of nodules per plant, nodule dry mass, shoot dry mass and symbiotic effectiveness as compared to the control treatments (Table 5). Likewise, measured parameters displayed significant variation among rhizobial isolates treated with two varieties (*Dosha* and *Degaga*) of faba bean plants $P \leq 0.05$. The inoculated plants formed red and pink nodules, which are indications for the formation of effective nodules (Somasgaran and Hoben, 1994) with effective N_2 fixation and for the presence of leghemoglobin (Amara et al., 1995).

The nodules number (NN) plant^{-1} ranged from 85 for isolate HUJM*Vf5* to 149 for HUCD*Vf5* with the variety *Degaga* and from 56 for isolate HUCO*Vf3* to 169 HUHM*Vf4* with *Dosha* variety. The mean NN plant^{-1} recorded in this study (which was 88 for *Degaga* and 92 for *Dosha*) were less than 98 NN plant^{-1} of faba bean on acidic soil reported by Girmaye Kenasa et al. (2014). Nodule dry weight (NDW) was between 71 for rhizobial isolate HUHC*Vf1* and 117 mg plant^{-1} for HUGA*Vf1* with *Degaga*. The corresponding range was 44 for isolate HUCO*Vf3* and 128 mg plant^{-1} for isolate HUHM*Vf4* with *Dosha*. The mean NDW recorded in this study was 79 mg plant^{-1} in both varieties. Therefore, we observed a slight higher mean nodules dry weight value than the 78 mg plant^{-1} which was reported by Girmaye Kenasa et al. (2014), but a lower than 145 mg plant^{-1} which was reported by Anteneh Argaw (2012).

It was also evident that, as compared to the control plants, inoculation induced significant improvement in the mean plant shoot height (SH) (Table 5). The highest plant SH 43.3 cm was recorded for isolate HUGA*Vf1* inoculated with *Degaga* varaiety and 45.3 cm recorded for HUJD*Vf2* inoculated with *Dosha* variety. These improvements in SH were equivalent to 32.94% over the negative control and 16.9% over the positive control (N treated plants) with *Degaga* and 29.3% over the negative control and 11.7% over the positive control (N treated plants) with *Dosha*. These results were more or less consistent with the results of Anteneh Argaw (2012) study on faba bean inoculation with *Degaga* variety which was noted 49.7 cm SH with rhizobial isolate NSFBR-48 collected from Central Ethiopia which showed pronounced improvement in shoot height i.e. 51 and 14% over negative and N treated plants, respectively. This enhancement of SH could be attributed to the fact rhizobia may augment plant growth by providing products of N_2 fixation (Kumar et al., 2011).

Based on shoot dry weight accumulation in reference to N fixing and control plants, All inoculated plants were symbiotic effective with *Degaga* and *Dosha* varieties and according to Purcino et al. (2000), 56% the isolates were highly effective in both *Degaga* and *Dosha* varieties. The best two isolates HUGA*Vf1* and HUCD*Vf5* with *Degaga* variety, showed effectiveness of 113.7 and 107.1%; whereas another best isolate HUJD*Vf2* with *Dosha* variety showed effectiveness of 102.3% as compared to the N treated plants (Table 5). In this experiment, more highly effective isolates were obtained compared to other investigator reports. Girmaye Kenasa et al. (2014) revealed that rhizobial isolates of faba bean collected from acidic soils of Wollega, Western Ethiopia were effective, from which 16% of the isolates were highly effective. This result underlines the importance for local screening of *Rhizobium* isolates which improve N_2 fixation in faba bean.

Conclusions

The rhizobial isolates tested in this study were phenotypically different representing very fast, fast and slow growing types with different colony morphology, EPS production, generation time, and even nutritional and eco-physiological characteristics. The variation among isolates for many important features is considered as important prerequisite for effective strain selection. Inoculation of faba bean with rhizobia isolates significantly improved all growth parameters on sand

Table 5. The effects of inoculation of phosphate solublizing rhizobia nodulating faba bean varieties (*Degaga* and *Dosha*) for different characteristics and symbiotic effectiveness under control condition by using sand culture.

Treatment*	Shoot length (cm)			No of nodules plant^{-1}			Nodule dry weight plant^{-1} (mg)*			Shoot dry weight plant^{-1} (g)			SE (%)		
	Degaga	***Dosha***	***Mean***	***Degaga***	***Dosha***	***Mean***	***Degaga***	***Dosha***	***Mean***	***Degaga***	***Dosha***	***Mean***	***Degaga***	***Dosha***	***Mean***
HUGE*Vf5*	41.3^{a-f}	42.7^{a-d}	42^{ab}	9.6^{g-n}	12.1^{a-c}	10.8^{bc}	9.1^{f-r}	10.0^{b-g}	9.6^{b-d}	1.88^{b-i}	2.08^{a-e}	1.98a-c	95.4	96.7	96.1
HUGA*Vf1*	43.3^{a-c}	39.7^{a-i}	41.5^{ab}	11.2^{c-f}	10.7^{d-g}	11.0^{bc}	10.8^{a-c}	10.8^{a-c}	10.8^{a}	2.24^{a}	2.06^{a-f}	2.15a	113.7	95.8	104.8
HUGI*Vf2*	36.0^{b-n}	31.3 $^{i-r}$	33.7^{d-h}	9.0^{h-p}	10.3^{e-i}	9.7^{d-f}	8.6^{h-t}	10.2^{a-f}	9.4^{c-e}	1.57^{i-s}	1.57^{i-s}	1.57^{e-h}	79.7	73.0	76.4
HUHC*Vf1*	32.0^{h-r}	41.3^{a-f}	36.7^{b-f}	10.4^{e-h}	10.1^{f-k}	10.2^{c-e}	8.4^{k-t}	9.0^{f-s}	8.7^{d-i}	1.42^{m-v}	1.41^{n-v}	1.42^{g-i}	72.1	65.6	72.1
HUHM*Vf4*	34.3^{d-r}	39.3^{a-i}	36.8^{b-f}	9.3^{h-n}	13.0^{a}	11.2^{ab}	9.2^{e-p}	11.3^{a}	10.2^{ab}	1.44^{k-v}	1.97^{a-h}	1.70^{d-f}	73.1	91.6	82.4
HUJD*Vf2*	41.0^{a-g}	45.3^{a}	43.2^{a}	10.2^{f-j}	12.1^{a-c}	11.2^{ab}	9.4^{d-n}	10.9^{a}	10.2^{a-c}	1.82^{c-j}	2.19^{ab}	2.01^{ab}	92.4	102.3	97.4
HUJM*Vf5*	29.3^{l-n}	35.3^{b-p}	32.3^{e-h}	9.2^{h-n}	8.6^{l-q}	8.9^{f-j}	8.7^{h-t}	7.9^{p-t}	8.3^{f-l}	1.66^{g-p}	1.22^{s-w}	1.44^{g-i}	84.3	56.7	70.5
HUCO*Vf3*	34.3^{d-r}	27.0^{p-r}	30.7^{gh}	9.4^{g-n}	7.5^{q-s}	8.4^{h-k}	8.5^{i-t}	6.6^{v}	7.6^{lm}	1.16^{t-w}	1.39^{n-v}	1.27^{i}	58.9	64.7	61.8
HUCD*Vf5*	38.3^{a-j}	37.7^{a-l}	38.0^{a-e}	12.2^{a-c}	11.5^{b-e}	11.8^{a}	10.4^{a-d}	10.8^{a-c}	10.6^{a}	2.11^{a-c}	2.09^{a-d}	2.10^{a}	107.1	97.2	102.2
N	36.0	40.0	38.0	0.0	0.0	0.0	0.0	0.0	0.0	1.97	2.15	2.06	-	-	-
Control	29.0	32.0	30.5	0.0	0.0	0.0	0.0	0.0	0.0	0.79	0.96	0.88	-	-	-
Mean	34.7	36.0	35.4	9.4	9.6	9.5	8.8	8.9	8.9	1.57	1.63	1.60	-	-	-
SEM (±)	17.17	17.17	17.17	0.4	0.45	0.45	0.38	0.38	0.37	0.034	0.034	0.034	-	-	-
CV (%)	11.7	11.7	11.7	7.05	7.05	7.05	6.9	6.9	6.9	11.5	11.5	11.5	-	-	-
LSD (0.05)	6.69	6.69	4.73	1.08	1.08	0.76	0.99	0.99	0.70	0.297	0.297	0.211	-	-	-

*Data transformed by square root, SE = symbiotic effectiveness SEM= Standard error of mean, CV= Coefficient of variation, LSD= least significant difference, Unit within a column followed by same letter(s) are not significantly different at LSD P < 0.05 level, Control = without chemical and inoculation, N=with optimum amount of N fertilizer

cultures. The results of this study enabled the identification of elite indigenous rhizobial isolates including HUGA*Vf1* and HUCD*Vf5* for further evaluation and verification under field condition. This study also indicated the need for the initiation of a planned strain collection and selection program from soil acidity-prone areas in Ethiopia.

Conflict of interest

The authors do not declare any conflict of interest.

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