Original Research Article

Isolation and Characterization of Phosphate Solubilising Rhizobia Nodulating Wild Field pea (*pisum sativum var. abyssinicum*) from Southern Tigray, Ethiopia

Comment [s1]: P

Comment [s2]: abyssinicum

Abstract

Phosphorus is the second limiting nutrients next to nitrogen as well as the least mobile element in the soil. This nutrient is one of the major constraint for low productivity of wild field pea in the study site. Hence, the development of environmental friendly and economically accepted to subsistent farmer is undeniably important. Thus, this experiment was initiated to isolate and characterize inorganic phosphate solubilizing rhizobia from root nodules of field pea (Pisum sativum var. abyssinicum) were characterized for their inorganic phosphate solubilisation ability on Pikovaskaya liquid and solid media. Results revealed that all isolates were gram negative, failed to grow on peptone glucose agar, ketolactose test and did not absorb congo-red upon incubation period. Results showed that phosphate solubilisation index of root nodulating bacteria on in vitro Pikovskaya's agar medium varied from 1.54 to 2.70. Inorganic phosphate solubilisation in broth medium dissolved insoluble $Ca_3(PO_4)_2$ *was within the range of 16.59-23.95 mg plant*$^{-1}$ *with pH drop from 7.01 to 5.33. Among the tested rhizobia isolates, HUDRI-8 and HUDRI-25 was found to be highest phosphate solubilisation compared to the remaining isolates, served as efficient phosphate solubilizers, and could be used for further test under field condition. Finally, those isolates effective in* N_2 *fixation and able to solubilise inorganic P were found to be effective in promoting nodulation and plant growth under greenhouse condition in soil having high and low background rhizobia nodulating wild field pea.*

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Keywords: Field pea, Phosphate solubilising rhizobia, *Pisum sativum* var. *abyssinicum*, Rhizobium

INTRODUCTION

Phosphorus (P) is a major growth limiting nutrient unlike nitrogen, there is no large atmospheric source that can be made biologically available (Ezawa *et al*., 2002). In most soils, its content is about 0.05% of which only 0.1% is plant available (Achal *et al*., 2007). Besides this, inorganic P fertilizer is the main sources of P in the agricultural soils, although 75 to 90% of the added P fertilizer is precipitated by iron, aluminium and calcium complexes present in the soil system (Turan *et al*., 2006). According to Antoun *et al*., 1998, report many soil bacteria and fungi have the ability to solubilize phosphorus (P) and make it available to plants. Microorganisms are central point to the soil P cycling and play a significant role in consent the conversion of the element between different inorganic and organic soil P fractions, then releasing available P for plant growth (Oberson, 2001). Phosphorus biofertilizers can help to increase the accessibility of accumulated phosphates for plant growth by solubilization (Gyaneshwar et al., 2002). The involvement of microorganisms in

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inorganic phosphates solubilization was reported as early as 1903 (Khan *et al*., 2007), and the presence of these microorganisms (PSMs) are everywhere, while their numbers are vary from soil to soil. Among the microbial populations present in the soil, P solubilising bacteria constitute 1-50% and P solubilizing fungi are 0.1 to 0.5% (Chen *et al*., 2006). The most important P solubilizing bacterial genera are *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Microccocus, Aereobacter, Flavobacterium and Erwinia* (Rodriguez and Fraga, 1999). This study found that out of 13 bacterial strains of different genera that screening on different insoluble mineral phosphate substrates were indicated that *Rhizobium, Pseudomonas* and *Bacillus* species were the most powerful P solubilizers. Tandon (1987) observed that in 10 out of 37 experiments phosphate solubilizing bacteria (PSB) inoculations resulted in 10-15% increment in crop yields. Khalil (1995) also investigated 10 bacteria and 3 fungi being able to solubilize phosphate on the basis of large clear zone on solid media. *Rhizobium leguminosarum* is involving in phosphate solubilization as well as biological nitrogen fixation (BNF) through the root nodules of bacteria (Gyaneshwar *et al*., 2002). During phosphate solubilization process, 2-ketoglucolnic acid is the most synthesized organic acid (Halder *et al*., 1990). Phosphate solubilizing rhizobia has been shown to increase the growth of maize and lettuce (Chabot *et al*., 1996). The multi-functionality exhibited by *R. leguminosarum* makes it important in food production in terms of reducing cost and improving efficiency of P fertilization, especially in P-limited soils (Jia Xie, 2008). So far, phosphate solbilizing of fababean and chickpea nodulating rhizobial isolates from Ethiopian soils have been done by several authors (Girmaye *et al.,* 2014, Assefa *et al*., 2010 and Mulissa *et al,* 2016). Feredegn, 2013 also assessed the phosphate solubilization of rhizosphere and endophytic bacteria from sugarcane (S*accharum officinarum* L.). Although the phenotypic and symbiotic effectiveness of rhizobia nodulating field pea (*pisum sativum var. sativum*) in Ethiopian soils were studied by Aregu *et al*., 2012; Fano, 2010 and Kassa *et al*., 2015), the phosphate solubilizing efficacy, symbiotic effectiveness of rhizobia nodulating field pea (*pisum satvum var. abyssinicum*) is not well investigated. Therefore, this study was designed to isolate and characterizing indigenous phosphate solubilizing root nodulating bacteria of field pea (*pisum sativum var. abyssinicum)* and their effect on converting insoluble P in to soluble P and effectiveness on soil culture.

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Material and Methods

Soil sampling site and sample collection

The soil samples for nodule trapping and physico-chemical analysis were collected from Emba-Alaje and Endamohoni districts of southern Tigray, considering long history of field pea growing and no history of rhizobium inoculation. The corresponding GPS data including altitude and soil pH were indicated in Table 1. Twenty two soil samples were separately collected from the depth of 0-20cm and stored at 4 ^{o}C refrigerator for further experimentation. Soil chemical properties were done following standard methods compiled in Sahlemedhin and Taye (2001).

Table 1. Sampling sites including geographical location and soil pH

District	Kebele	Longitude	Latitude	Elevation (m.a.s.l)	Cropping History	Soil pH H_2O(1:2.5)
Emba-Alaje	Betmera	12^{0}58.787’	039^{0}32.116’	2925	Field pea	6.6
	Betmera	12^{0}58.822’	039^{0}32.069’	2923	Field pea	7.47
	Atsela	12^{0}55.615’	039^{0}32.040’	2471	Field pea	7.37
	Atsela	12^{0}58.408’	039^{0}31.722’	2989	Field pea	7.85
	Ayba	12^{0}53.589’	039^{0}30.811’	2745	Field pea	6.6
	Ayba	12^{0}53.660’	039^{0}30.818’	2709	Field pea	6.59
	Ayba	12^{0}53.611’	039^{0}30.872’	2722	Field pea	5.91
	Ayba	12^{0}53.973’	039^{0}31.501’	2725	Field pea	6.48
	Ayba	12^{0}52.584’	039^{0}33.239’	2765	Wheat	7.22
	Ayba	12^{0}52.614’	039^{0}33.325’	2777	Field pea	6.76
	Ayba	12^{0}52.077’	039^{0}33.750’	2889	Barley	7.52
	Tekea	12^{0}54.954’	039^{0}28.254’	2592	Field pea	6.75
	Tekea	12^{0}55.104’	039^{0}29.343’	2651	Field pea	7.75
EndaMohoni	E/hasti	12^{0}51.481’	039^{0}33.920’	2955	Field pea	7.41
	E/hasti	12^{0}51.488’	039^{0}33.899’	2952	Field pea	7.36
	E/hasti	12^{0}51.477’	039^{0}33.895’	2951	Field pea	7.88
	E/hasti	12^{0}51.514’	039^{0}33.981’	2944	Field pea	7.75
	E/hasti	12^{0}50.720’	039^{0}34.006’	2935	Field pea	8.11
	Tsibet	12^{0}50.549’	039^{0}33.844’	2964	Field pea	7.89
	Tsibet	12^{0}50.537’	039^{0}33.873’	2965	Fababean	7.58
	Tsibet	12^{0}50.533’	039^{0}33.856’	2958	Wheat	6.3
	Sh/gaze	12^{0}50.514’	039^{0}33.383’	2956	Field pea	6.28

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Where; E/Alaje= Embaalaje, E/Mohoni= Endamohoni, H/T/hanot= hazeboteklehaymanot, E/hasti=Enbahasti

Nodule collection and Isolation of Rhizobia

After 45 days of growing period, well grown, large and pink colour nodules were uprooted carefully so as to get intact nodules. The nodules were thoroughly washed with distilled water and surface-sterilized briefly with 70% ethanol and 3% (v/v) solution of hydrogen per oxide (H_2O_2) for 10 sec. and 3 min. respectively (Howieson and Dilworth, 2016). They were then more than 5 times with sterile distilled water, and transferred into sterilized Petri dishes and crushed with flamed glass rod in 0.1 N NaCl. One loop full of the nodule suspension were streaked on freshly prepared Yeast Extract Manitol Agar (YEMA) plates containing 0.0025% Congo red (CR) with pH of 6.8±0.2, and the plates were incubated at 28 ± 2 °C for 3-5 days. After 5 days of incubation, single colonies were picked and purified by re-streaking on newly prepared YEMA plates. The pure isolates were temporarily preserved at 4 ^{o}C on YEMA slants containing 0.3 % (W/V) $CaCO_3$ until further analysis.

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Presumptive tests and colony characterization of the isolates

All isolates was examined for presumptive purity using YEMA-CR medium, Gram staining, peptone glucose Agar (PGA) and ketolactose Test (KLT) following the procedures indicated in Somasegaren and Hoben, (1994). The isolates were characterized by colony morphology and acid/base production on YEMA plus 25μgml^{-1} Bromothymole blue (BTB) media (Ahmed *et al*., 1984).

Authentication and preliminary screening of symbiotic effectiveness (SE) of isolates on sand culture

Seeds of the same variety Raya one (R-1) was surface sterilized as before and five pre-germinated seeds were sown on 1.5 kg surface sterilized capacity pots filled with acid washed sand (95% sulphuric acid). The seedlings were thinned down to three per pot after few days, and inoculated with 1 ml active cells (undiluted cells) grown on YEM broth as the exponential of 108 visible cells ml-1. The experimental set up was arranged in a Complete Randomized Block Design (RCBD) with three replications including the positive control (N supplied with 5ml/pot as 1% KNO3 (w/v)) solution once a week, and un-inoculated unfertilized pots as negative control under semi-controlled greenhouse conditions at Haramaya University. All pots were supplied with quarter strength N-free nutrient solution once a week (Somasegaran and Hoben, 1994) and washed with sterilized distilled water as required to control salt accumulation. After 45 days of growing period, all plants were uprooted and washed carefully with tap water. The nodules were cut off from the plant roots to count and then dried at 70 ^{o}C for 24hrs until constant weight. The rhizobia infectiveness based on the presence and absence f nodules on seedling root were investigated.

Qualitative Phosphate Solubilization Test

The potential of Rhizobium strains for solubilization of insoluble phosphates were checked on the Pikovskaya's agar medium (Pikovskaya, 1948), containing 10g glucose, 0.5g yeast extract, 0.5g NH_2SO_4, 0.1g Magnesium Sulphate ($MgSO_4$ $7H_2O$), 5g Calcium Phosphate ($Ca_3(PO_4)_2$), 0.2g $NaCl_2$, 0.2g KCl_2, 0.001g $MnSO_4$ $2H_2O$, 0.001g $FeSO_4$ $7H_2O$ and 15g Agar medium per litter of distilled water. Three days old culture isolates with 10^8 viable cells ml^{-1} were streaked on the medium and incubated at 28 ± 2 ^{o}C for 7 days. After 7 days of incubation, clear halo zone diameter and colony diameter were measured and microbial phosphorus solubilisation index (SI) was calculated following the formula indicated in Edi-Premono *et al*. (1996)

$$\mathrm{SI} = \frac{Colony\ Diameter + Hallo\ zone\ diameter}{\mathrm{Colony\ Diameter}}$$

Quantitative Phosphate Solubilisation test

Five pure and best rhizobial isolates were selected based on their solubilization index in Pikovskaya agar medium. 100ml of Pikovskaya broth was prepared without phosphate source and dispensed in 250 ml Erlenmeyer flasks. In each flask, about 0.5g of tri-calcium phosphate ($Ca_3(PO_4)_2$) was added and sterilized at 121 ^{o}C at 15 psi for 15 minutes. Then 1ml of culture containing about 10^8 cells ml^{-1} suspensions of each isolates was inoculated into the medium and kept at 28 ± 2 ^{o}C in shaker incubator for about 12 days. All the experiments were carried out in triplicate. 10ml of each isolate was withdrawn at regular intervals of 3 days and was examined for soluble phosphate and pH changes using spectrophotometer and digital pH meter, respectively, following the method cited in Subba Rao (1993).

Screening Effective Isolates Under Soil Pot Experiment

Two bulky soils collected from filed pea growing areas of southern Tigray were grounded, sieved in to 2 mm size particles and filled into 3 kg capacity surface sterilized as before polyethylene plastic pots, and the experiment were set as randomized complete block design (RCBD) in three replications. Five effective rhizobial isolates based on their symbiotic effectiveness on sand culture were selected including N treated pots supplied with 5ml/pot of 1% KNO_3 (w/v) solution once a week as positive control, and un inoculated unfertilized pots as negative control. All pots were treated once a week with stock solutions of 12.5 mg/kg urea, 20 mg P_2O_5/kg, 10 mg/kg KCl_2, 5 mg/kg $ZnSO_4$, 5 mg/kg $NaMoO_4$ and 5 mg/kg $FeSO_4$ (Somasegaren and Hoben, 1994). After 45 days of planting shoot and root fraction were separated to determine nodule number and dry weight, shoot dry weight and total nitrogen.

Statistical Analysis

The collected data was subjected to analysis of variance (ANOVA) using SAS ver. 9.1 (2002) and the differences tested for significance was faced to Fisher method using the least significant differences (LSD) test at 0.05 probability level.

Result and discussion

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Qualitative Phosphate Solubilization

All the tested isolates induced nodulation on the host plant indicating that the tested isolates are the root nodulating bacteria of field pea (*pisum sativum var. abyssinicum*).

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The qualitative phosphate solubilisation showed a clear halo zones around their colonies. The phosphate solubilisation index was ranged from 1.10 to 2.67 and soil pH of moderately acidic to moderately alkaline (5.90 to 8.11) which is the optimum pH for growth of the isolates. Of the tested isolates, five of them showed greater solubilization index (SI) ranging from 1.5 to 2.7 (Table 2). Isolates HUDRI-8, HUDRI-25 and HUDRI-26 were scored the highest solubilisation index at soil pH range of (6.75-7.75) neutral to slightly alkaline.

Table 2. Growth of isolates on Pikovaskaya's agar medium

Isolates	Soil pH (1:2.5)	CD (mm)	HD (mm)	SI
HUDRI-8	7.75	3.0	5.0	2.7
HUDRI-18	6.59	9.3	7.0	1.8
HUDRI-25	7.47	4.3	6.7	2.5
HUDRI-26	6.75	3.0	4.0	2.3
HUDRI-30	6.76	5.7	3.0	1.5

Key word(s): CD-colony diameter, HD- holo zone, SI- solubilisation index

This indicates that some rhizobial isolates had the capacity to mobilize phosphates from in organic tricalcium phosphate (TCP). Similar results were found from *Vicia faba* L. of Ethiopian soils, with soil pH (4.8-6.3)as well as SI in the range of 1.25 to 2.10 (Girmaye *et al.,* 2014). Mulissa *et al*. (2016) also obtained related results from *Cicer aeritinum* L. in the range of 1.40 to 3.06. Superior solubilisation index was obtained by Alia *et al*. (2013) from phosphate solubilizing bacteria associated with roots of vegetables that found within the range of 1.8 - 5.0.

Quantitative Phosphate Solubilisation

The quantitative phosphate solubilisation efficacy of selected rhizobial isolates were further evaluated by measuring the soluble P (mg L^{-1}) and the changes in pH as presented in Table 3. Accordingly, the amount of solubilised P released by the isolates exhibited wide variation ranging from 16.59 to 23.95 mg L^{-1}, with a significant drop in pH from 7.13 to 5.23. Similar results were obtained by Assefa *et al*. (2010), all bacterial isolates of faba bean (*vicea faba*) were solubilized TCP in the range of 5-39 mg/50ml with a drop in pH ranging from 6.8-4 after 20 days of incubation. Various phosphate solubilization values were obtained by incubating them at different incubation period.

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The ANOVA result showed a significant difference ($P < 0.05$) at the first 3 days incubation. The highest phosphate solubilizations were recorded from treatments inoculated with HUDRI-30 (21.84 mg L^{-1}) followed by HUDRI-25 (21.72 mg L^{-1}), and the lowest P solubilizations (3.43 mg L^{-1}) were recorded from un-inoculated treatment (Table 3). Phosphorus solubilization in the inoculated treatment was 537%

higher than the un-inoculated one, which is seven fold. The same treatments incubated for the next 6 days had also significantly higher P discharge over the un-inoculated one by 413%. The highest amount of P discharge 23.95, 23.48 and 23.00 mg L^{-1} were recorded by isolates HUDRI-8, HUDRI-25 and HUDRI-26, respectively. After 9 days of incubation, the highest P solubilizations (22.83 mg L^{-1}) were recorded by HUDRI-26. Incubation of isolates for uninterrupted 12 days, the highest P solubilization was found by inoculating HUDRI-8 (23.32 mg L^{-1}) followed by HUDRI-26 (22.02 mg L^{-1}); resulting in 354.58% and 329.24% over the un-inoculated. With regard to the incubation period, the highest P solubilisation (23.95 and 23.48 mg L^{-1}) was found at the sixth day, while the lowest P discharge (16.59 mg L^{-1}) was recorded at the first 3 days of incubation. The current result was significantly lower than the results obtained by Assefa *et al*. (2010) (39 mg/50ml). Other researches were done by Sharma *et al*. (2012), isolates from tea rhizosphere, Qian *et al.* (2010) from shallow eutrophic lake and Feredegn (2013), isolates from rhizosphere and endophytic of sugarcane solubilized TCP within the range of 40.62-136.73 mg L^{-1}, 4-170 mg L^{-1} and 45.12- 88.41 mg L^{-1}, respectively.

The pattern of interaction between phosphate discharge and pH at different incubation period had a strong negative correlation (r= -0.613 and r= -0.542) from day 6 and 9, respectively, followed by day 3 and 12 with r= -0.517 and r= -0.202 (Table 4). This result was corresponding to Assefa *et al*. (2010), inverse correlation between the amounts of P solubilize and reduction in pH (r≥ -0.93). Alia *et al*. (2013) also found negative correlation (r = -0.862), (r= -0.94) correlation from bacterial growth on mung bean by Buddhi and Min-Ho (2013) also found similar trend.

Table 3. Tri-calcium phosphate solubilization efficiency of selected isolates

Isolates	3 days		6 days		9 days		12 days	
	pH	P (mg L^{-1})	pH	P (mg L^{-1})	pH	P (mg L^{-1})	pH	P (mg L^{-1})
HUDRI-8	5.93±0.214^{bc}	16.59±7.123^{b}	5.54±0.015^{b}	23.95±0.767^{a}	5.37±0.164^{b}	20.41±8.911^{a}	5.25±0.069^{b}	23.32±8.100^{a}
HUDRI-18	5.93±0.263^{bc}	16.81±0.966^{b}	5.27±0.136^{c}	22.77±2.915^{a}	5.53±0.045^{b}	20.72±1.015^{a}	5.38±0.217^{ab}	19.76±1.127^{b}
HUDRI-25	5.61±0.063^{bc}	21.72±0.981^{ab}	5.49±0.029^{b}	23.48±0.214^{a}	5.59±0.017^{b}	20.67±0.563^{a}	5.97±0.351^{a}	21.41±0.374^{ab}
HUDRI-26	6.21±0.316^{b}	19.17±2.072^{a}	5.45±0.051^{bc}	23.00±2.951^{a}	5.40±0.220^{b}	22.83±6.639^{a}	5.23±0.261^{b}	22.02±9.374^{ab}
HUDRI-30	5.31±0.144^{c}	21.84±2.302^{a}	5.26±0.058^{c}	21.18±1.128^{a}	5.24±0.089^{b}	20.17±0.893^{a}	5.23±0.031^{b}	21.27±0.225^{ab}
Control	7.01±0.00^{a}	3.43±0.00^{c}	6.97±0.00^{a}	4.67±0.00^{b}	7.21±0.00^{a}	5.49±0.00^{b}	7.13±0.00^{b}	5.13±0.00^{c}
G mean	6.00	16.59	5.66	19.84	5.72	18.38	5.69	18.82
CV (%)	5.77	14.74	2.01	8.11	3.62	8.63	6.49	8.07
LSD(0.05)	0.62	4.35	0.20	2.86	0.37	2.82	0.62	2.70

Where; Means followed by the same letters are not significantly different at $p < 0.05$ (Fisher's LSD test)

Table 4. Correlation coefficients of P and pH parameters on phosphate solubilizing bacteria

	Day 3		Day 6		Day 9		Day 12	
	pH	P	pH	P	pH	P	pH	P
pH		-0.52*		-0.6**		-0.54*		-0.20*
P (<0.05)		0.03		0.01		0.02		0.42
P	-0.52*		-0.61**		-0.54*		-0.20*	
P (<0.05)	0.03		0.01		0.02		0.42	

Symbiotic Effectiveness of Isolates on Unsterilized Soil:

The physico-chemical properties of the soils are presented in Table 5. The textural class of the districts were classified as sandy clay loam. Similar results were found by Amanuel *et al*., 2015, from Tekea and Shimta kebeles with particle size distribution of 50-54% sand, 18-17% silt and 35-30% clay fractions, respectively. The pH of the two districts was slightly acidic (6.38-6.42) according to the ratings of Tekalign (1991), which is the optimum pH range for bacterial growth. Low organic matter (1.7-2%) and low to medium total nitrogen (0.01-0.14%) was found according to Murphy (1968). This lower soil organic matter could be due to the presence of continuous cropping system, cultivation and intensive tillage practice.

Table 5. The soil physico-chemical properties

Parameters	E/Alaje	E/Mohoni	Status	Refference
OM (%)	1.72	1.96	Low	Murphy (1968)
Available P (mg/kg)	18.78	17.7	high	Olsen *et al*. (1954)
Total N (%)	0.09	0.14	low to medium	Murphy (1968)
pH	6.42	6.38	slightly acidic	Tekalign (1991)
EC(mhos/cm)	0.09	0.09	low	Horneck *et al*. (2011)
CEC (meq/100g soil)	40.20	43.40	very high	Landon (1991)
Textural Class	Sand 52% Silt 18% Clay 30%	Sand 59% Silt 16% Clay 30%	Sandy clay loam	

High available P (18-19 mg kg^{-1}) and very high CEC (40.2-43.4 meq/100gsoil) was found from the study area according to the ratings of Olsen *et al*. (1954) and Landon (1991), respectively. This is in agreement with the findings of (Amanuel *et al.*, 2015) who reported the characterization of agricultural soils of southern Tigray, in capacity building for scaling up of evidence-based best practice in Ethiopia (CASCAPE) intervention woredas. According to Horneck *et al*. (2011), soil test interpretation guide the electrical conductivity was low.

After nodulation test on sand culture, five symbiotically effective isolates (HUDRI-15, 26, 28, 43 and 44) were selected and further tested for their performance on a soil pot culture. The data showed that the inoculated plants produced significantly (P<0.05) higher nodule number (NN), nodule dry weight (NDW), shoot dry weight (SDW) and total plant nitrogen (TN) (Table 6). The highest nodule numbers (156 and 145) were found from HUDRI-15 and HUDRI-28 isolated from E/Alaje and E/mohoni soils, respectively. The current result was higher than the number of nodules found by Asrat (2017) (112 NN/plant) for field pea treated with commercial strain 1018. The lowest nodule number per plant was recorded from un-inoculated plants (31 NN/plant) (Table 5). N treated plants also reduced nodule number per plant by 36% (156-100 NN/plant) and 42% (145-84 NN/plant) compared to other treatments from the two soils, respectively. This result indicates that application of nitrogen somehow inhibited nodule development in field pea. Anteneh and Abere (2017) also reported that application of N reduced nodule number (62 NN/Plant and 20.00NN/Plant) in 2012 and 2013 cropping season.

Inoculation of the host plant also significantly (P < 0.05) affected nodule dry weight. The highest nodule dry weight (NDW) was recorded from HUDRI-15 (0.189 g $plant^{-1}$) and HUDRI-28 (0.117 g $plant^{-1}$) relative to the other inoculants and control treatments on both soils (Table 6). This result was in agreement with Asrat (2017) (0.094 and 0.009 g $plant^{-1}$) of field pea *rhizobium* inoculation. However, it was slightly lower than the results obtained by Anteneh and Abere (2017) (0.552 and 0.140 g $plant^{-1}$) two years report. This might be due to the ecological factors, which are tested on field condition.

The effect of inoculation on shoot dry weight (SDW) was found significant (P < 0.05) and values were superior to the positive and negative control. Isolates HUDRI-15 and HUDRI-28 gave the highest shoot dry weight (1.64 g $plant^{-1}$) and (1.42 g $plant^{-1}$) on both soils, and it was advanced by 43 and 25% over the negative control (Table 6). In contrary to this result Asrat (2017) was found higher shoot dry weight in the range of 14 to 29 g $plant^{-1}$. Anteneh and Abere (2017) also reported that field pea rhizobium inoculation increased shoot dry weight on the range of 57 to 87 g $plant^{-1}$.

A significant effect of *Rhizobium* inoculation on the plant N accumulation of field pea was observed among the treatments including N treated and un-inoculated (Table 6). The highest total N accumulation was obtained from plants treated with HUDRI-15 (3.67%) and HUDRI-15 (3.53%) on the two districts, respectively. This result was in agreement with Asrat (2017) found in the range of 3.5-4.1% total N from inoculated field pea. The total N accumulation was found to be 70% and 89% increment over the negative control.

Table 6. Evaluation of symbiotic effectiveness of isolates on soil culture

Treatment	Nodule number		Nodule dry weight(g plant^{-1})		Shoot dry weight (g plant^{-1})		Total Nitrogen (%)	
	E/Alaje soil	E/Mohoni soil	E/Alaje soil	E/Mohoni soil	E/Alaje soil	E/Mohoni soil	E/Alaje soil	E/Mohoni soil
HUDRI-15	156.00±3.46^{a}	103.33±2.40^{c}	0.189±0.03^{ab}	0.089±0.03^{c}	1.64±0.13^{a}	1.41±0.17^{ab}	3.67±0.135^{a}	3.53±0.098^{a}
HUDRI-26	111.67±3.84^{d}	86.00±3.46^{d}	0.097±0.01^{bc}	0.092±0.00^{bc}	1.31±0.14^{ab}	1.28±0.16^{ab}	3.36±0.120^{ab}	3.05±0.034^{ab}
HUDRI-28	138.67±1.76^{b}	145.33±2.91^{a}	0.109±0.03^{a}	0.117±0.00^{a}	1.51±0.17^{ab}	1.42±0.11^{ab}	2.48±0.057^{c}	3.08±0.045^{ab}
HUDRI-43	150.00±7.64^{ab}	126.00±3.46^{b}	0.097±0.00^{bc}	0.108±0.00^{ab}	1.61±0.17^{a}	1.28±0.23^{ab}	3.40±0.038^{ab}	2.84±0.038^{b}
HUDRI-44	125.67±3.48^{c}	96.00±2.08^{c}	0.121±0.01^{ab}	0.100±0.00^{abc}	1.53±0.05^{a}	1.34±0.21^{ab}	3.48±0.038^{ab}	3.07±0.070^{ab}
N^{+}	100.00±3.06^{d}	84.33±1.66^{d}	0.046±0.00^{bc}	0.005±0.00^{bc}	1.22±0.01^{a}	1.24±0.02^{ab}	2.60±0.027^{c}	2.42±0.039^{c}
N^{-}	31.00±1.15^{e}	36.33±2.40^{e}	0.065±0.02^{c}	0.014±0.02^{d}	1.15±0.09^{b}	1.14±0.04^{ab}	2.16±0.05^{d}	1.87±0.226^{d}
CV (%)	5.875	5.045	34.85	10.87	14.30	19.84	4.65	5.51
LSD (0.05)	11.95	8.51	0.071	0.017	0.37	0.47	0.21	0.27

Where: CV= coefficient of variation, LSD= least significant difference, values are ±SE, numbers in the same column followed by the same letter(s) are not significantly different at $\alpha<0.05$

Conclusion

It can concluded that the phosphate solubilizing rhizobia exhibited a broad range of ability of solubilizing TCP *in vitro*. Most of the isolates originated from Emba-alaje are generally able to solubilise inorganic TCP. Among all the isolates, maximum potential to solubilize tri-calcium phosphates are HUDRI-8 and HUDRI-25. Results found an inverse correlation between amount of solubilized phosphate and pH of the culture medium. Isolate that are effective in N_2 fixation and able to solubilise TCP are found to be effective in improving nodulation and plant growth under greenhouse condition. Further research is recommended to investigate its efficacy under field trials in diverse soil types having different amount of soil P.

Comment [s13]: Conclude

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