Preliminary Characterization of Soybean Nodulating Rhizobia in Ethiopian soils

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Introduction

Soybean (*Glycine max* L. (Merrill)), the 'golden bean', is the world's most valuable oilseed crop, and is an excellent source of plant protein (34–40%) and oil (20%) (Vasilia and Boerma, 2009), and supplies high protein levels for livestock (Robaina *et al*., 1995). However, the plant requires large quantities of N which must be obtained from the biological nitrogen fixation (BNF), soil or supplied in the form of fertilizer. Nitrogen is often the major crop growth limiting nutrients in most soils of Ethiopia. But, nitrogen fertilizer was not commonly used to fertilize legumes under smallholder farmers in Ethiopia. Hence, BNF remains an alternative and environmentally friendly source nitrogen for growing legumes.

In Ethiopia soybean is cultivated mainly in the southern and western parts of the country around Hawassa, Jimma, Bako, Pawe and Assosa (Aregu, 2012). According to CSA (2011) report the national average yield of soybean in Ethiopia is about 1.4 tons/ha, lower than the national average for other countries (for instance 2.84 in Brazil). The low average yield compared with potential productivity may be due to several reasons, which could include poor soil fertility or lack of compatible rhizobia.

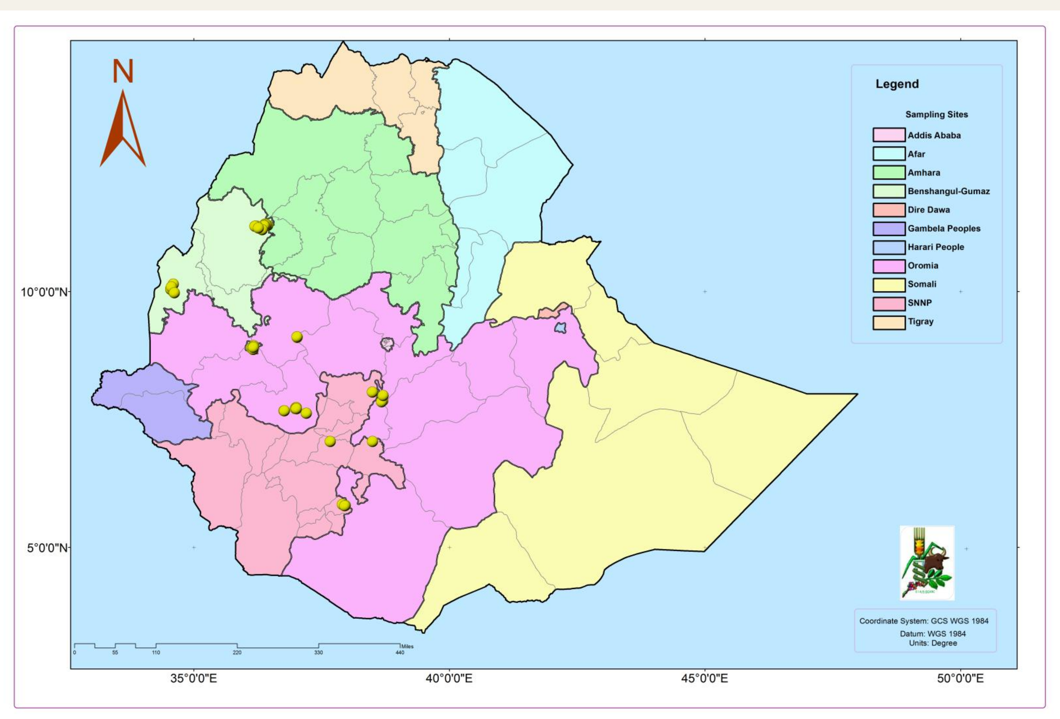
Soybean can establish effective nitrogen fixing symbiosis with species of slow growing bradyrhizobia, like *Bradyrhizobium japonicum* (Jordan, 1982), *B. elkanii* (Kuykendall *et al*., 1992), *B. liaoningense* (Xu *et al*., 1995) and *B. Yuanmingense* (Yao *et al*., 2002; Appunu *et al*., 2008) as well as with species of fast-growing rhizobia such as *Ensifer* (*Sinorhizobium*) *fredii* (Scholla and Elkan, 1984; Chen *et al*., 1988) and *Ensifer xinjiangense* (Peng *et al*., 2002).

Nevertheless, the process of BNF is sensitive to various environmental stresses such as soil salinity, acidity, alkalinity, temperature which leads to unsuccessful legume nodulation. Hence, identification of bacterial strains and host cultivars that are tolerant to these stresses (Appunu and Dhar, 2006) will give rise to a more sustainable agriculture and it would open the way for alternate, lower cost solutions to these problems.

However, knowledge about the diversity and symbiotic efficiency of rhizobia nodulating soybean in Ethiopian soils is scanty. Therefore in this study, rhizobia nodulating soybean isolated from major soybean growing areas of Ethiopia are characterized based on morphological, physiological, biochemical and symbiotic effectiveness.

Material and Methods

Study area: Forty-two soil samples were collected from different districts that grow soybean in Ethiopia.



Soybean rhizobia were trapped using two soybean varieties; Awassa-95 and Clark-63K and one cow pea variety (Bole) from 42 soil samples collected from major soybean growing soils of Ethiopia. Root nodules were collected from trap plants after 60 days of growth in the lath house and isolated by a routine method (Vincent, 1970). The purity of the cultures was confirmed by repeatedly streaking the bacteria on yeast extract-mannitol agar (YMA) medium (Somasegaran and Hoben, 1994) and verifying a single type of colony morphology, absorption of Congo red (25 µg ml^{-1}) and the Gram stain reaction.

These isolates were characterized on the basis of colony morphology, growth on Peptone-Glucose Agar (PGA) Medium, tolerances to extremes of pH, salt concentration and resistance to different heavy metals. The isolates were classified as fast (medium turn yellow) and slow growers (medium turn blue) based on their reaction on the yeast extract mannitol agar media supplemented with bromothymol blue.

Production of 3-ketolactose was determined by the method of Bernaerts and De Ley (1963).The symbiotic ability of each isolate was verified by a nodulation test (Vincent, 1970) using clark-63K soybean variety.

Results

Cowpea is nodulated in all soil samples, whereas soybean is nodulated in 85% of soil samples collected. A total of 67 bacterial isolates were obtained from the soybean growing soils collected around Jimma, Assosa, Pawe, Bako, Chewaka, Hawassa and Adamitulu. Out of these, 47 isolates were obtained using soybean as trap host while the remaining 20 isolates were obtained using cowpea variety 'Bole' as a trap host. All of the isolates were subjected to gram staining and microscopic observation showed that all are gram negative rods.

The isolates varied in morphology, i.e. 14.9% formed small colonies (≤0.5 mm), whereas the majority of the isolates (49.3%) had intermediate colony size, i.e. between 1 to 2 mm diameters and 35.8% had diameters ≥2 mm.

Almost all isolates did not absorb Congo red under dark condition except isolates SB-Y-04 and SB-Y-06 which absorbed it slightly implying that they could be rhizobia. While further confirming these isolates, out of 67 isolates, 8 isolates showed no growth on the glucose peptone medium and the remaining showed slight growth indicating character of rhizobia as a conventional rule.

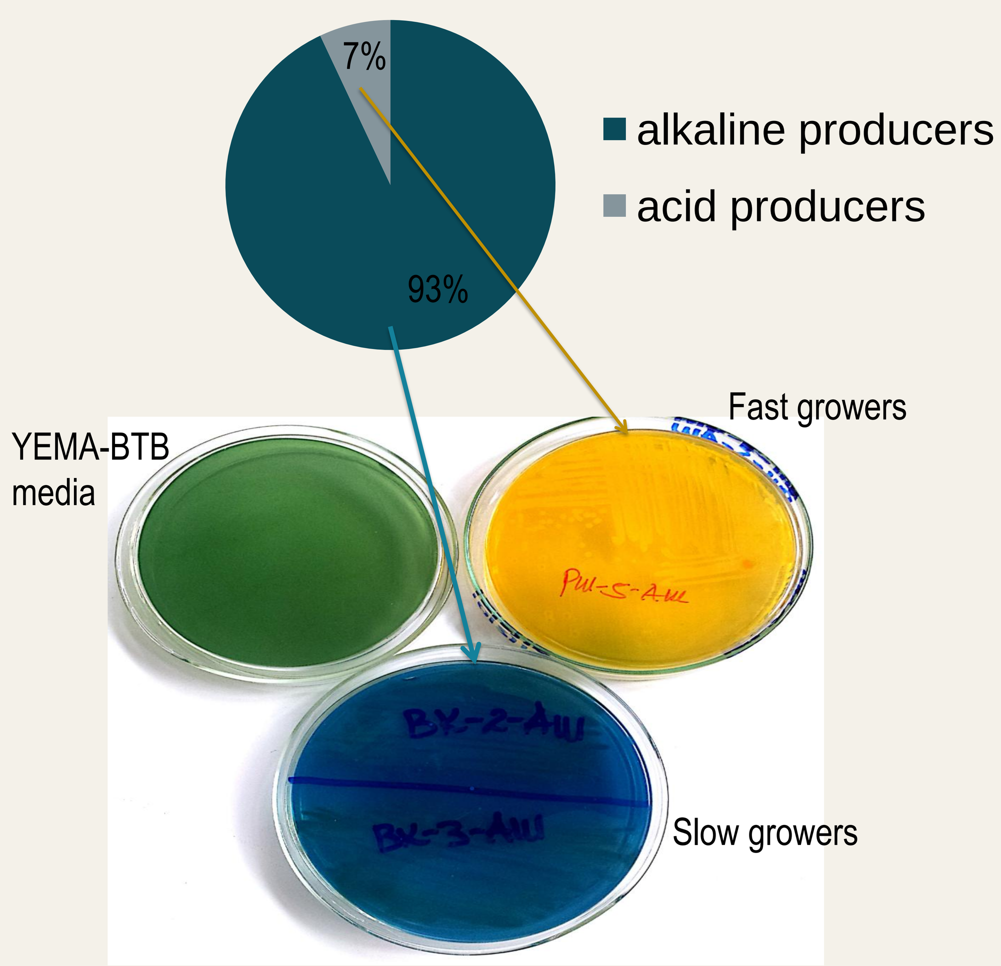


Figure 1. The pH reaction of isolates on YEMA-BTB medium

Moreover, when ketolactose test was performed, all of the isolates were found to be negative for the production of 3-ketolactose from lactose, which confirms the rhizobial colonies.

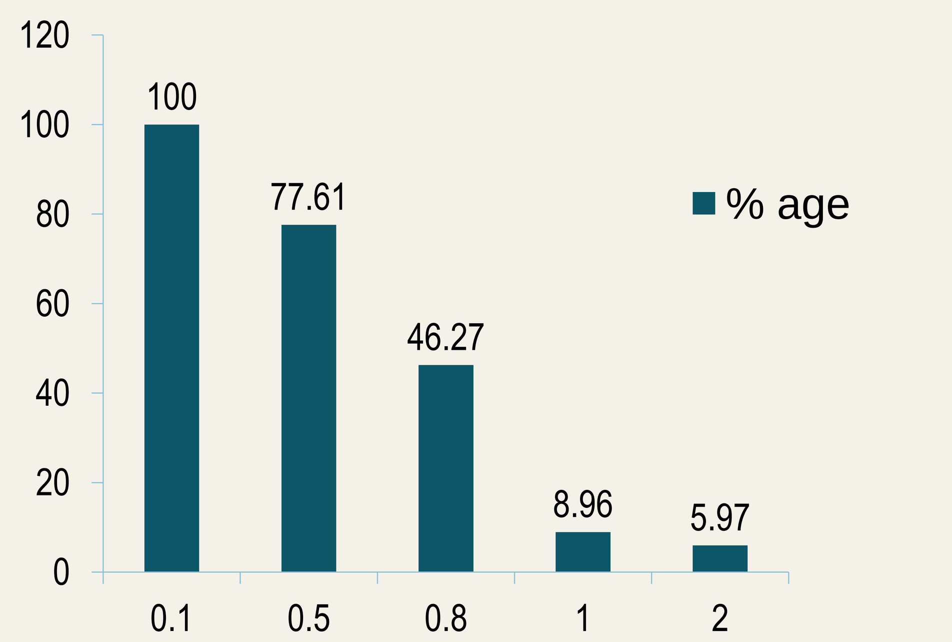


Figure 2. tolerance of isolates to different salt concentration (%)

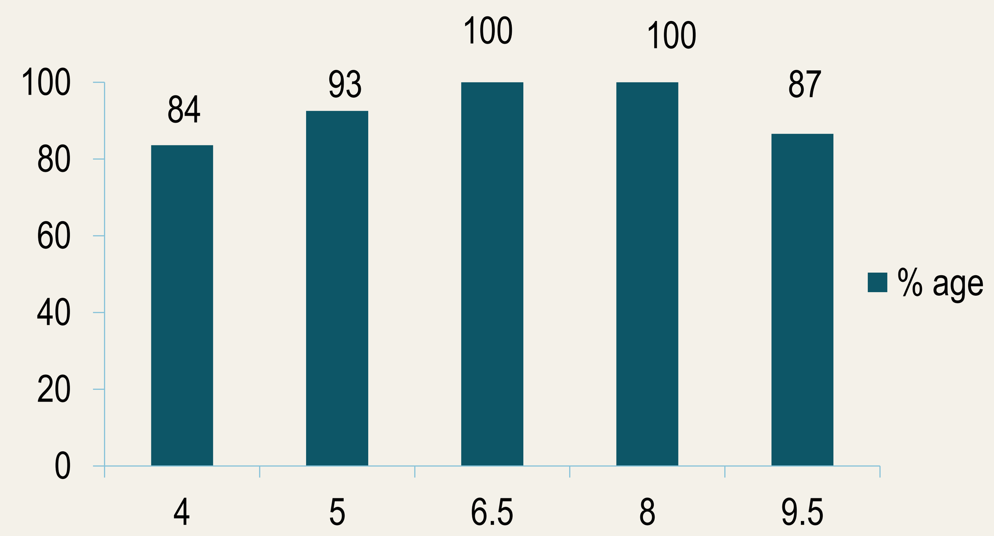


Figure 3. tolerance of isolates to extreme pH ranges

Most of the isolates tolerated Al, Mn,Cu, Co and Zn. But, only few of the isolates tolerated Fe.

Authentication and symbiotic effectiveness of the isolates

In a greenhouse experiment, nodulation was not observed for the control treatments (without inoculation and 70 µg N ml^{-1} applied as KNO_3 solution), indicating the absence of contamination. All of the isolates formed nodules on soybean and hence authenticated as rhizobia nodulating soybean. The nodules were pink indicating the presence of leghemoglobin and the color of the leaves of inoculated treatments are green to deep green, while the uninoculated unfertilized control plants were yellow.

Out of the 67 isolates tested, about 12% of the isolates produced significantly higher ($P < 0.05$) nodule number than MAR 1495. Similarly, 61% of the isolated produced significantly higher ($P < 0.05$) nodule dry weight than MAR 1495. Ten isolates (Bk-3-Aw, Aw-2-Aw, Pw-10-Bo, Cw-6-Aw, Pw-11-Aw, Pw-9-Bo, Bk-2-Aw, As-7-Cl, Pw-3-Cl and Pw-12-Aw) produced the highest shoot dry weight which is significantly at par with the nitrogen supplied treatment. The majority of these isolates (50%) were obtained from soils of Pawe.

Relative effectiveness (%)	Number of isolates	Category
50-80 %	57	Effective
> 80%	10	Highly effective

Conclusion

The majority of soybean rhizobia in Ethiopian soils are slow growers (*Bradyrhizobium* species).and produced alkaline reaction in YEMA medium containing bromothymol blue. The isolates were diverse with respect to their physiological and biochemical properties as well as their symbiotic effectiveness. Particularly, their tolerance to extreme pH is of greater value for future selection program. Ten of the isolates could the best candidates for future inoculants technology after testing their effectiveness under field condition.

Acknowldgements

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