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Exploring the potential of three *Rhizobium* strains from Peruvian soils as biofertilizers for the common bean (*Phaseolus vulgaris*)

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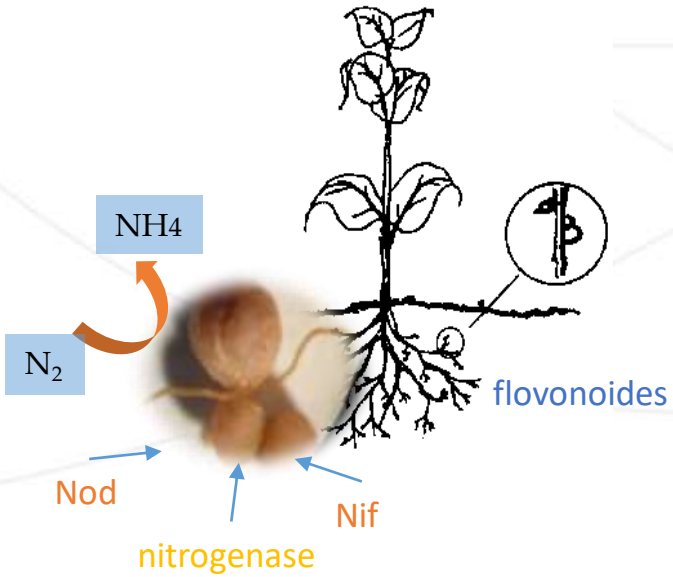
Introduction

Fijación biológica de nitrógeno



- The common bean (Phaseolus vulgaris) is a plant legume grown worldwide for human consumption
- Soil nitrogen is a limiting growth element for plants
- The NH4+ ion is assimilated by the plant which in return provides carbon sources to rhizobia.
- Rhizobia can be used as inexpensive and ecologically-friendly fertilizers for legume production in agriculture.

The objectives of this study were to determine the species affiliation of the selected strains as well as their in vitro plant growth promoting (PGP) activities and effects of bean growth as single or dual strain inoculants.

in vitro plant growth promoting (PGP) activities

Production of indole

Bacterial broth in YEM broth supplemented with L-tryptophan (1 g/l) 24 a 72 h at 28 °C

A 500 ml aliquot was centrifuged at 12000 rpm for 8 min

Colorimetric detecction: 250 ul of the supernatant was mixed with 1 ml of Salkowsky reagent. Reaction proceeded in the dark for 30 min

Cuantification: measures were taken at 530 nm and compared to a standard curve

Elaboration own

For di-calcium phosphate solubilization

Selectet strains

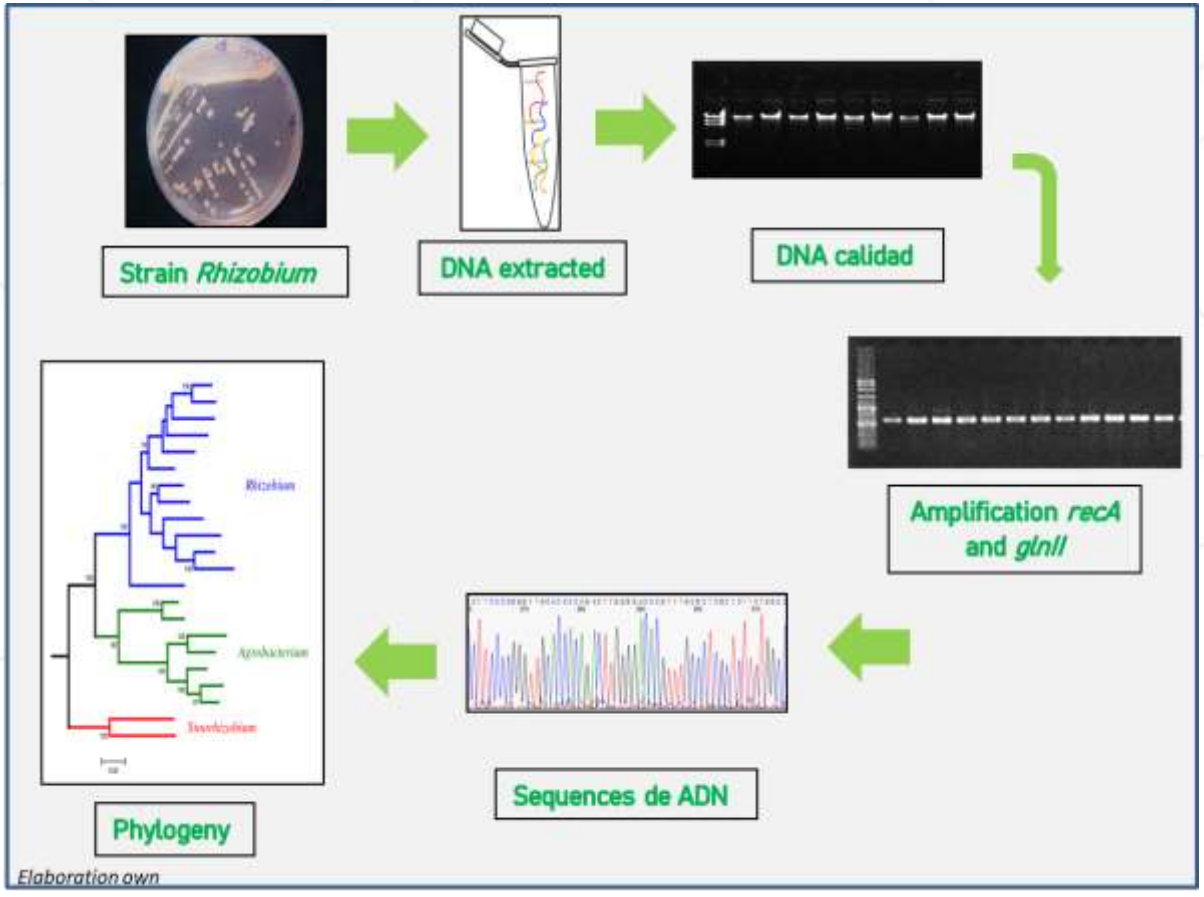
Bacteria grown in YEM broth until an OD600 of 0.8

Were inoculated in discs which were placed in plates of NBRIP medium (Shekar, 1999)

Elaboration own

✓ incubated at 28 ° C for 15 d

Phylogenetic analysis



DNA was extracted with the GeneJet Genomic DNA purification kit (Thermo Fisher Scientific).
Fragments of the recA and glnII genes were amplified as previously described (Aguilar, et al., 2004; Lloret, et al., 2007; López- López, et al., 2010). sequences were aligned to those of rhizobial type strains retrieved from the GenBank database. phylogeny with Tamura Nei distances was constructed with the concatenated alignment of recA and glnII using Mega v7 (Tamura, et al., 2013).

Effects of the symbiosis on bean growth

Pregermination → Inoculation → Instalation

1 ml of bacterial cultures grown until an OD600 of 0.8 inoculated.

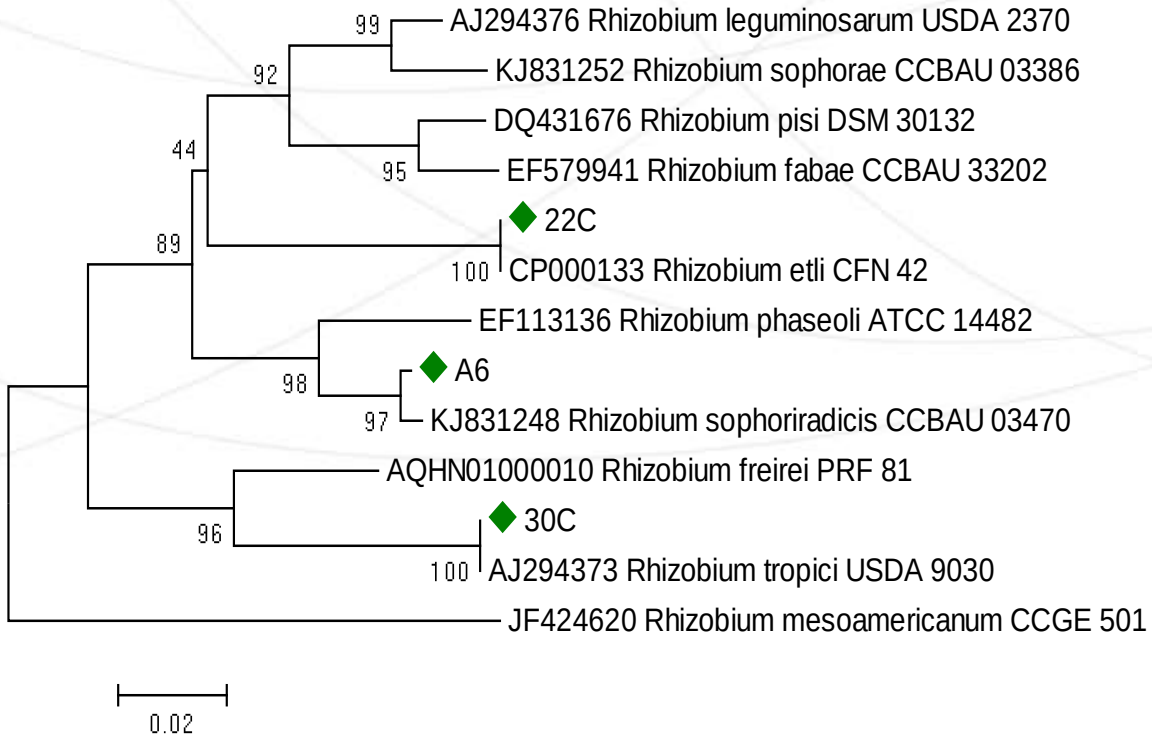
seeds disinfected in 70% alcohol and 3% sodium hypochlorite, then rinsed ten times with sterile distilled water

used completely randomized experimental design with three replicates per treatment (DCA).

Treatmen	Inoculated
T1	*R. tropici*
T2	*R. etli*
T3	*R. sophoriradicis*
T4	*R. tropici* + *R. etli*
T5	*R. tropici* + *R. sophoriradicis*
T6	*R. sophoriradicis* + *R. etli*
testigo	*N+*

Pots were watered with nitrogen-free Broughton & Dilworth solution (Broughton & Dilworth, 1971) except the control that received nitrogen fertilization.

Species affiliation of native *P. vulgaris* rhizobia isolated from Peruvian soils



The results showed that three Rhizobium species able to stablish symbiosis with bean are present in Peruvian soils, none of these species has been described before in Peru.

Plant growth activities

Table 1: AIA production and di-calcium phosphate solubilization by three *Rhizobium* strains from Peruvian soils

Species	Strain	IAA concentration (µg/ml)	Solubilization index (mm)
Rhizobium tropici	30C	38,10	1,46
Rhizobium etli	22C	38,29	1,40
Rhizobium sophoriradicis	A6	36,22	0

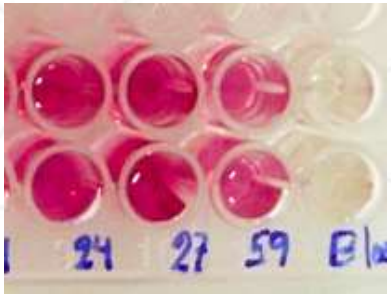


Figure 1: AIA Colorimetric observation

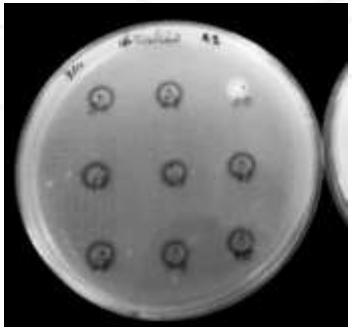


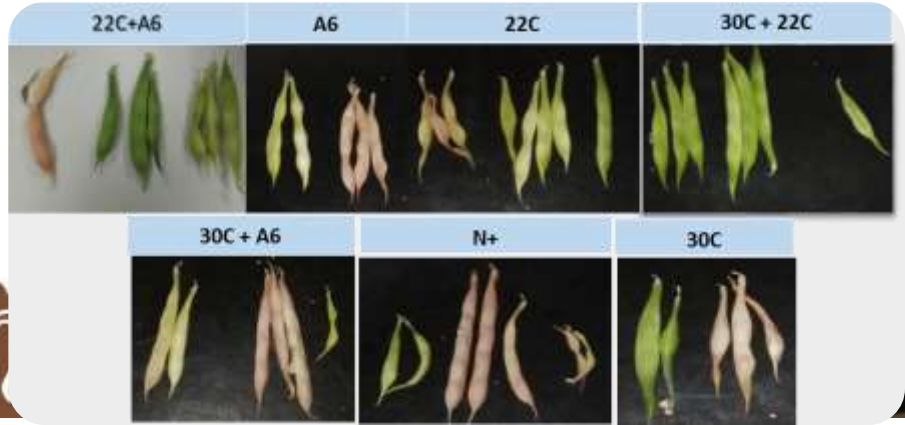
Figure 2: Halos of solubilization of di-calcium phosphate by rhizobium

Analysis of the inoculation effects of *Rhizobium* strains on bean plants

Table 2: Bean response to rhizobia inoculation

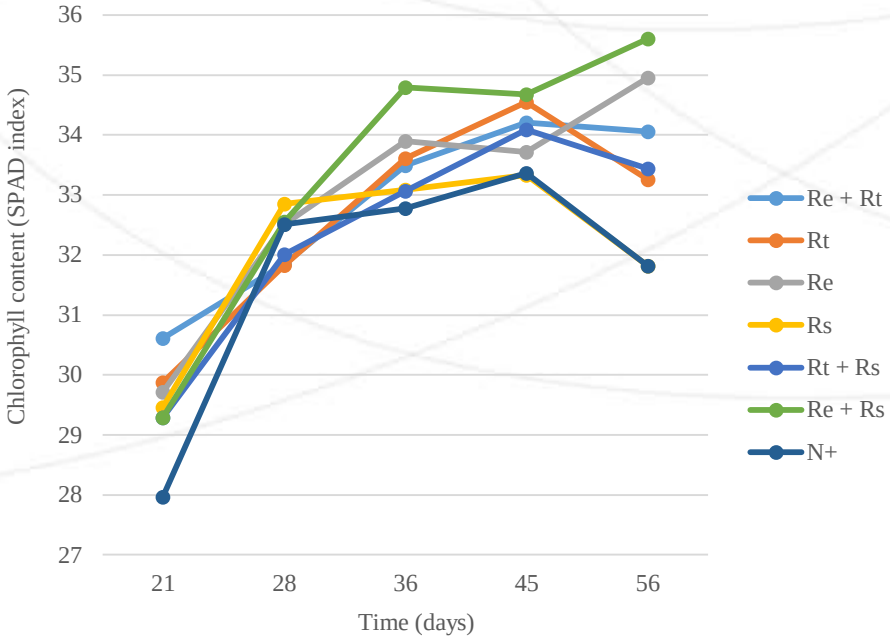
Treatment	Shoot dry weight (g)		Pod fresh weight (g)		Pod dry weight (g)		Nodule dry weight (g)	
	Mean	Groups	Mean	Groups	Mean	Groups	Mean	Groups
R. tropici	3,603	A	6,04	B	1,357	C	0,2175	A
R. etli	2,350	B	9,10	AB	3,787	AB	0,1300	AB
R. sophoriradicis	2,720	AB	5,627	B	2,273	BC	0,1225	AB
R. tropici + R. etli	3,185	AB	10,63	AB	3,572	AB	0,1825	AB
R. tropici + R. sophoriradicis	2,965	AB	9,41	AB	3,337	AB	0,1750	AB
R. sophoriradicis + R. etli	2,6825	AB	13,540	A	3,902	A	0,0900	BC
Control N+	2,4875	AB	7,93	B	2,505	ABC	0	C

Means in the same column followed by the same letter did not significantly differ according to the Tukey test ($p<0.05$)



Analysis of the inoculation effects of *Rhizobium* strains on bean plants

Figure 3: Chlorophyll content (SPAD index) of bean plants inoculated with rhizobial strains. Re, *R. etli*; Rt, *R. tropici*; Rs, *R. sophoriradicis*



it was observed that the R. etli + R. sophoriradicis treatment had the best values at 36 and 56 d followed by the treatment with R. etli alone

Plants inoculated with the R. tropici strain reached their maximum values at 45 d.

R. sophoriradicis-inoculated plants showed a similar behavior as the control plants

Results and discussion

The R. tropici strain stands out in shoot and nodule dry weight but not in pod weight.

Chlorophyll content the R. tropici strain in plants inoculated with that strain increased until day 45 but then decreased, this may explain its better performance in shoot versus pod weight

the R. etli + R. sophoriradicis treatment showed a good correspondence between a high pod weight and better and steady increase in chlorophyll content over time.

R. tropici + R sophoriradicis strain combination did not significantly differed from most other treatments in dry nodule weight which suggest that strain effectiveness did not always correlate with nodule quantity.

Conclusions

- Three species of the bacterial genus *Rhizobium* able to nodulate the common bean were found in Peruvian soils.

- Although all strains were able to promote the growth of bean, a combination of the species *Rhizobium etli* and *R. sophoriradicis* stands out in chlorophyll content and pod yield.

- More studies are recommended to test the rhizobial strains under field conditions.

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