

Isolation and
Characterization of
Rhizobacteria from
Raphanus sativus

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Biology 112-
14

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Introduction

- Radish rhizobacteria can increase root mass (Kravchenko, 2004).
- Two bacterial isolates from roots of *R. sativus* (the radish) tested on cowpea and radish seeds.



Fig. 1: *Raphanus sativus*

Background information about 14KA01

- 14KA01 increased growth of cowpea.
- 14KA01- 97% identity with *Asticcaucalis taihuensis*.
- 14KA09- unidentified.

14KA01 increased the shoot dry weight of cowpea

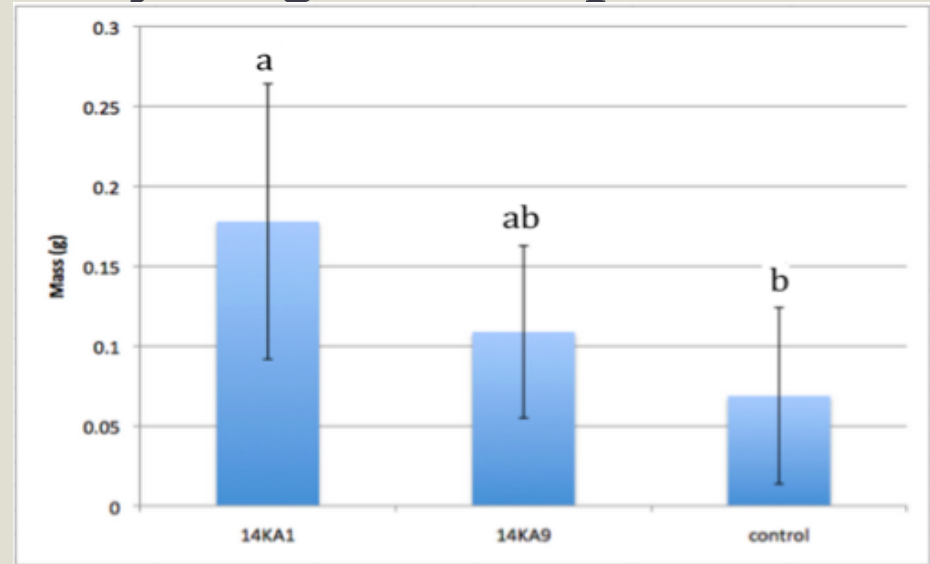


Fig. 2: Dry weight. Shoot 14KA01 vs. control: $p=0.0467$

Hypothesis

- Plants inoculated with 14KA01 will have significantly higher shoot dry masses.
- Predict an increase in root mass in radish.

Methods

- Gram stain to confirm same isolates
- Inoculate cowpea seeds to confirm results
- Inoculate *Raphanus sativus* seeds
 - “quickly appearing” and refers to the rapid germination
- Perform PCR in order to obtain sequence for 14KA09 and confirm the identity of 14KA01

Gram Staining Results

- Gram staining confirmed initial results.

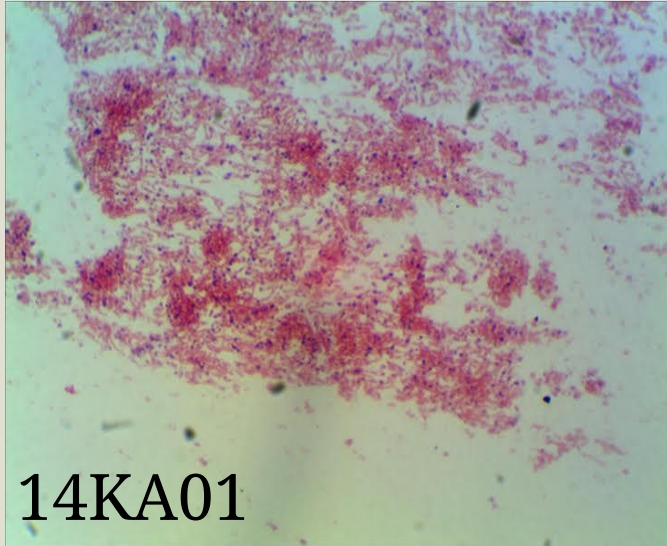


Fig. 3: Gram negative staphylococcus

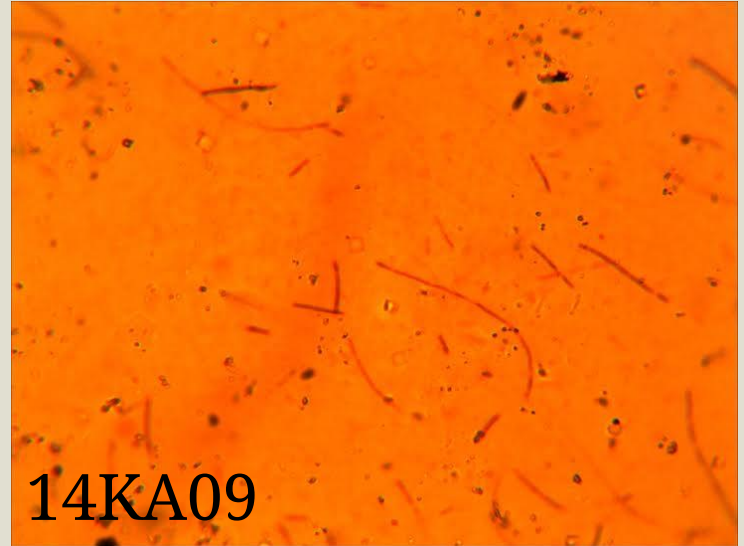


Fig. 4: Gram positive streptobacillus

14KA01 did not increase growth of cowpea plants

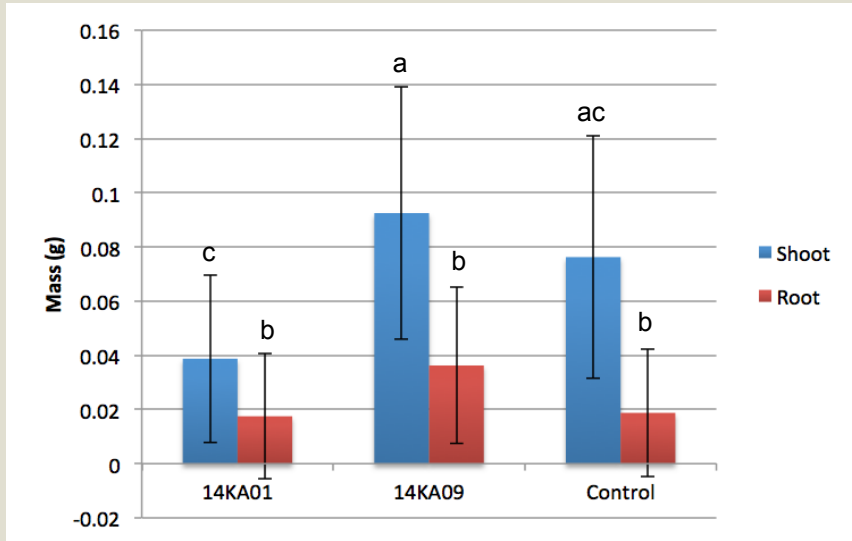


Fig. 5: Dry weight. Shoot: 14KA01 vs 14KA09: $p=0.0467$; Roots: $p>0.05$. 14KA01 decreased growth.

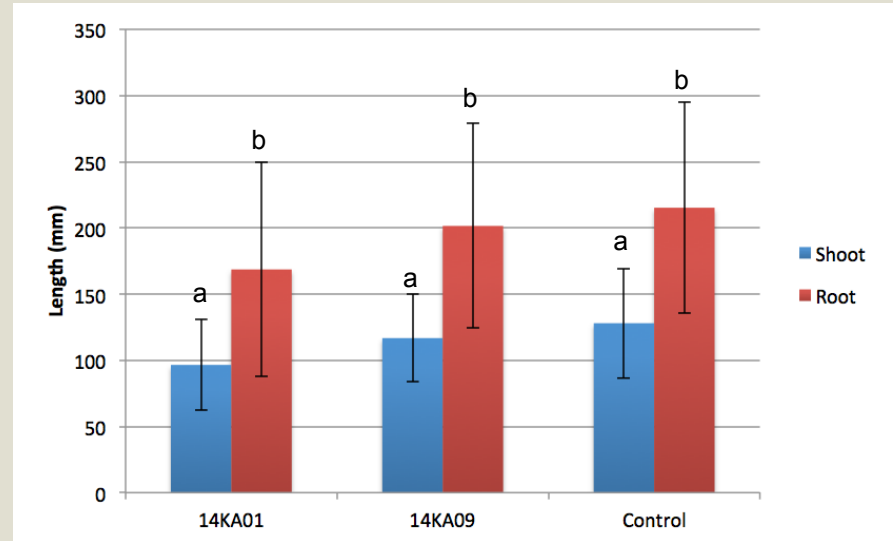


Fig. 6: Length of cowpea. Shoot: $p>0.05$; Roots: $p>0.05$.

Isolates did not increase growth of *Raphanus sativus*

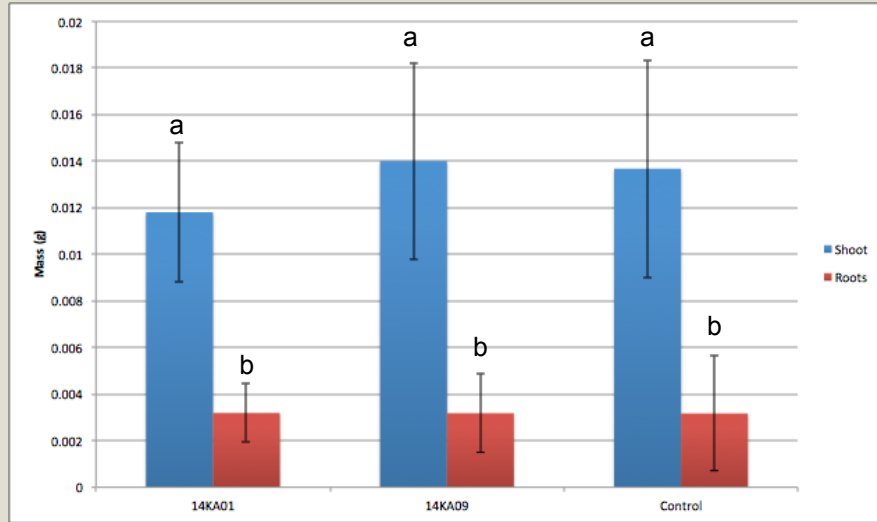


Fig. 7: Dry weight of radish. Shoot: $p > 0.05$; Roots: $p > 0.05$

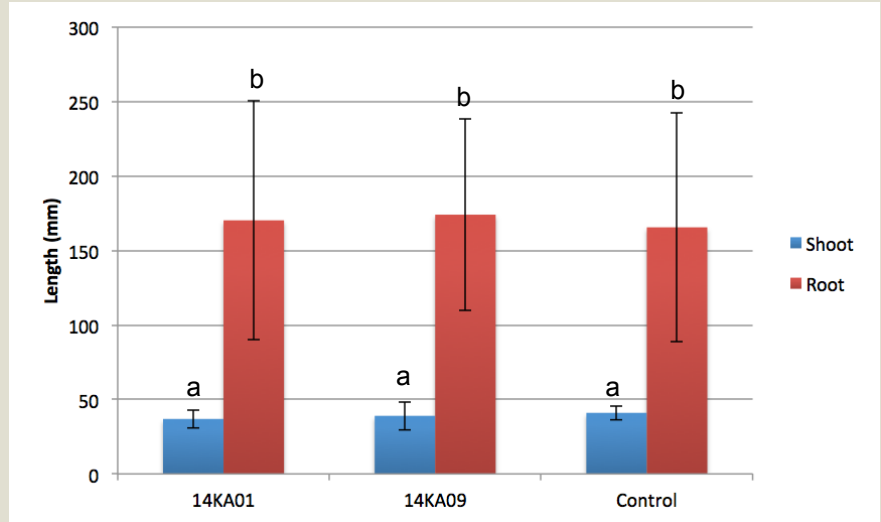


Fig. 8: Length of radish. Shoot: $p > 0.05$; Roots: $p > 0.05$

Identifying Isolates

- 14KA01 had a 97% similarity with *Asticcacaulis taihuensis* the first time and a 95% similarity the second time.
- Both times 14KA09 could not be sequenced.

<input type="checkbox"/> Uncultured Asticcacaulis sp. clone CH2-25 16S ribosomal RNA gene, partial sequence	684	684	100%	0.0	95%	JX079480.1
<input type="checkbox"/> Uncultured Asticcacaulis sp. clone CH2-108 16S ribosomal RNA gene, partial sequence	684	684	100%	0.0	95%	JX079462.1
<input type="checkbox"/> Uncultured Asticcacaulis sp. clone CH2-101 16S ribosomal RNA gene, partial sequence	684	684	100%	0.0	95%	JX079455.1
<input type="checkbox"/> Asticcacaulis taihuensis strain T3-B7 16S ribosomal RNA gene, partial sequence >gb AY500141.1 Asticcacaulis taihuensis strain T3-B7 16S ribosomal RNA ge	684	684	100%	0.0	95%	NR_042933.1
<input type="checkbox"/> Uncultured Caulobacteraceae bacterium 16S rRNA gene, clone HrhB88	684	684	100%	0.0	95%	AM159303.1

Overall Results

- No effect seen in radish seeds - need more time.
- Opposite effect as expected in cowpea seeds inoculated with 14KA01.
- 14KA01 appears to be *Asticcacaulis taihuensis* based on sequencing
 - phenotypically does not match

Discussion

- Chemical exposure to the plants could have altered results by affecting the mass and growth of the plants.
 - Cowpea shoot mass not consistent with first trial.
 - Radish root data not consistent with Kravchenko (2004).
- 14KA09 could not be sequenced either time most likely due to PCR error.

Acknowledgments

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References

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