



Role of Pectinase in *Agrobacterium tumefaciens* Virulence and Binding in Tomato Fruits and Roots



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Abstract

Agrobacterium tumefaciens is a gram-negative soil bacterium found in the rhizosphere. If a plant is wounded, the bacteria infects the wound and transfers a piece of DNA to the host cells to induce the formation of crown gall tumors. Pectin is a polysaccharide found in the cell walls of plants. Pectin plays roles in intracellular adhesion, cell fate specification, and intracellular communication. Pectinase, an enzyme found in the wild type agrobacterium C58, breaks down pectin in plant cell walls. The role that pectinase plays in agrobacterium infection of a plant cell is unknown. A mutant of C58 called 3129/4560 had both genes involved in pectinase production mutated. Tomato roots and tomato fruits were infected with C58 and with 3129/4560. Both C58 and 3129/4560 were able to infect tomato fruit but 3129/4560 was not able to bind to tomato roots as well as C58. This suggests pectinase might be necessary for proper binding to tomato roots.

Methods

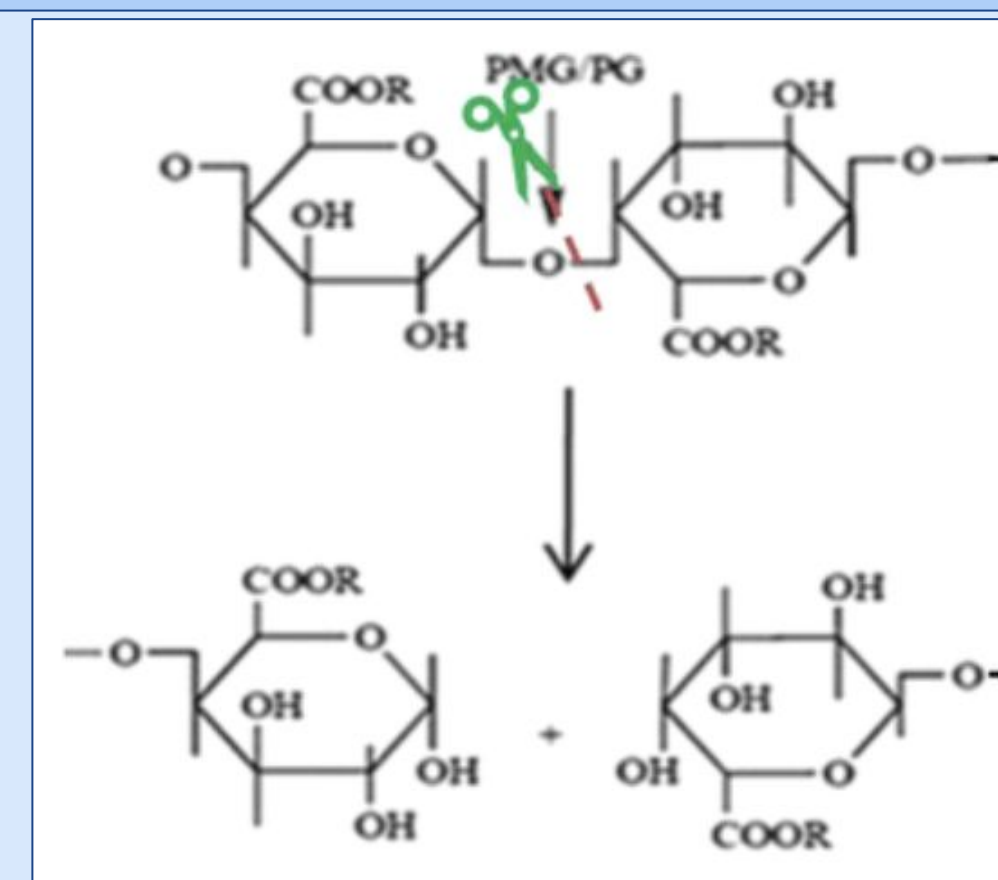
Germination of Plant Seeds: Rutgers tomato seeds were surface sterilized with 80% ethanol and bleach. They were then rinsed with sterile water and germinated in a sterile petri dish with sterile water and kept in a dark drawer at room temperature.

Preparation of Bacterial cultures: Bacterial cultures were obtained using an inoculation loop to transfer colonies from a Luria agar plate to a screw capped tube with 2 mL of Luria broth. The cultures were grown overnight on a roller drum at room temperature.

Inoculation of Tomato Roots: 2 mL of 1/4 MS salts + 0.1% glucose were added to each of 8 35 x 10 mm petri dishes, 4 for each bacterial strain. Under a fume hood, a scalpel and forceps were used to cut about 5 cm segments of roots and transfer them into 4 of the petri dishes, where the roots were then cut into about 1 cm segments. A sterile nylon thread was then added to 2 of these dishes and 2 sterile nylon threads were added to 2 of the empty petri dishes. A crochet hook was then used to move an intact root into the last 2 empty petri dishes. 0.1 mL of each bacteria culture was added to 1 mL of 0.9% NaCl and 0.1 mL of this mixture was added to the appropriate petri dishes. A day 0 viable cell count was performed for each of the petri dishes. On day 7, another viable cell count was performed and the roots and threads from each of the dishes were viewed under a microscope to look for *Agrobacterium* binding and infection.

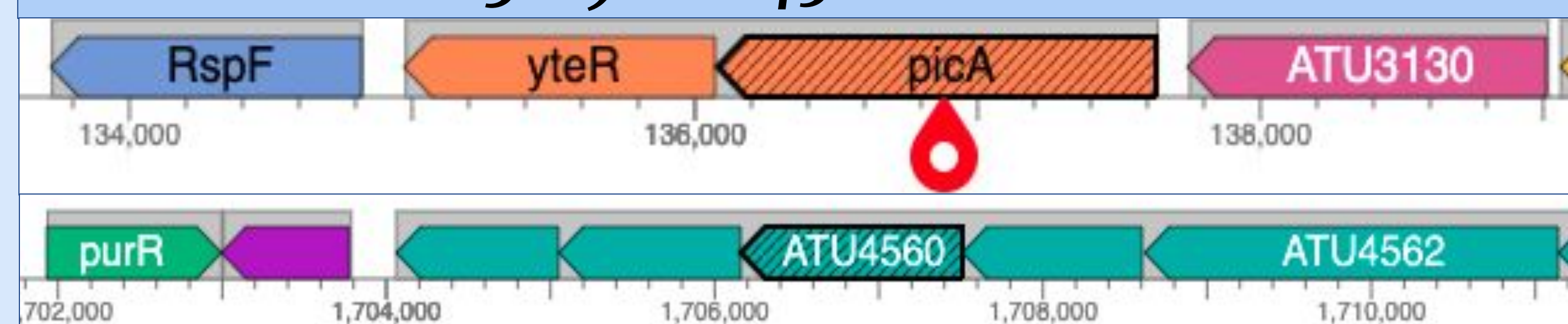
Inoculation of Tomato Fruit: 3 grape tomatoes were cleaned using ethanol and sterile water and were then placed in a plastic zip-lock bag along with 10 mL of sterile water and 0.1 mL of a bacteria culture. This process was then repeated for the other culture. The bags were left in a dark drawer at room temperature. After 3 days, 10 µL were taken from each bag, diluted, and plated on an L agar plate with kanamycin. 1 tomato from each bag was placed into a UV sterilized beaker with 25 mLs of sterile water which was then placed in a sonicator with about 200 mL of sterile water and was run for 1 tomato at a time for 2 minutes. 10 µL from the beaker in the sonicator was also plated on the L agar plates with kanamycin. The tomato was then placed into a sterile petri dish and cut using forceps and a scalpel. 10 µL was taken directly from the interior of each tomato and plated directly.

Exo-polygalacturonase Cuts Pectin



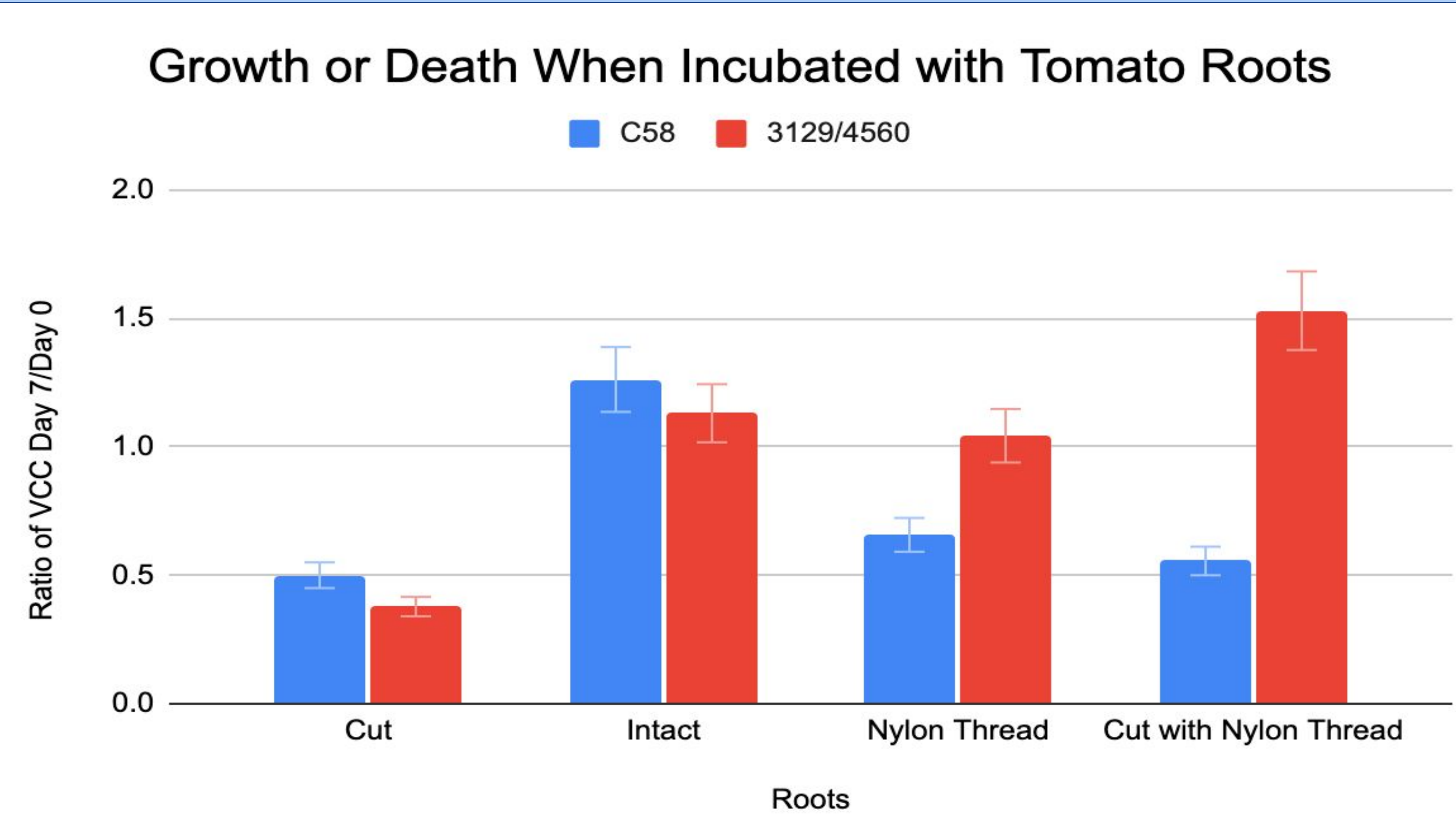
The structure of pectin and the site where pectinase cuts. Pectin is made of galacturonic acid chains, and pectinase cuts the chain. Shrestha et al. 2021

3129 and 4560 Genes



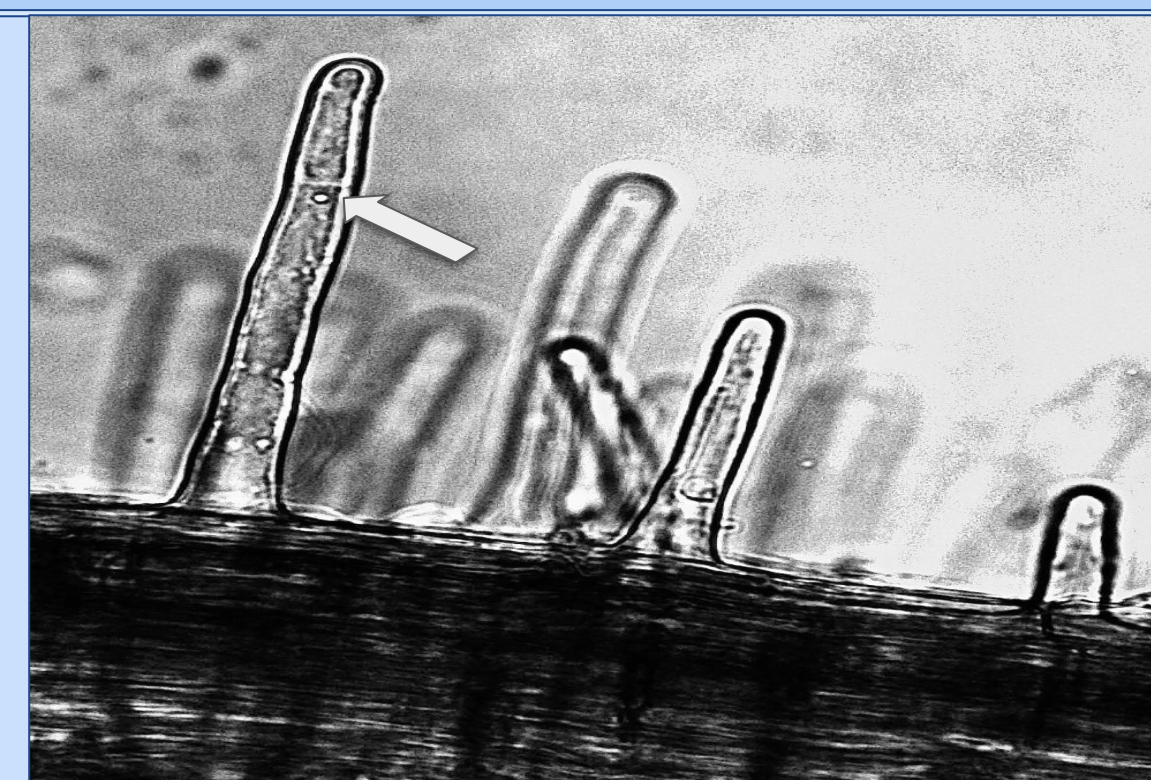
Segments of the linear chromosome showing the position of *Atu3129* (*picA*) and *Atu4560*. *Atu3129* is in an operon with genes of unknown function. The *4560* gene is in an operon with a beta-galactosidase gene, which is required for virulence, and the 5 genes of an ABC transporter. *purR* which encodes a DNA binding protein may regulate the expression of this operon. Images from Biocyc

Growth or Death When Incubated with Tomato Roots

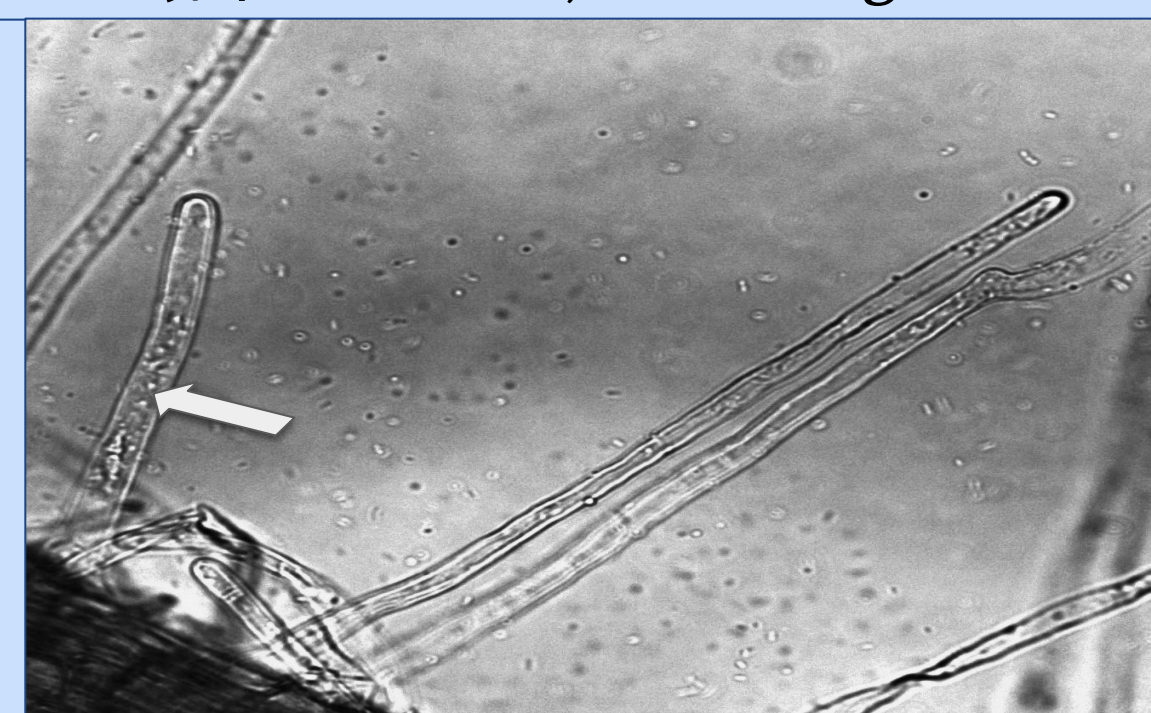
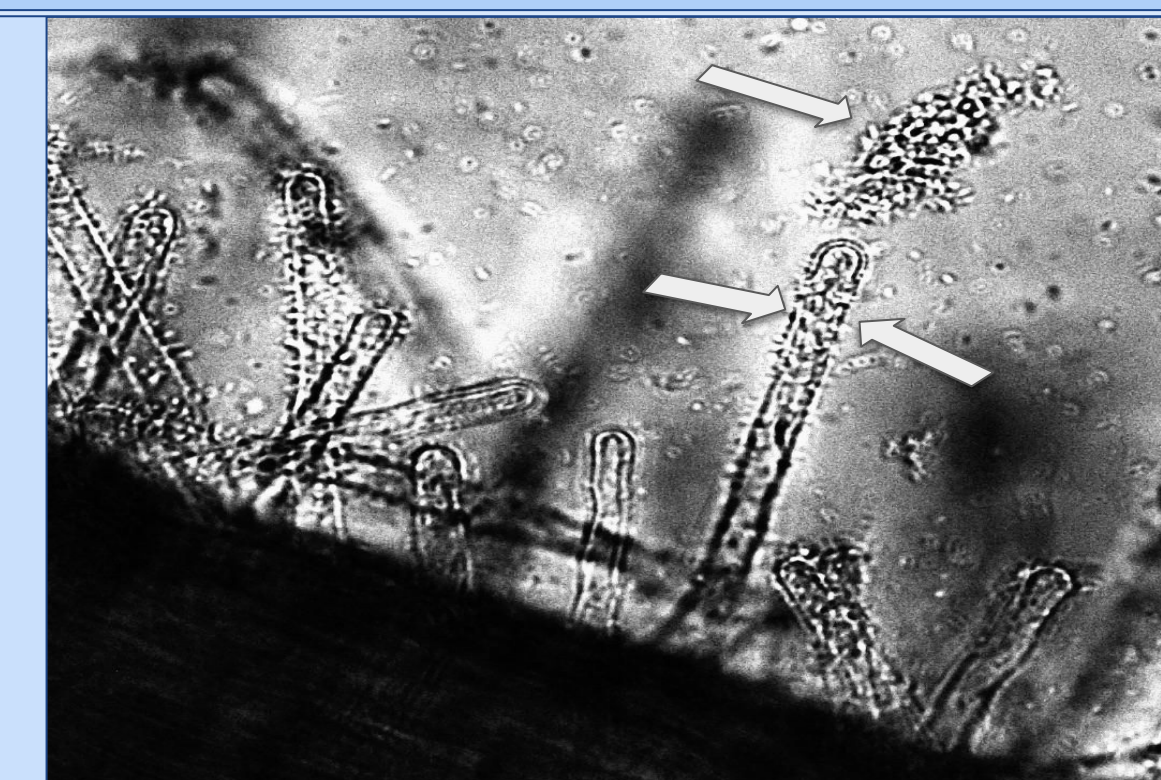


C58 typically has a ratio over 1

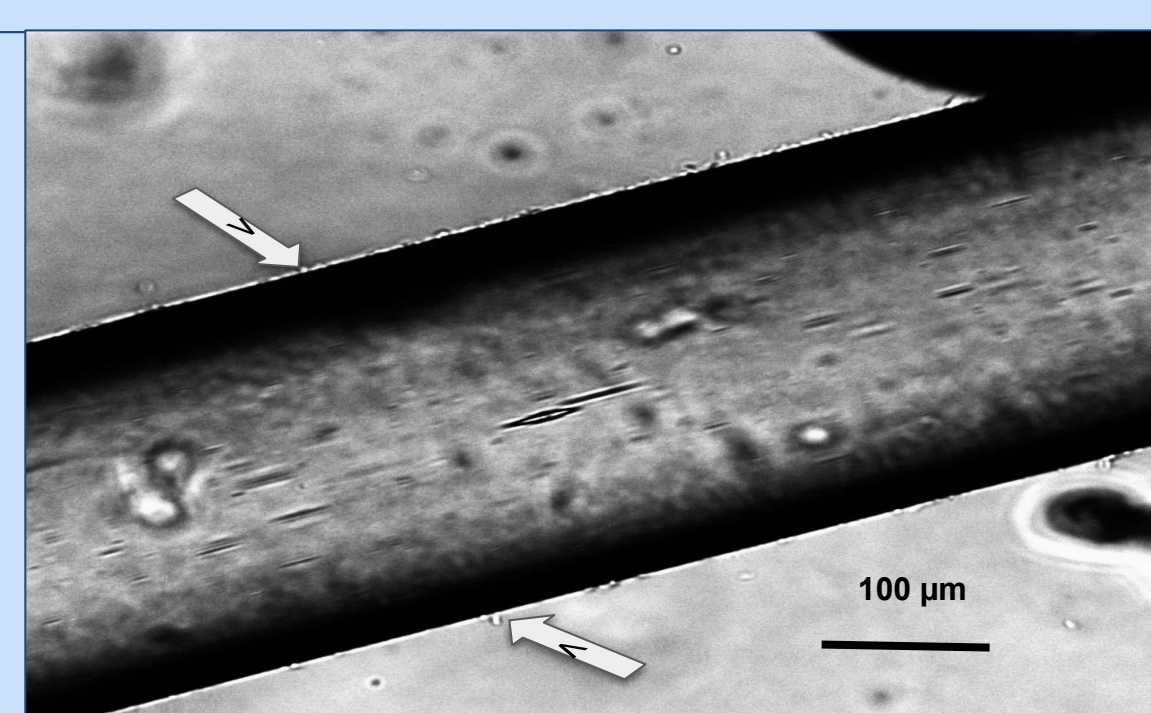
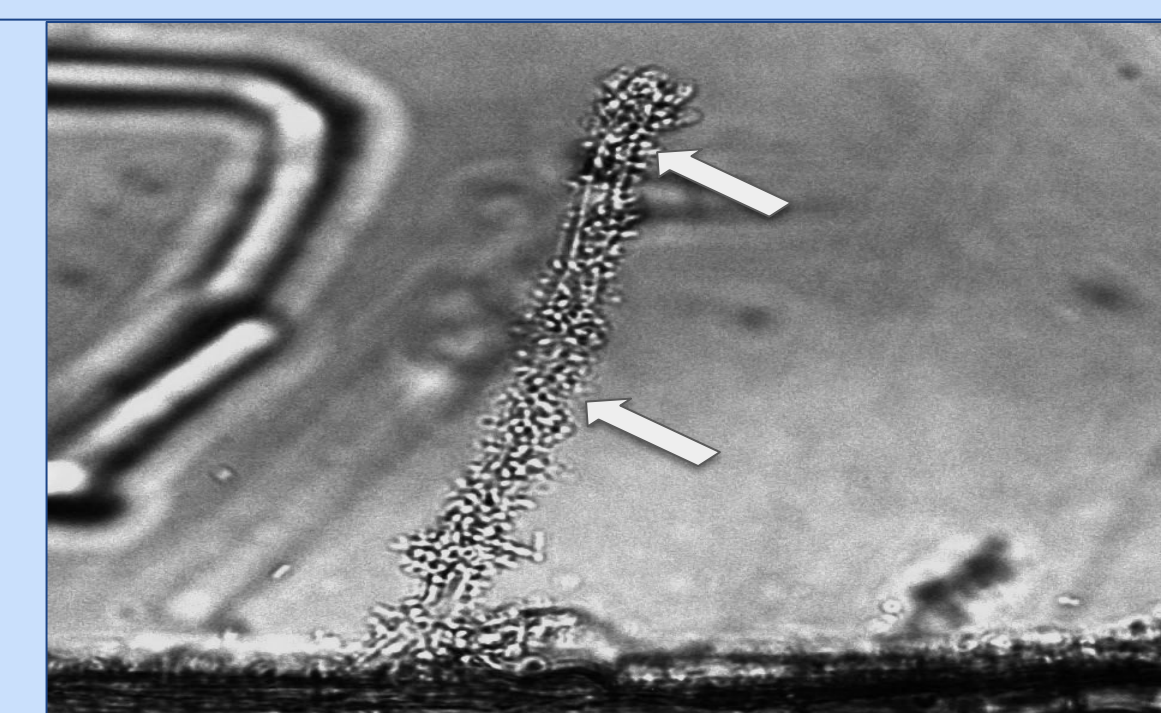
C58 vs 3129/4560 Binding on Tomato Roots



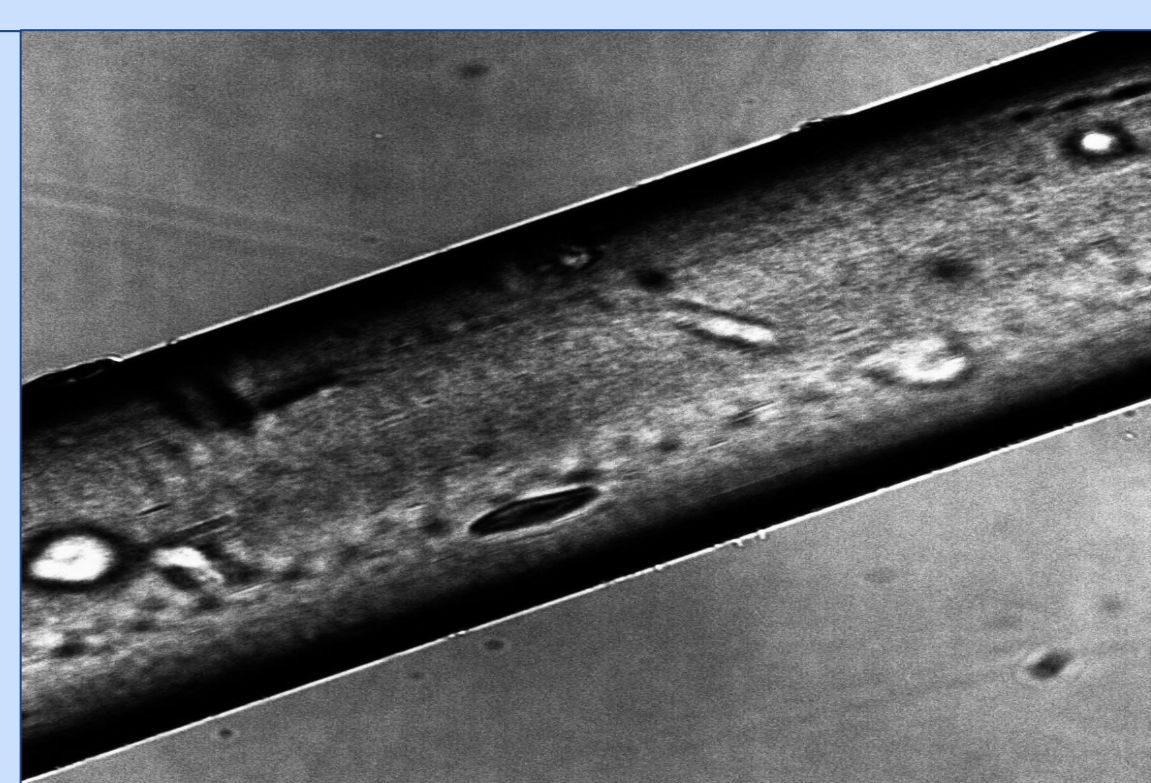
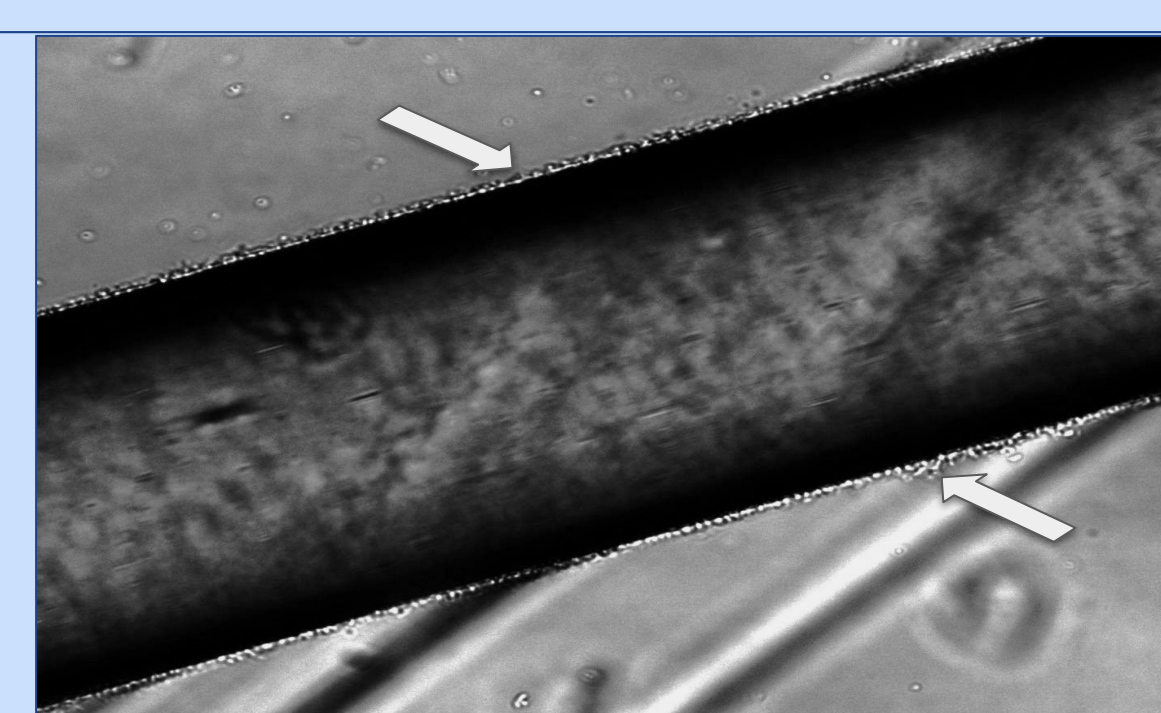
Cut: 3129/4560 on left, C58 on Right



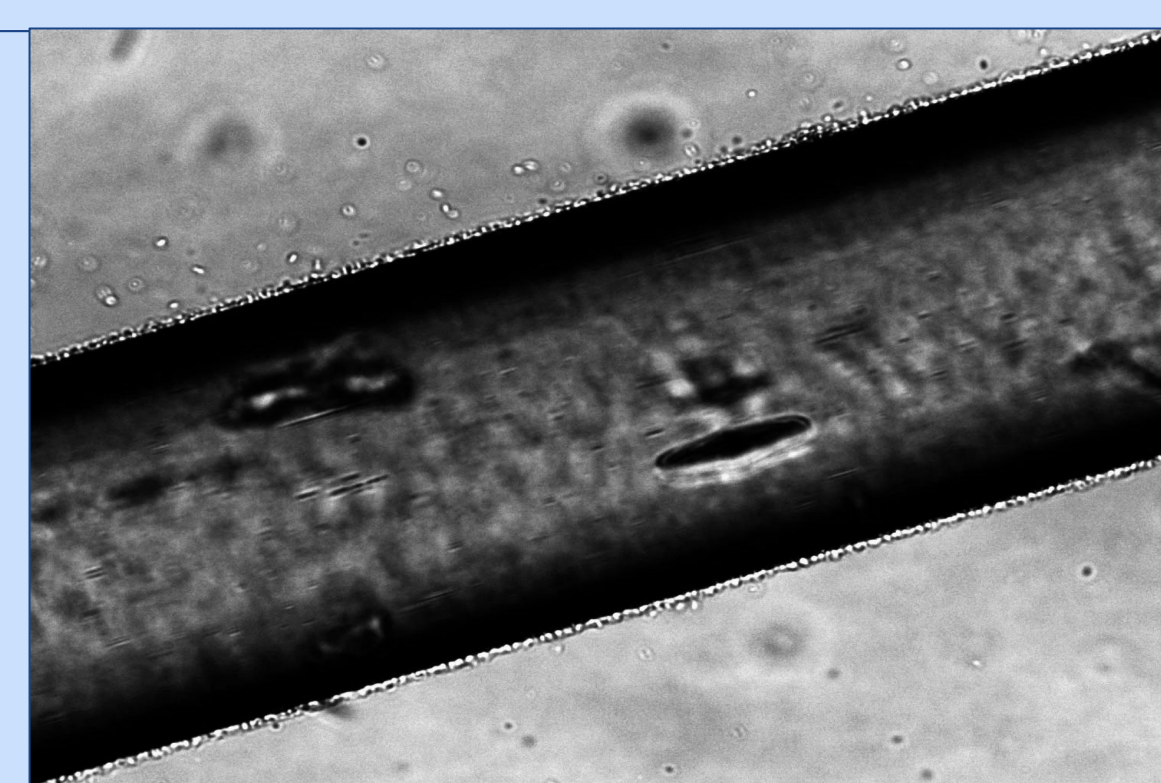
Intact: 3129/4560 on left, C58 on Right



Nylon Thread: 3129/4560 on left, C58 on Right
3129/4560: About 6 bacteria/70 µm
C58: About 36 bacteria/70 µm (arrow)



Nylon Thread: 3129/4560 on left, C58 on Right



C58 and 3129/4560 Binding to Roots

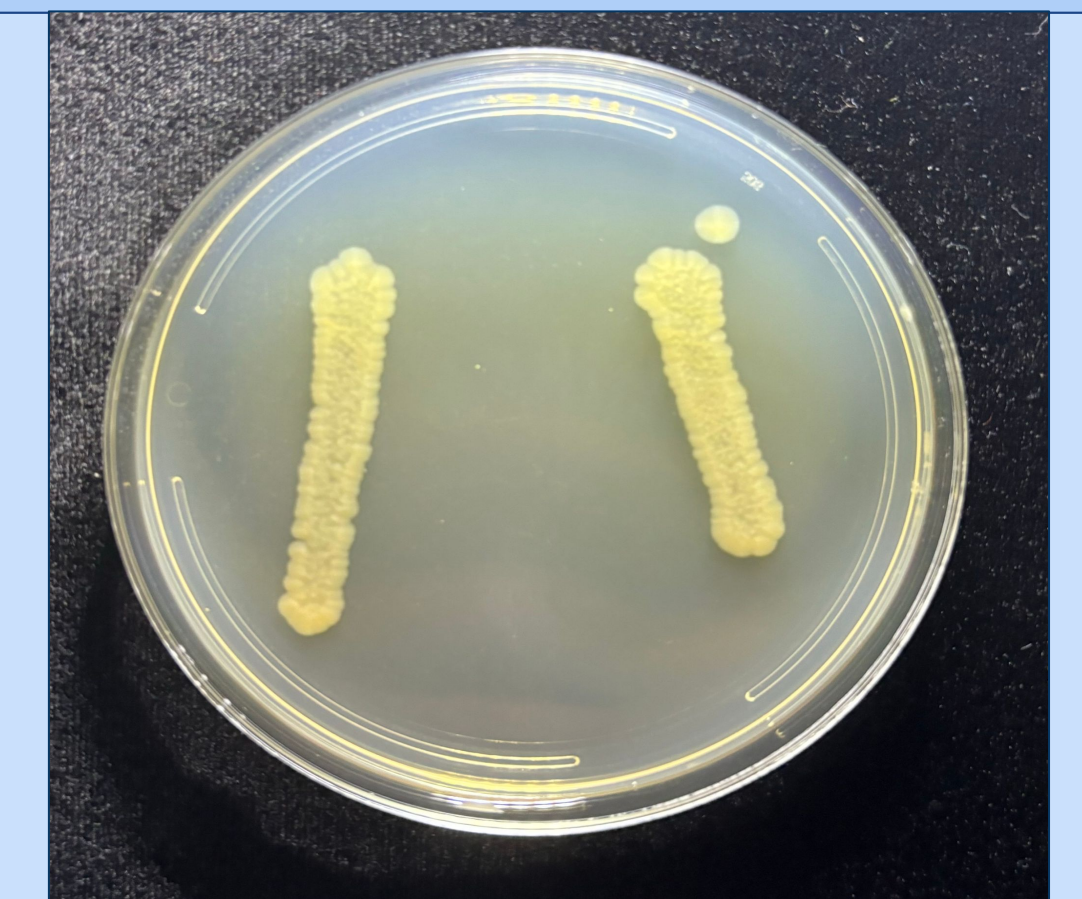
	C58	3129/4560
Cut	1	0.4
Intact	1	0.4
Nylon Thread	1	0.3
Cut with Nylon Thread	1	0.3

Each trial was ranked on a scale from 0-1 and an average was taken.

C58 and 3129/4560 Infection of Tomato Fruit

	C58	3129/4560
Infection of Tomato Fruit	Yes	Yes

C58 and 3129/4560 Sample From Inside Tomato



3129/4560 on left, C58 on right. On left side of the plates: sample taken from sonicator. On right side of the plates: sample taken from inside the tomatoes.

Conclusions

- 3129/4560 is able to infect the stem scars of tomato fruits the same as C58
- The double mutant 3129/4560 is not able to bind to tomato roots as well as the wild type C58
- This could indicate that pectinase is necessary to break down the pectin in the cell wall of the tomato roots in order to bind to the roots but not necessary for infection of the stem scar of a tomato fruit

Future Directions

- Begin to look at tomatoes after they have been sitting in the bacteria for 7 days
- Isabelle: Continue to perform trials until graduation to gather more data
- Ava: Take over performing the research and begin independent trials

References

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