

Isolating and mapping suppressors of the Δ SMc02230 morphologic mutation in *Sinorhizobium meliloti*

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ABSTRACT

Specific proteins control the three-dimensional organization of cells but may behave differently in cells that are morphologically distinct. We are using two related model bacteria, *Sinorhizobium meliloti* and *Caulobacter crescentus*, to investigate how conserved proteins contribute to the synthesis and localization of specific organelles to programmed subcellular sites. In *C. crescentus*, the protein PodJ acts as a subcellular localization factor required for chemotaxis and biogenesis of polar organelles. Two homologs of the *podJ* gene have been identified in *S. meliloti*: SMc02230 and SMc02231. Mutants with a deletion of the SMc02230 gene form smaller swarms on soft-agar plates compared to wild-type, indicating a chemotaxis defect, and have elevated levels of exopolysaccharides. In liquid media, we observed that the Δ SMc02230 strain grown in M9 minimal media and in Luria Bertani broth with low salt concentrations (LBLE) display branched and aberrant morphologies. In contrast, the wild-type strain grown under the same conditions has a rod-shaped morphology. In addition, the mutant exhibits a novel defect: it is unable to grow on LBLE plates. We subsequently isolated ten colonies of the Δ SMc02230 mutant that were able to grow on LBLE plates, apparently due to acquisition of suppressor mutations. Using transposon mutagenesis and arbitrary PCR, we are mapping and identifying these suppressor mutations to help elucidate the defect caused by the Δ SMc02230 mutation. Functional comparison of conserved proteins in related species will contribute to an understanding of how cellular factors evolved to suit the particular needs of different cell types.

INTRODUCTION

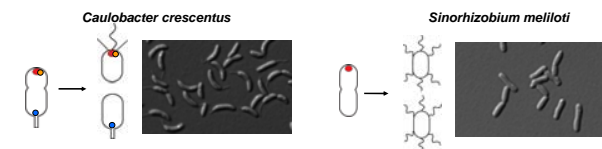


Fig 1. Diagrams and microscopic images of *C. crescentus* and *S. meliloti*

C. crescentus is found in fresh water environments, and *S. meliloti* is a soil bacterium that has a symbiotic relationship with legume plants. The two species appear quite different under the microscope—*C. crescentus* has a single polar flagellum in the swarmer cell and divides asymmetrically, whereas *S. meliloti* has peritrichous flagella and divides symmetrically—but many genes that contribute to cell morphology are shared between the two genomes.

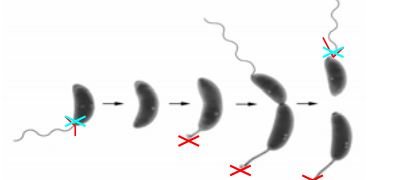


Fig 2. Deletion of *podJ* gene has pleiotropic effects in *C. crescentus*.

podJ mutants are unable to form pili in the swarmer cell and holdfast in the stalked cell; they also have a chemotaxis defect.

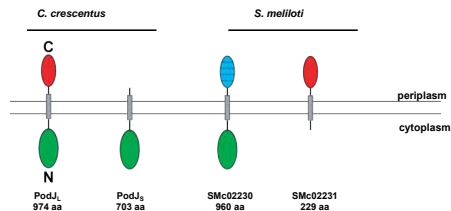


Fig 3. Schematics of PodJ protein in *C. crescentus* and two homologs in *S. meliloti*

Through DNA sequence analysis, two homologs of PodJ were found in *S. meliloti*. SMc02230 encodes a homolog of the N-terminal domain of PodJ, and SMc02231 encodes a homolog of the C-terminal domain of PodJ.

DEFECTS OBSERVED IN Δ SMc02230

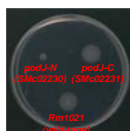


Fig 4. Effect of deletion of SMc02230 and SMc02231 on the swarming ability of *S. meliloti*.

Mutants with a deletion in SMc02230 demonstrate a swarming defect on soft agar plates, indicating that SMc02230 may be involved in chemotaxis.

DEFECTS OBSERVED IN Δ SMc02230

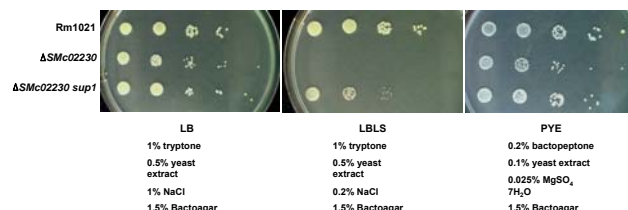


Fig 5. Media-dependent growth defects of Δ SMc02230.

Δ SMc02230 is unable to grow on LB plates containing 0.2% NaCl. This defect is not observed on LB plates containing 1% NaCl nor on PYE plates. The wild-type and Δ SMc02230 *sup1* mutants are able to grow on all three types of media.

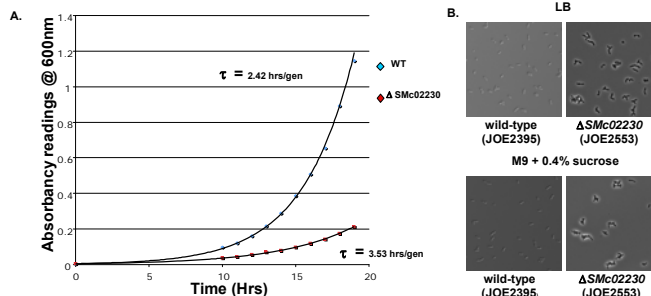


Fig 6. Growth of Δ SMc02230 on LBLE liquid medium and morphology in LB and M9 + 0.4% sucrose liquid medium

6A shows that the Δ SMc02230 mutant grows poorly, if at all, in liquid LBLE. 6B shows that in liquid LB and M9 + 0.4% sucrose liquid medium, the mutant cells often clump together, resulting in a "T-shaped" morphology. The wild-type strain exhibits the normal, rod-shaped morphology on both types of media.

ISOLATION OF Δ SMc02230 SUPPRESSOR MUTANTS

We were able to isolate 10 colonies of Δ SMc02230 that grow on LBLE plates. We hypothesized that these colonies have gained additional mutations that allow them to grow on LBLE.

Comparing colony forming units (CFU) on different media, we were able to determine that the rate at which suppressor mutations arise is 10^{-6} – 10^{-7} .

HYPOTHESIS

Because the *S. meliloti* Δ SMc02230 mutant shows no growth on LBLE plates, our hypothesis is that the growth that is observed in LBLE liquid media is due to suppressor mutant strains that have gained an additional mutation, allowing the Δ SMc02230 mutant to grow on LBLE.

ASSESSING LOCATION OF SUPPRESSOR MUTATION

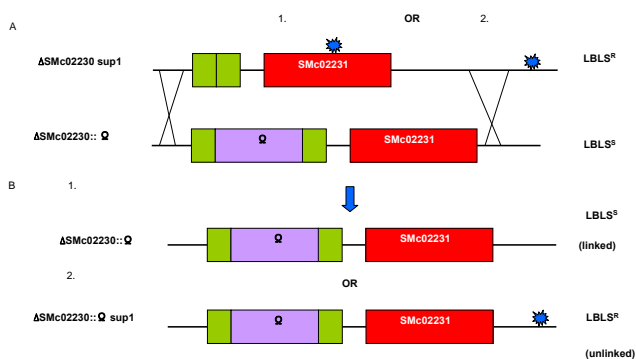


Fig 7. Diagram of possible outcomes from transducing cassette insertion into suppressor strains.

2A depicts the homologous recombination event that will occur after introduction of phage. The star represents the suppressor mutation which may or may not be linked to SMc02230. 2B depicts the two possible outcomes: In outcome 1, the mutation is closely linked and the resulting strain becomes LBLE^S. In outcome 2, the mutation is not linked and the strain stays LBLE^R.

MAPPING Δ SMc02230 SUPPRESSOR MUTATION

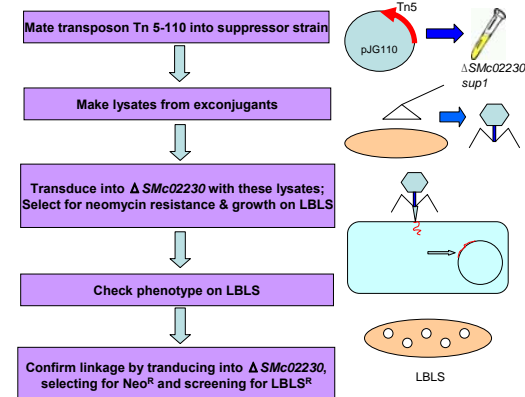


Fig 8. Experimental scheme for mapping the suppressor mutation.

We utilized transposon mutagenesis to link a transposon to the suppressor mutation in *S. meliloti*, performed a series of transductions to confirm that the transposon is linked to the mutation, and performed arbitrary PCR to locate where the transposon landed in the genome of *S. meliloti*.

PERFORM PCR TO LOCATE WHERE TRANSPOSON LANDED

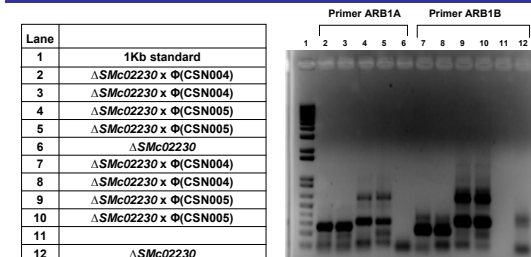


Fig 9. PCR result for two Tn linkage candidates: CSN004 and CSN005

Using two isolates of the candidate strains and two different primers, we found transposon insertions in the same general location. In CSN004, Tn is in position 1540194 and interrupting gene SMc01015. In CSN005, Tn is in position 1546862 and interrupting gene SMc01021.

GENERATING LBLE^R PHENOTYPE DUE TO TRANSPOSON INSERTION

To determine if there are additional loss of function mutations that lead to the LBLE^R suppressor phenotype, we will mate a transposon into the Δ SMc02230 mutants, make lysates from these exconjugants, and transduce the phages into Δ SMc02230 mutants. The experimental methods that we will use are similar to those utilized in mapping the suppressor mutation.

CONCLUSIONS

- Δ SMc02230 mutants exhibit a growth defect on LBLE plates and LBLE liquid media.
- Ten suppressor colonies of Δ SMc02230 mutants were isolated that are able to grow on LBLE plates.
- The spontaneous suppressor mutation rate is 10^{-6} – 10^{-7} .
- Using transposon mutagenesis and arbitrary PCR, we determined that the suppressor mutation is within the range of 1459639 and 1603294 base pairs.
- Some transposons were inserted in the range of 1107356 and 1107487 base pairs. The LBLE^R phenotype in these strains may be due to the introduction of the transposon itself.

FUTURE DIRECTIONS

We will sequence the genome of the suppressor mutants in the range where the transposon inserted to determine whether there are candidate mutations in the regions where the suppressor mutations should be. We will also be using integrating plasmids to make directed insertions in locations where the transposon itself may be causing suppression on Δ SMc02230 mutant strains and checking the resulting strain on LBLE to determine if the suppressor phenotype can be recapitulated.

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