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7.13 Experimental Microbial Genetics  
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## Care and Handling of PA01 *fur* mutants.

Cultures are being sent to you as growing organisms on Brain Heart Infusion Agar. Streak them out as soon as they arrive and check their phenotypes as described below. Cultures of the mutants should be stocked in 12.5% glycerol in a nutrient broth such as BHI and frozen at -70C. Use a fresh overnight culture if possible for all your experiments.

1. All of these *fur* mutants are point mutations and they can and do revert. They revert at a higher frequency in media rich in iron. Their phenotype should be checked as often as possible (i.e. whenever you are doing an important experiment). There are two ways to check them that are relatively easy. Their phenotype on CAS agar plates with high and low concentrations of iron should be consistent with the photograph enclosed. The formula for the CAS plates is on the following pages. They can also be checked by doing a disc diffusion assay with filter discs impregnated with Manganese Chloride. Make a suspension of a fresh culture from a plate or broth to an A600 ~.1-.2. Using a cotton swab thoroughly swab a Tryptone agar plate to make a uniform lawn for each organism to be tested. 4g Bacto Tryptone, 2.5 g NaCl, 16 g agar, 1L H<sub>2</sub>O. Make small filter paper discs ~7-8mm diameter. Put 15 µL of a 1M solution of MgCl<sub>2</sub> on the disc. Put disc on Tryptone agar plate. Incubate 37C overnight. PA01 has a zone size of ~17-18mm while the C6 mutant has a zone size of 10mm on these plates. You may come up with simpler variations of this method but basically the *fur* mutants will be more resistant to manganese than the wildtype. **Note: do not use organisms from plates that contain MnCl<sub>2</sub> for experiments since that may cause additional mutations.**
2. These *fur* mutants are not easily transformed with plasmids. One problem is that they do not seem to take up DNA very easily the other problem is that the manipulations that are required for mating or transformation are conditions that will select for reversions. We have not tried electroporation with these mutants.

**Remember: We have determined that a *fur* null mutation in *P. aeruginosa* is lethal and they do not like having a poorly functional *fur* either. What appears to happen is that revertants occur and then they seem to outgrow any of the *fur* mutants in the culture. However, we have successfully done RNase protection assays and we have gotten plasmids into them, but after you do so, you need to check the phenotype to be sure that you still have a *fur* mutant.**

**PA01wt**

**PA06261 anr°**

**PA06261-C6 anr° *fur-6* (Mutation is Ala-10-Gly)**

**PA06261-C64 anr° -revertant of *fur-6* to *fur+* (Reversion is Gly-10-Ser)**

**PA01-C6Tc *fur-6*.** This mutant was generated by cointegration of pSUP203 carrying a 5'fragment of *fur* containing the C6 mutation. It has all the phenotypes of the PA06261 with regard to Mn<sup>r</sup> and Siderophore synthesis on CAS plates.

The phenotype of C6 with respect to siderophore synthesis is that it constitutively produces siderophores on CAS plates and it normally regulated for exotoxin A synthesis in 20% O<sub>2</sub>. This phenotype for exotoxin A does not have anything to do with the anr null mutation in PA06261 since the PA01-C6Tc mutant has the same phenotype.

**PA01 A2 *fur-2* Mutation His-86-Arg.** This seems to be a mild mutation since siderophore synthesis on CAS plates is still repressed with iron.

**PA01 A4 *fur-4* Mutation is His-86-Tyr.** This mutation causes constitutive siderophore synthesis on CAS plates but exotoxin A synthesis is not affected.

**Failure to follow these instructions may result in irreproducible results.**

**Note: Plates will look very similar at 37°C as well.**

