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7.13 Experimental Microbial Genetics

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Characterization of phenazines in *Pseudomonas*

Concept

P. aeruginosa produces a number of small molecules known as phenazines. They are often redox active, and can be detected through spectroscopic analysis.

Protocols

Run through these protocols with wild type PA14, the melanin overproducing mutant Δ hmgA and Big Blue to establish the assay methods before starting with the CF isolate library. All of these strains can be found in the lab's strain collection. See the stellar site for the list of strains and ask a TA to help you get these strains from the lab stock.

LB Media

Liquid LB: 25 g Difco LB /liter deionized water. Autoclave for 35 min on fluid cycle.

Plates: add 15 g bacto agar to 1 liter liquid LB before autoclaving. Let cool to $\sim 50^{\circ}\text{C}$, then pour.

NOTE: You don't have to make a liter of plates if you don't need that many!!! Just make what you need.

Pyocyanin detection

1. Pipette 0.5ml LB into the wells of a sterile deep well plate (one for each culture you plan to grow).
2. Inoculate each well with a strain from the CF library.
3. Incubate at 37°C with shaking overnight.
4. The next day, some of the wells should look blue. This is the pyocyanin.
5. Pipette 30 ul of each culture into a fresh microtiter plate. Set aside.
6. Spin the deep well plate for 5 min at $6000\times g$ to pellet the cells.
7. Transfer 30 ul of the supernatant to a fresh microtiter plate. Include a blank of the same LB you used to start the cultures.
8. Take an absorbance reading at 691 nm to quantify the pyo present in the culture. The extinction coefficient of pyo at this wavelength is $4.31\times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.

Growth defect check

This should be done to ensure that differences in phenazine production are not due to a simple growth defect.

1. Read absorbance at 600 nm of both microtiter plates you prepared earlier.
2. Subtract the values of the plate you prepared after spinning the cultures (supernatant only) from the absorbance of the first plate you prepared (unspun culture).
3. Different cell densities should be accounted for in phenazine production calculations. How do you do this?

Melanin detection

Look for colonies showing red pigment. To determine whether this pigment is melanin, add several drops of concentrated HCl to a small volume of supernatant (3 drops in 500 ul is good). Melanin will form clumps of dark precipitate.

“Mystery compound” detection

Tryptone agar:

10 g/L tryptone

10 g/L agar

Autoclave, cool to 50°C, then pour the plates.

1. Spot 5 ul of liquid culture from your deep well plate onto tryptone plates (note: do this before you spin it down).
2. Incubate at room temperature for several days.
3. Look for orange or red coloring of agar around the colonies. See example results on last page of this protocol.

This compound has a color similar to melanin, but does not precipitate when acidified.

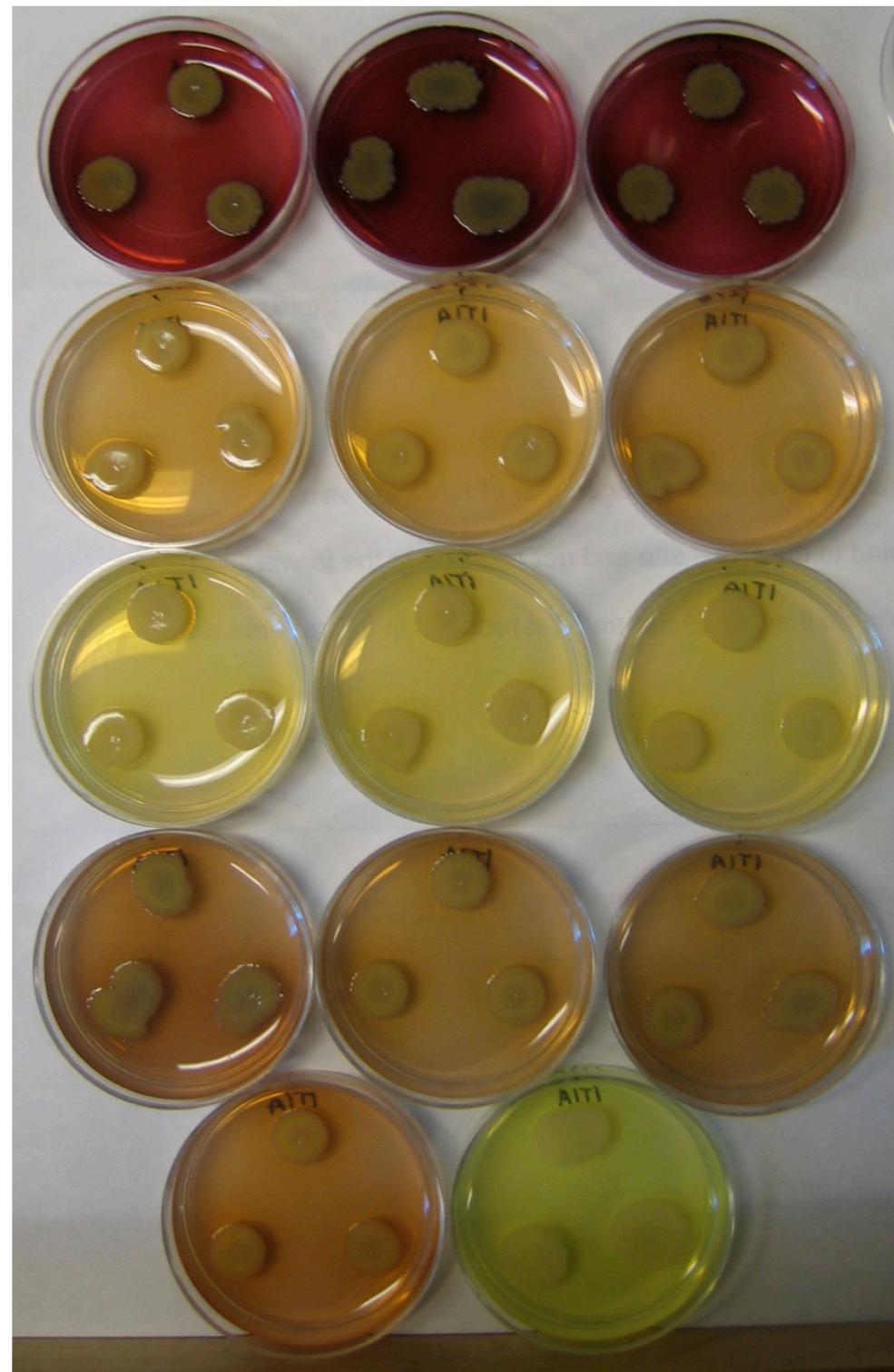
Medium: 1% tryptone, 1% agar

“Big Blue”
(contains extra copy of *phzM*)

wild type

$\Delta mexGHI-opmD$

$\Delta soxR$



$\Delta phsS$ $\Delta phzA1-G1$
 $\Delta phzA2-G2$

(the first three strains are shown in triplicate; the last two are controls so are shown one replicate each)