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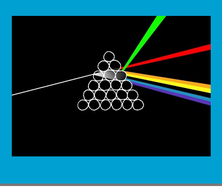
Finding the link between bacterial predation and structural colour using genetics



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Introduction

Flavobacteria colonise and move over environmental surfaces where competitive interactions with other microorganisms are inevitable.
Flavobacterium IR1 is a gliding bacterium with a high degree of colonial organisation; the cells create a 2D photonic crystal, resulting in vivid structural colouration.
IR1 is a predator, it moves over surfaces and consumes other bacteria. There is a link with a 2D photonic crystal, this colony structure seems necessary for predation – but we don't know why. This project creates mutants in IR1 which are altered in predation to try to explain this question.

Plan of Action

Create mutants in IR1 using the HiMar transposon mutagenesis system that knocks out genes at random.

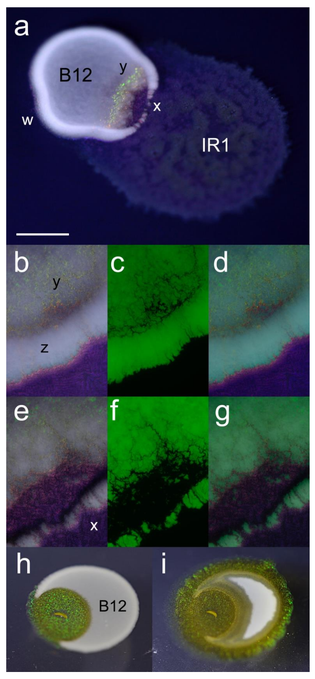
Screen these mutant libraries for mutants altered in predation using the GFP expressing strain of *Enterobacter cloacae* as a prey bacterium.

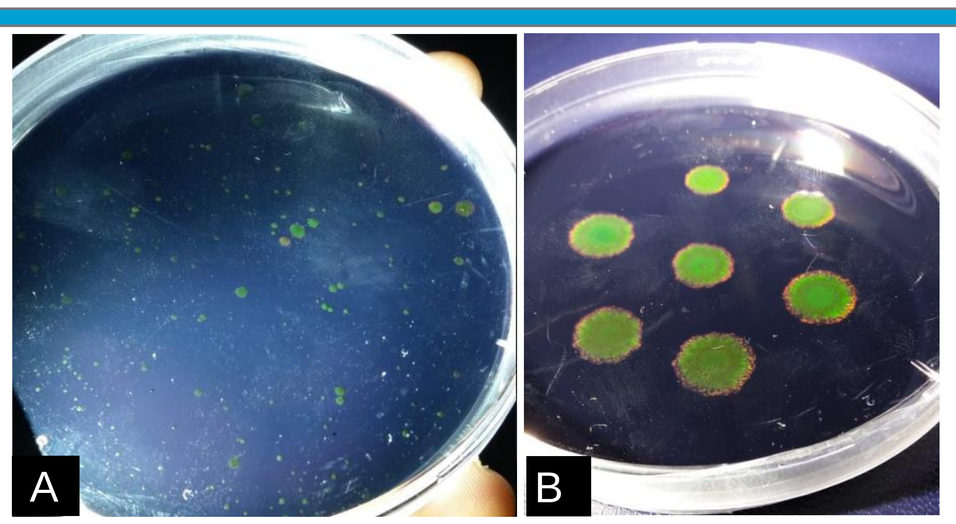
Characterise mutants and sequence the genomes to find which genes are involved.

Materials & Method

IR1 Invades and Predates Adjacent Colonies of the prey bacterium B12.
Inoculation of IR1 adjacent to B12 (pGFP) on agar plates containing a black dye, showing the result 10 h after contact between the spreading colony of IR1 and the static mass of B12. IR1 surrounds the B12 colony (w) and creates breaches (x) in the thicker edge of the B12 colony and a shift from dull purple/red structural colour typical of growth of ASWF to green (y). IR, IR1 colony; B12, B12 colony. Panels **b.** to **d.** Images 4 h after contact with invading IR1. (b) Illumination from side showing white B12, with a thicker colony at the periphery (z) and structural colour from IR1 (bright pinpoints of colour including deep within the B12 colony) (y). (c) Fluorescence image showing GFP expressed by B12. (d) Merged panels b and c. (e-g) are similar to b-d but after 9 h showing more extensive clearing of B12 cells and major breaches at periphery of the B12 colony (x). Panels h and i show an experiment where B12 is inoculated in a droplet on starvation medium, allowed to dry and then IR1 inoculated inside B12. (h) Result after 4 days showing expansion of the IR1 colony (IR1, showing predominantly green structural colour) to breach the periphery of the B12 colony (opaque white) from within. (i) Result of the same colony as panel **h** after 8 days showing progressive destruction of the B12 colony and movement around the periphery of B12 to engulf it. Size bar in panel a indicates 0.4 mm, 0.15 mm for panels b-g and 0.5 mm for panels h and i.

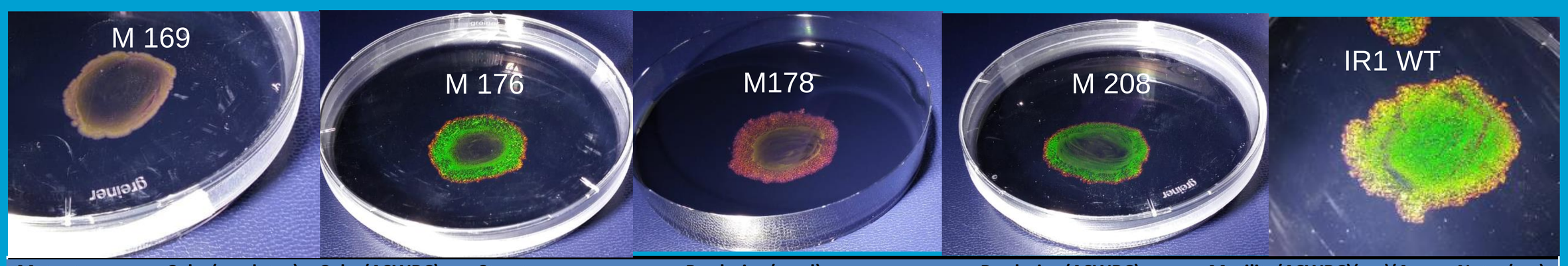
Mixing predator and prey bacteria and looking at the survival of the prey Bacteria form the basis of the predation assay used in this project.





A: Mutants on predator agar after conjuagtion of the HiMar-transposon.
B: Motility test of M208, 5ul of bacteria solution on ASWBC-plate.
Making mutants of IR1 by inserting the HiMar-transposon through conjugation from e.coli to IR1. This transposon shall randomly insert in the genome, causing knock-outs of genes. Those mutants will be tested at their ability of predation, motility an coloration on different agars.

Results



Mutant nr:	Color(predator)	Color(ASWBC)	Structure	Predation(pred)	Predation(ASWBC)	Motility(ASWBC)(cm)(4 days)	Notes(cm)
169	Pink/Red	Green/Red	Lost crystal structure	Up regulated	Wild type	Down regulated	0,80
176	Green	Green	Lost crystal structure	Up regulated	Down regulated	Down regulated	1,00
178	Green/Red	Green/Red edge's	Lost crystal structure	Down regulated	Down regulated	Down regulated	0,92
181	Green	Green	Lost crystal structure	Up regulated	Down regulated	Down regulated	0,98
202	Green/Blue	Green	Lost crystal structure	Down regulated	Wild type	Down regulated	0,86
208	Less Green	Green	Lost crystal structure	Down regulated	Wild type	Down regulated	1,08
IR1 (Wildtype)	Green	Green	Crystal structure	Wild type	Little predation	1,38	1,38

Conclusion

It is possible to identify mutants affected in predation. From sequencing these mutants it will be possible to identify the genes involved. Some Mutants are more predatory than the WT which might be more useful as a biological control agent.

Possible application

As a biological control agent instead of antibiotics – for example to control pathogenic bacteria in a fish farm.