Predatory Bacteria a New Ally in the Fight Against Infection

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Disclaimer

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"Despite the World Health Organization listing antibiotic resistance as one of the top three health threats the world faces, as early as two year ago, little has been done to address the issue."

Prof. Laura Piddock, President of the British Society for Antimicrobial Chemotherapy (UK Daily Telegraph 2011)

Friday 14 June 2013

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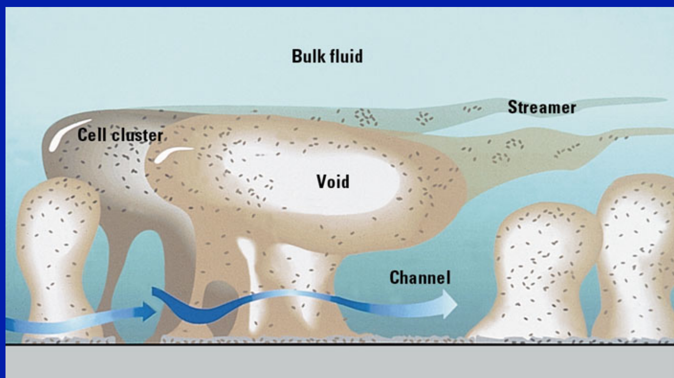
News > Science

Drug-resistant bacteria as big a threat as climate change and water shortages for future generations, warns science minister David Willetts

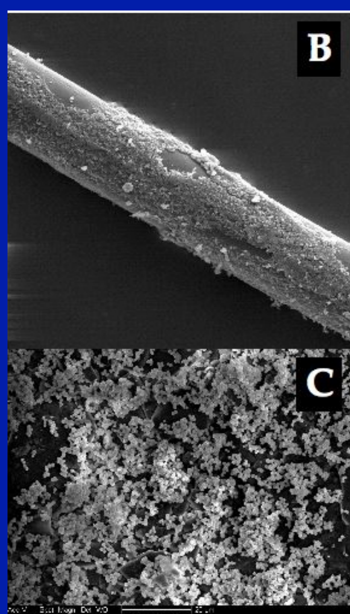
Fears over antibiotic usage leading to lethal infections will be discussed at G8 meeting

Biofilms

Community of microorganisms attached to a surface



Wounds and burns



Zegans *et al.*, 2005

Dental plaque stained with Gram's iodine

Biofilm control

- Reducing planktonic cells in the system.
- Reducing cell adhesion to the surface.
- The use of various treatments to reduce or destroy already existing biofilm.

    Physical treatment (heat, laser, sonication).

    Chemical treatment (all types of antimicrobial agents).

    Biological and biocontrol agents.

Biocontrol agents

• Direct feeding (bacteria / biofilms as a food source).

• Competition for biological niche with limited resources (establishment by means of using the niche resources faster, or secretion of antimicrobial compounds).

• The use of biological derivative compounds to destroy biofilms (enzymes, quorum-sensing molecules, lectins etc).

Direct feeding on biofilms

- Grazing by invertebrates.
- Grazing by protozoa.
- Bacteriophages.

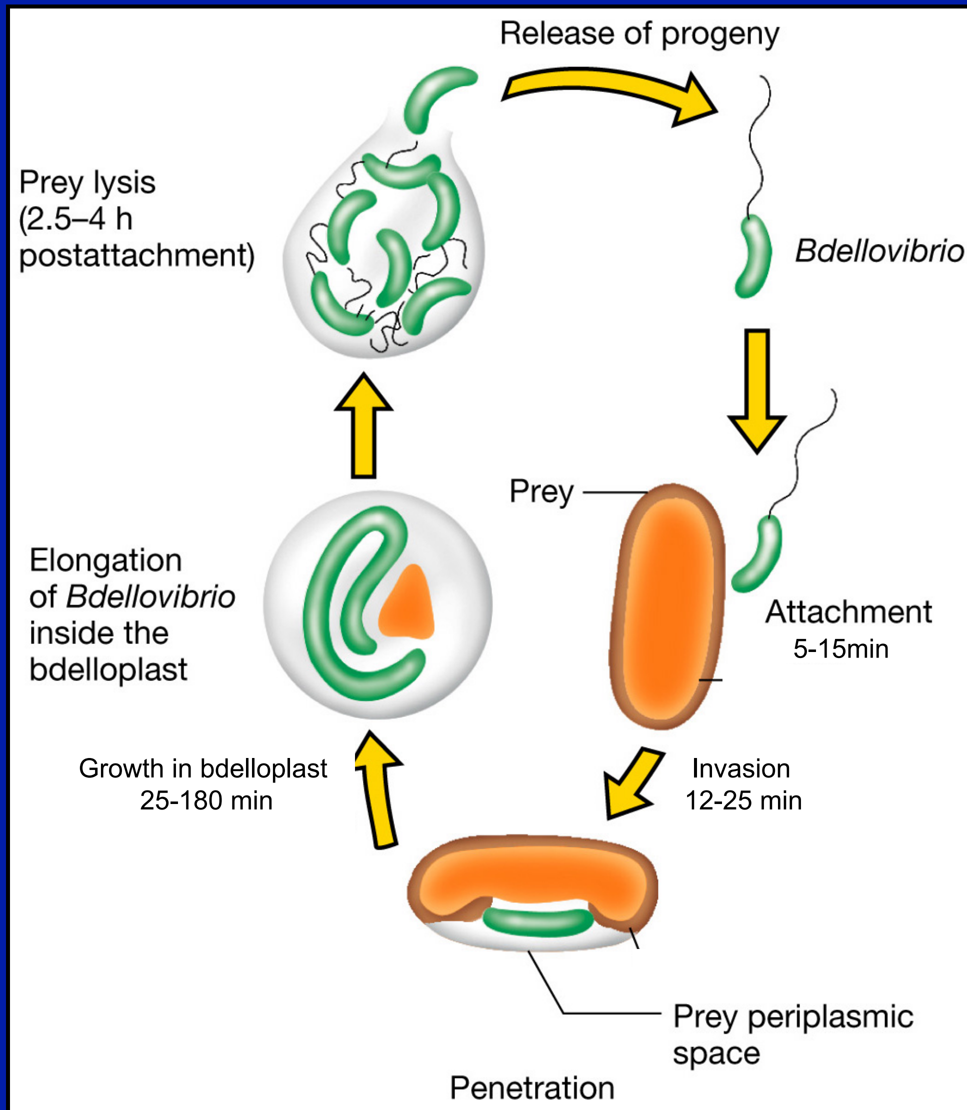
Predatory Prokaryotes

Bdellovibrio spp.

Order: *Bdellovibrionales* **Family**: *Bdellovibrionaceae* **Genus**: *Bdellovibrio* **Species**: *Bdellovibrio bacteriovorus*

- **Gram negative, motile, and uniflagellated bacteria.**
- **The genus has been placed within the δ *Proteobacteria.***
- **First isolated from soil (Stolp and starr, 1963).**
- **The genera are characterized by predatory behavior.**
- ***Bdellovibrios* are found in wet, aerobic environment. Also recovered from biofilms, soils, and the rhizosphere.**
- **The genome of strain HD100 consists of 3,782,950 bp predicted to code for 3584 proteins.**

Bdellovibrio life cycle



http://people.sju.edu/~jtudor/undergrads/docs/tudorweb/tudorresearch.htm

A day in the life of *Bdellovibrio*

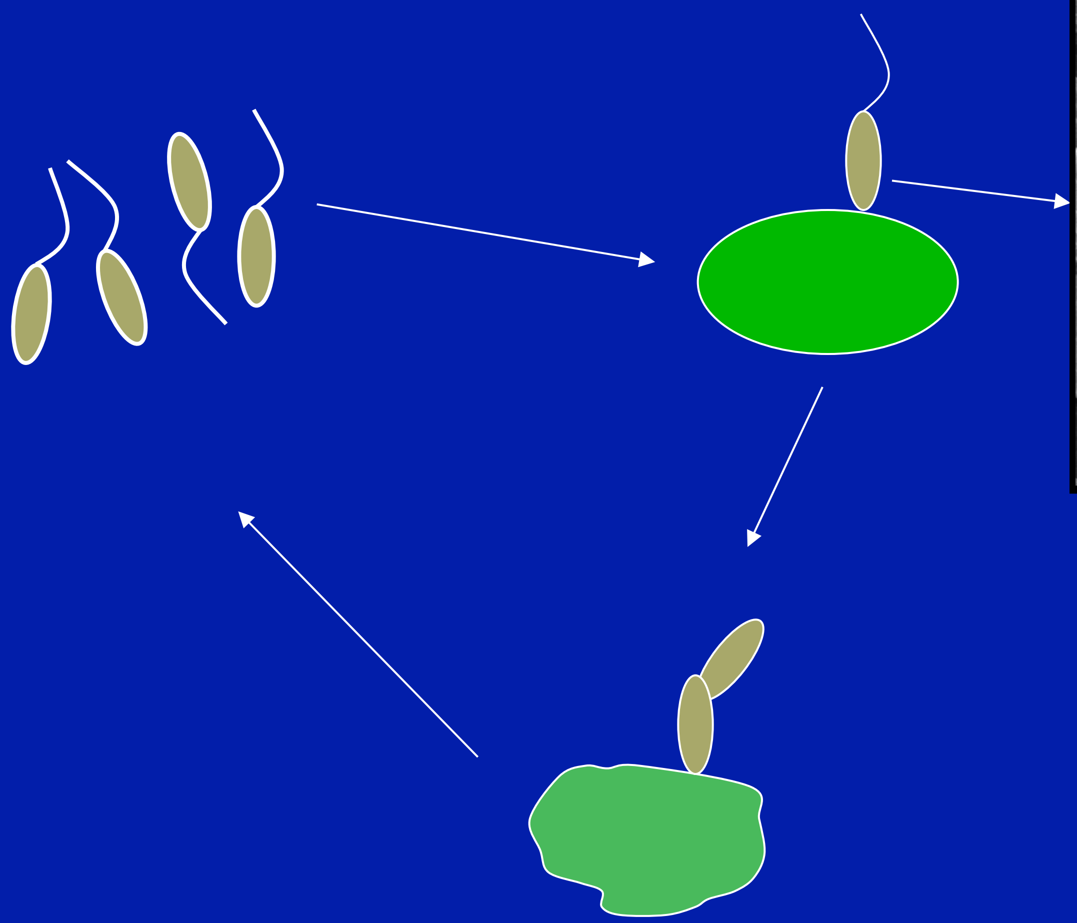
Micavibrio spp.

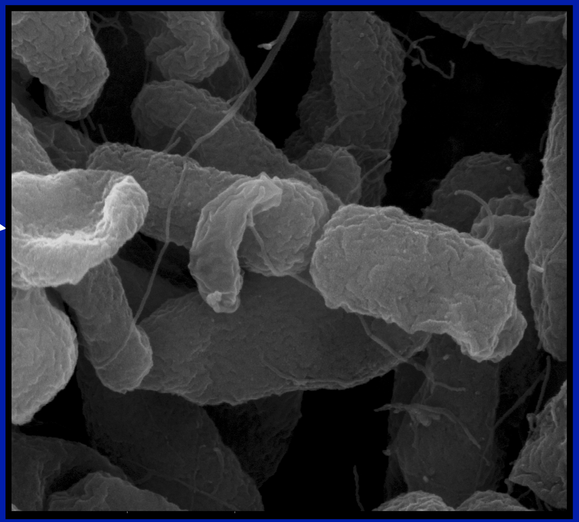
- **Gram negative, motile bacteria.**
- **The genus has been placed within the α *Proteobacteria.***
- **First isolated from sewage water (Lambina *et al.,* 1983).**
- **The genera are characterized by exoparasitic behavior.**
- **Narrow host range.**

•The genome of *Micavibrio aeruginosavorus* ARL-13 consists of 2,481,983 bp predicted to have 2432 open reading frames .

Wang *et al*., 2011. PMC 3189940

Micavibrio attacks a specific Gram negative host → attaches to the host → growth → lysis of the host and binary fission → start a new life cycle.

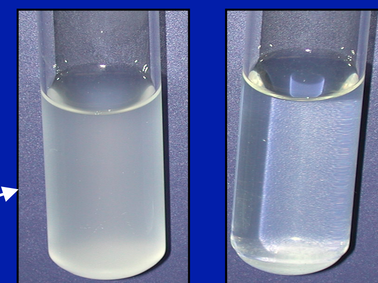




Growing predatory bacteria

Plaque of *Bdello/Mica*

Prey



0.45μm

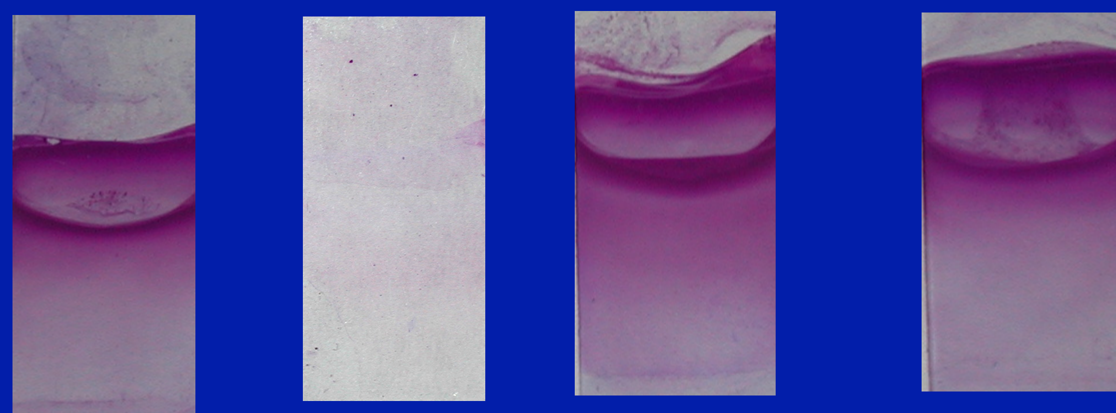
Purified
Bdellovibrio /
Micavibrio

0.22μm

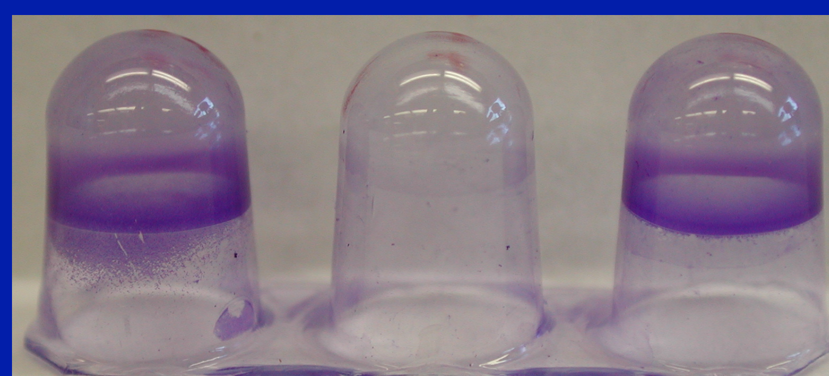
Control

Biological control of biofilms by predatory bacteria

Reduction of *E. coli* biofilm by *B. bacteriovorus*



Overnight Treated Control Control dead *Bdellovibrio*



Kadouri and O'Toole. 2005. PMC 1169041

Reduction of *P. aeruginosa* PA14 biofilms by *M. aeruginosavorus* ARL-13

ON Treated Control

Initial screening has shown that *M. aeruginosavorus* ARL-13 was successful in reducing 104 out of 120 *P. aeruginosa* clinical isolates tested.

Kadouri *et al*., 2007. PMC 1796979

Controlling biofilms by predatory bacteria

• A reduction in biofilm biomass was observed as early as three hours after exposure to the predator.

• An initial titer of *Bdellovibrio* as low as 1 pfu/ml is sufficient to reduce a preformed biofilm.

• Microscopy studies had confirmed that predatory bacteria have the capability to access the biofilm during predation and are not restricted to the surface of the biofilm.

Kadouri and O'Toole. 2005. PMC 1169041

Predatory bacteria can attack human pathogens

B. bacteriovorus 109J host range specificity

B. bacteriovorus was able to prey, attack and reduce 117 of the 135 examined bacteria in both single and multi-species culture suspension (representing 20 different genera).

Predatory bacteria attacked 99% of drug resistant *A. baumannii* bacteria isolated from Wounded Warriors.

Dashiff *et al*., 2011. PMID 21114596

B. bacteriovorus 109J host range specificity

-Acinetobacter

A. baumannii

-Aeromonas

-Aggregatibacter

-Bordetella

B. bronchiseptica

-Burkholderia

B. cepacia

-Citrobacter

-Eikenella

-Erwinia

-Enterobacter

E. aerogenes

-Escherichia

E. coli

Fusobacterium

-Klebsiella

K. pneumoniae

-Morganella

-Proteus

-Pseudomonas

P. aeruginosa

-Salmonella

-Shigella

S. sonnei

-Serratia

S. marcescens

-Vibrio

V. cholerae

-Yersinia

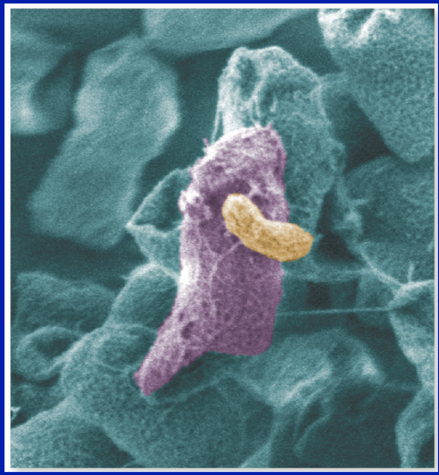
Y. pseudotubercu

Dashiff *et al*., 2011. PMID 21114596

M. aeruginosavorus ARL-13 host range specificity

Micavibrio was able to prey, attack and reduce 145 of the 177 examined bacteria grown in culture suspension (Representing 6 different genera).

Micavibrio was able to prey, attack and reduce 131 of the 168 examined bacteria grown as biofilms.



Dashiff *et al*., 2011. PMID 21114596

M. aeruginosavorus prey range specificity

<u>Good predation capability (3-4 log)</u>

Burkholderia
**Escherichia*
**Klebsiella*
Pseudomonas
**Shigella*
**Yersinia*

<u>Moderate predation capability(0.5-2 log)</u>

Acinetobacter
**Enterobacter*
**Proteus*

<u>No predation</u>

Bordetella
**Citrobacter*
Enterococcus
**Erwinia*
**Morganella*
Mycobacterium
**Serratia*
Stenotrophomona
Vibrio

**Enterobacteriaceae* family

Dashiff *et al.*, 2011. PMID 21114596

molecular oral microbiology

molecular oral microbiology

Predation of oral pathogens by *Bdellovibrio bacteriovorus* 109J

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ORIGINAL ARTICLE

Predation of human pathogens by the predatory bacteria *Micavibrio aeruginosavorus* and *Bdellovibrio bacteriovorus*

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Predatory bacteria can prey on multidrug-resistant Gram-negative human pathogens

OPEN ACCESS Freely available online

PLOS | ONE

Predatory Bacteria: A Potential Ally against Multidrug-Resistant Gram-Negative Pathogens

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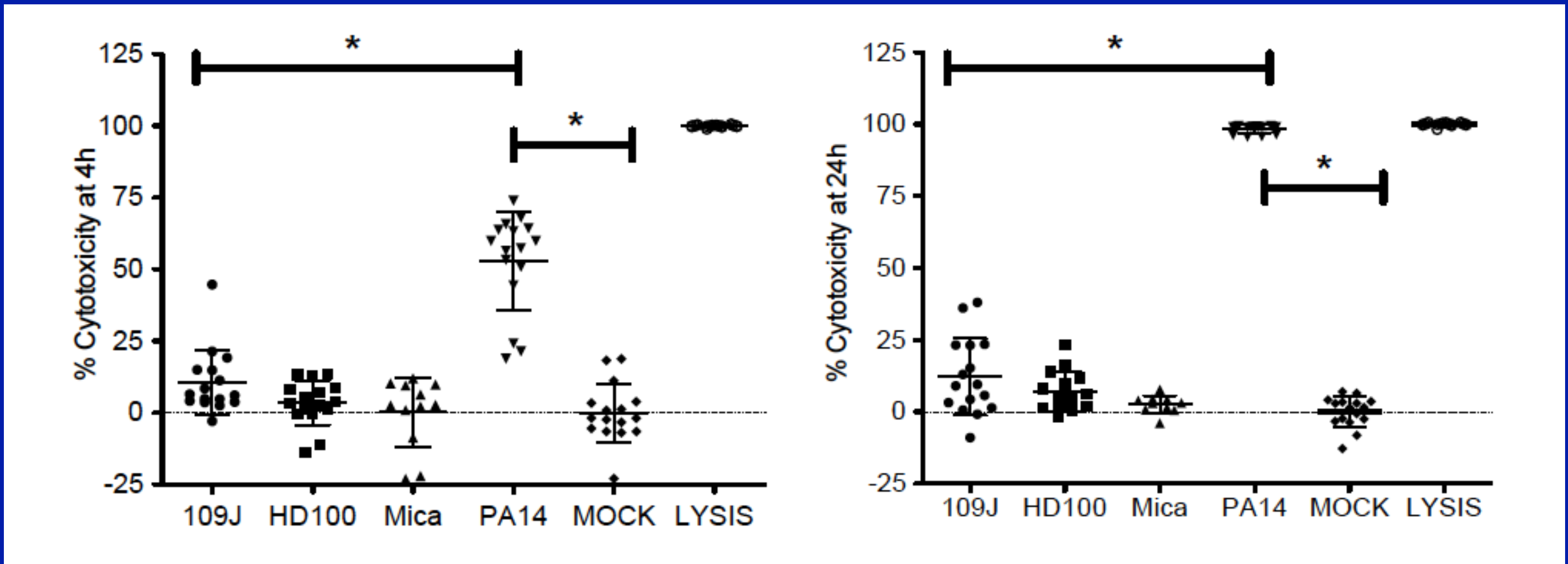
The effect of predatory bacteria on eukaryotic cells, *ex-vivo* models

Cytotoxicity of predatory bacteria on human corneal-limbal epithelial (HCLE) cells

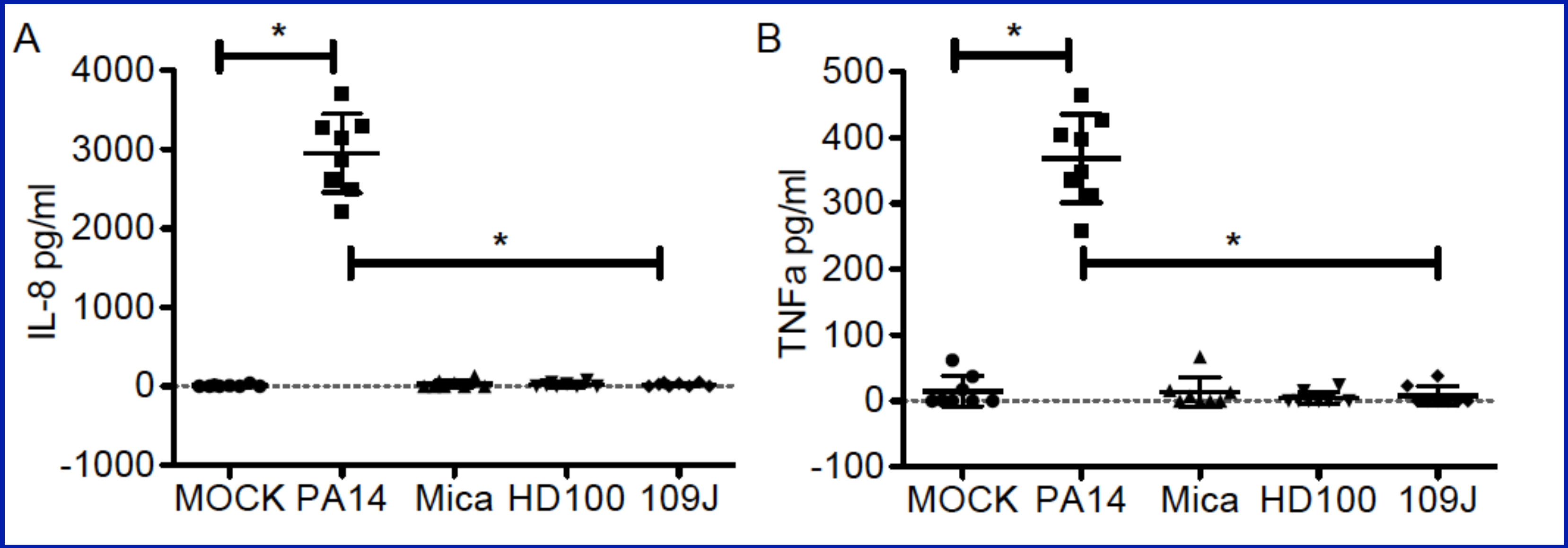
4 hr Exposure **24 hr Exposure**



Average predatory bacteria in well ~1x10^8 PFU/ml
Average *P. aeruginosa* PA14 in well ~5x10^7 CFU/ml

Shanks *et al*., 2013. PMC 3688930

Inflammatory response of human corneal-limbal epithelial (HCLE) cells to predatory bacteria



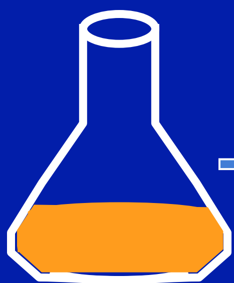
Average predatory bacteria in well ~1x10^8 PFU/ml
Average *P. aeruginosa* PA14 in well ~5x10^7 CFU/ml

Shanks *et al*., 2013. PMC 3688930

The effect of predatory bacteria in *in-vivo* models

Galleria mellonella model for microbial pathogenesis

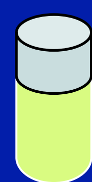
Experimental procedure



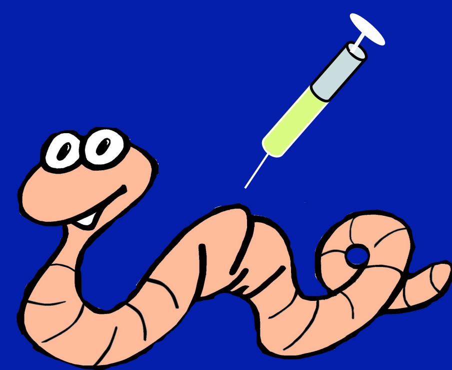
Grow the predators

0.45μm

Purified predators

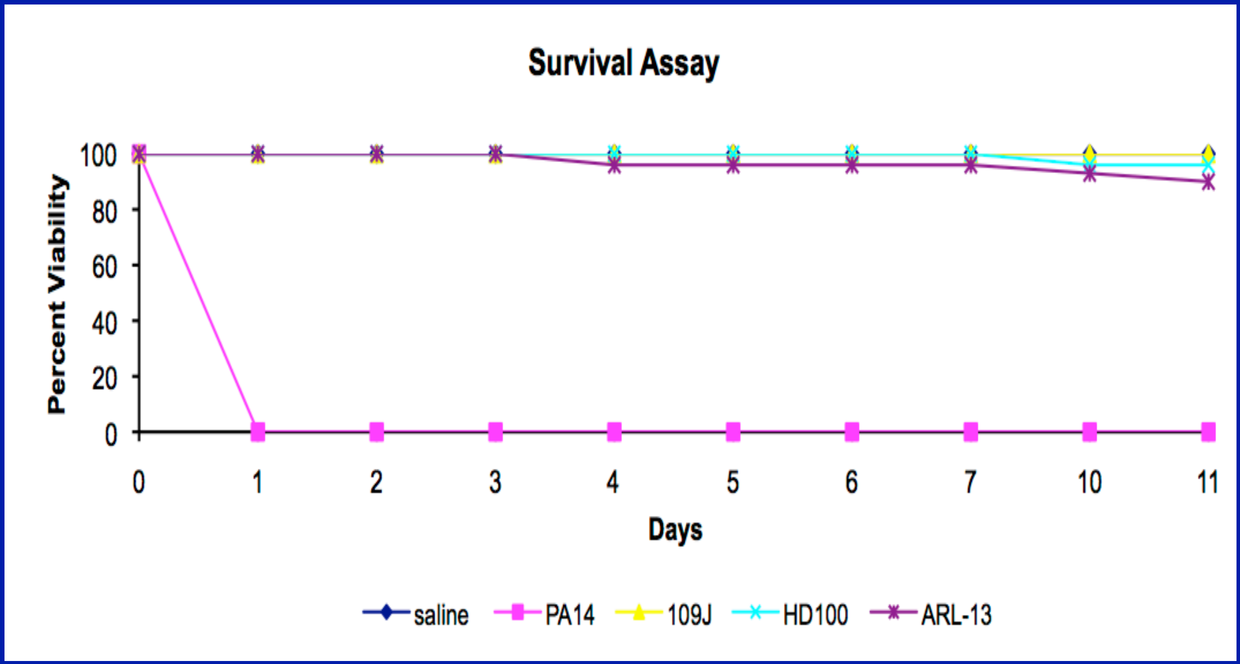


Cells were washed and resuspended in 10mM saline



G. mellonella caterpillars in the final larva stage were used in this experiment. A 10 μl Hamilton syringe, with a 30.5 gauge needle, was used to inject 5 μl aliquots of each inoculum into the hemocoel of each larva.

Predatory bacteria are non-virulent in *Galleria mellonella*



P. aeruginosa PA14 10^3 CFU/worm
Predatory bacteria 10^7 PFU/worm

Shanks *et al*., 2013. PMC 3688930

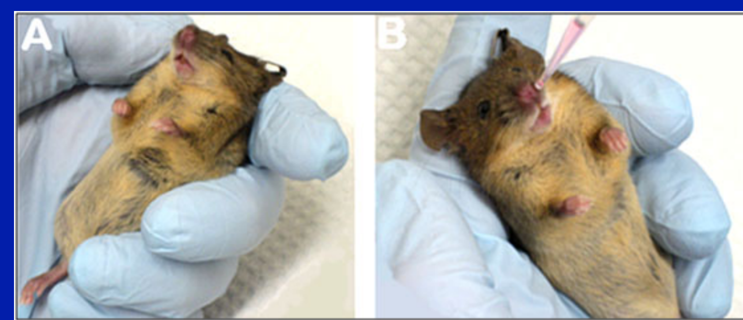
Determining the toxicity of predatory bacteria in a mouse respiratory model

Experimental procedure

Grow the predators

0.45μm

Purified predators



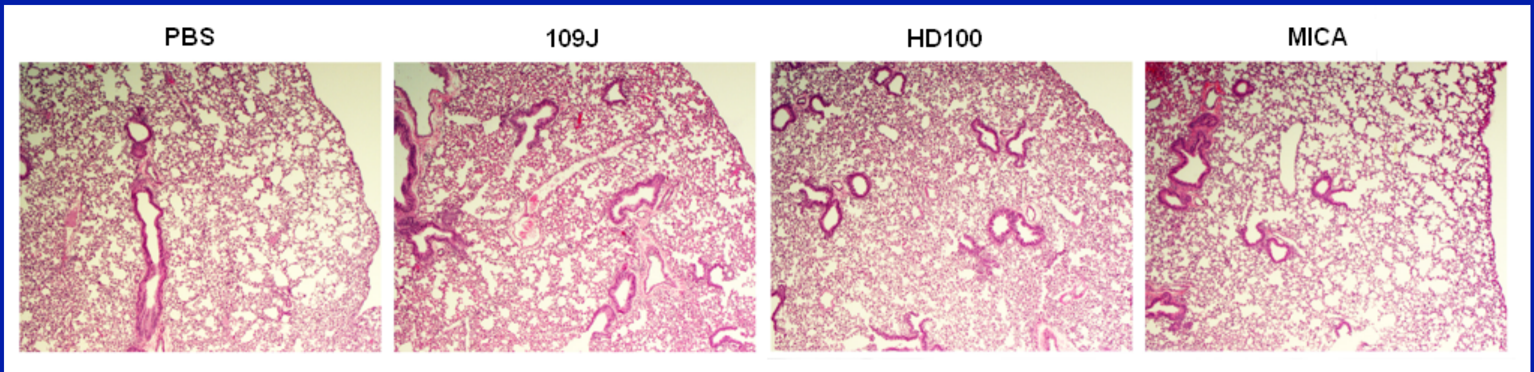
C57BL/6 mice were used.
Intranasal inoculation of bacterial suspension.

Determining the toxicity of predatory bacteria in a mouse respiratory model

Mice viability after intranasal inoculation of predatory bacteria.

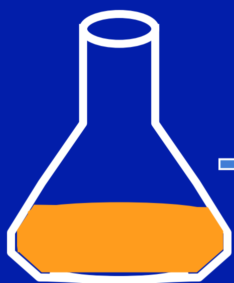
Treatment	# of Mice	% Viable on Day 5	% Viable on Day 50*
Control (PBS)	5	100%	100%
B. bacteriovorus 109J	5	100%	100%
B. bacteriovorus 109J (HK)	5	100%	100%
B. bacteriovorus HD100	5	100%	100%
B. bacteriovorus HD100 (HK)	5	100%	100%
M. aeruginosavorus ARL-13	5	100%	100%

Average *Bdellovibrio* used ~1x10^9 PFU/mouse.
Average *Micavibrio* used ~1x10^6 PFU/mouse.



Determining the toxicity of predatory bacteria
Intravenous inoculation

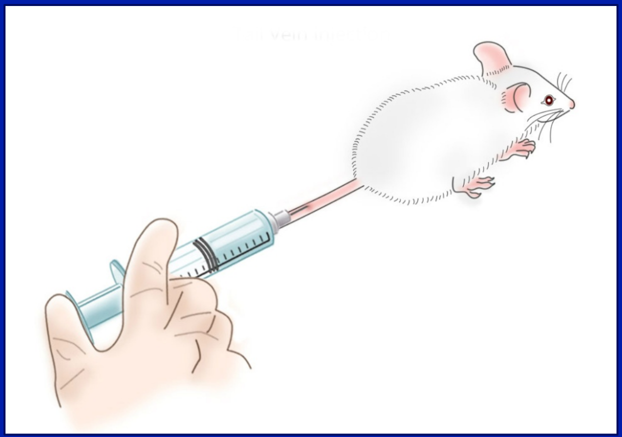
Experimental procedure



Grow the predators

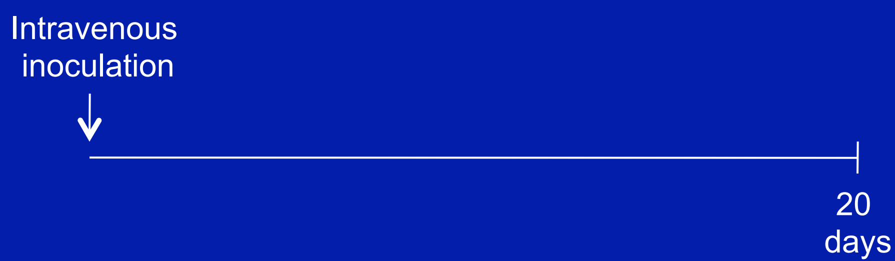
$0.45\mu m$

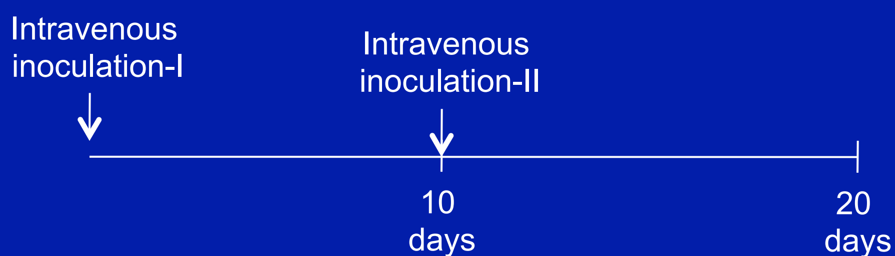
Purified predators



C57BL/6 mice were used. Intravenous inoculation of bacterial suspension.

Determining the toxicity of predatory bacteria intravenous inoculation

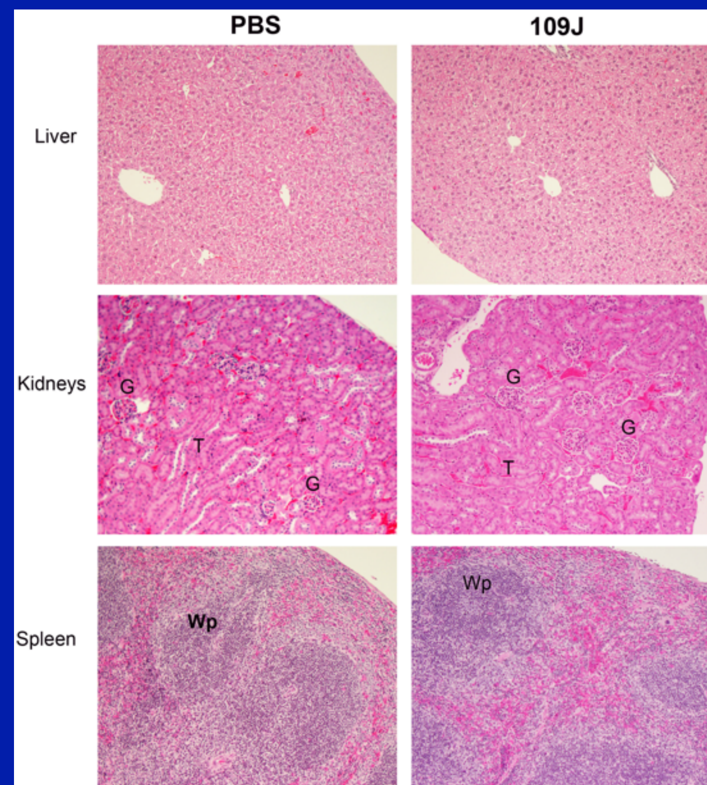




Mice viability after intravenous inoculation of predatory bacteria.

Treatment	# of Mice	% Viable on Day 20
Control (PBS)	5	100%
– Re-inject at 10 days	5	100%
B. bacteriovorus 109J	5	100%
– Re-inject at 10 days	5	100%

Average *Bdellovibrio* used ~$1x10^8$ PFU/mouse.



Images are taken at 20 days post-injection

Applications

•**Therapy**: Treating infection in humans and livestock. Delivered as a live antibiotic or probiotic (urinary-tract infection, CF, wounds and burns).

•**Food industry**: Equipment cleaning, *Bdellovibrios* can prey on many human food-born pathogens such as *E.coli, Salmonella.*

•**Water purification**
•**Industry**
•**Agricultural**
•**Aquaculture**
•**A source for novel enzymes**

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