Magnetic Cell Capture for Isolation of Microorganisms from Natural Samples

Quintin Davis and Dr. Naomi L. Ward

Overview

- Background: The challenge of isolation
- The Application: Detecting and predicting unique pathogenic potentials
- Magnetic Cell Capture: Current techniques and uses
- Improving MCC protocols for increased yield, decrease possibility of false positives.

Tried and True, Isolation by Culture…

- First culture media were undefined broths developed by Spallanzani and refined by Pasteur
  - No way to isolate individual organisms from each other
- Solid media needed to isolate clonal colonies
- First solid media: boiled potato slices
  - Even at early stage evident to Koch that many microbes were not growing. [3]

…But Not That True

- It's estimated that only about 2% of environmental bacteria can be cultured with current techniques[2]
- Some of these are important!
    - e.g. *Treponema pallidum*, causes syphilis
- Culture of slow-growing bacteria
- PCR, molecular techniques allow study of unculturable bacteria.
    - But how can we apply Koch's postulates without culturing?

Koch's Postulates

- The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy animals.
- The microorganism must be isolated from a diseased organism and grown in pure culture.
- The cultured microorganism should cause disease when introduced into a healthy organism.
- The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.
    - -aka-
- Must be present when disease is cause and cause disease when present.

RVA+Magnetic Cell Capture

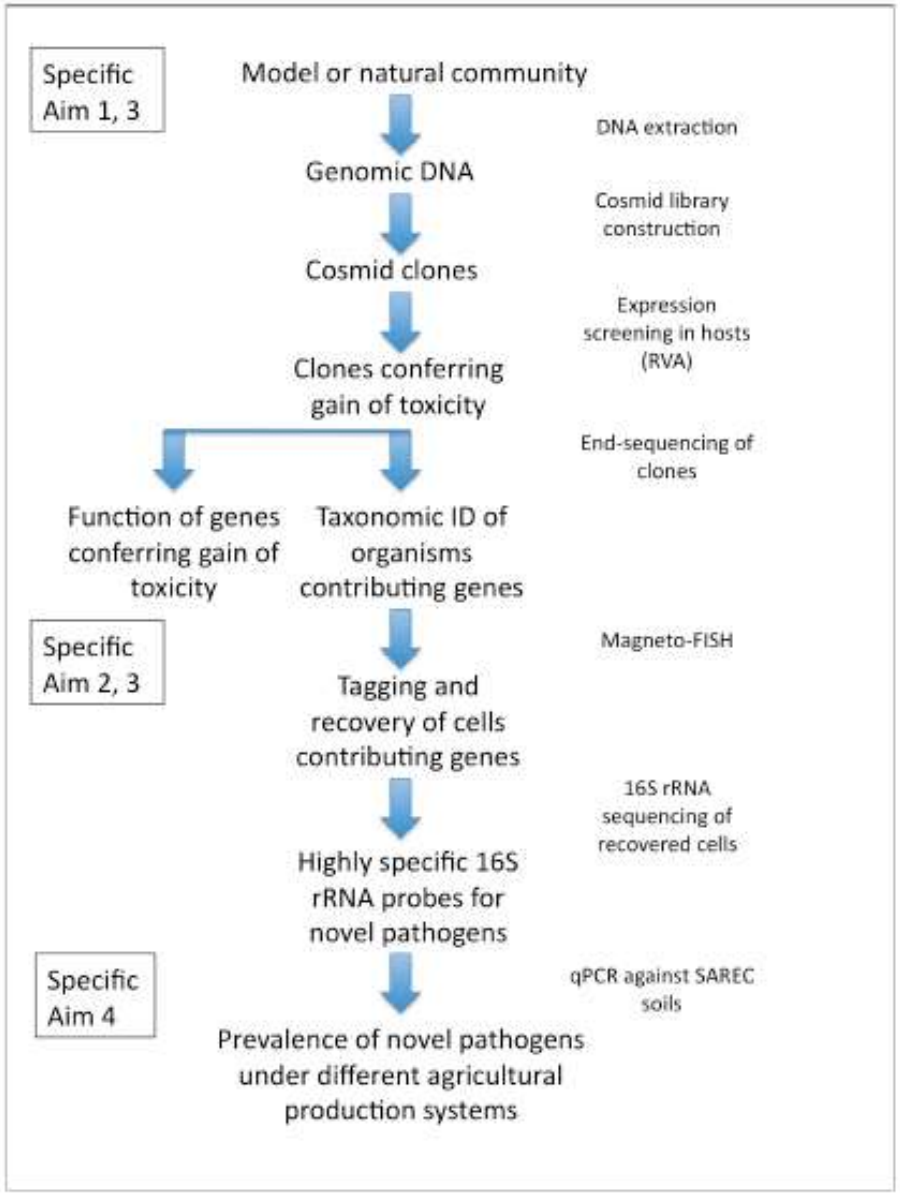
- RVA: Rapid Virulence Annotation
- Break up DNA and insert into ~30 kb cosmids
- Express in *E. coli,* grow colonies in microtiter plates
- Introduce model organism, incubate.
- Determine gain of toxicity in *E. coli* expressing cosmid DNA fragments.

Specific Aim 1: Develop a functional screen for virulence genes using a model of mixed microbial cultures.

Specific Aim 2: Recover cells from the model system using a magnetic tagging approach

Specific Aim 3: Apply the functional screen and cell recovery approach to characterize both recognized and novel pathogens in natural microbial communities from agricultural soils.

Specific Aim 4: Determine the prevalence of tagged pathogens in soils subjected to different production approaches.

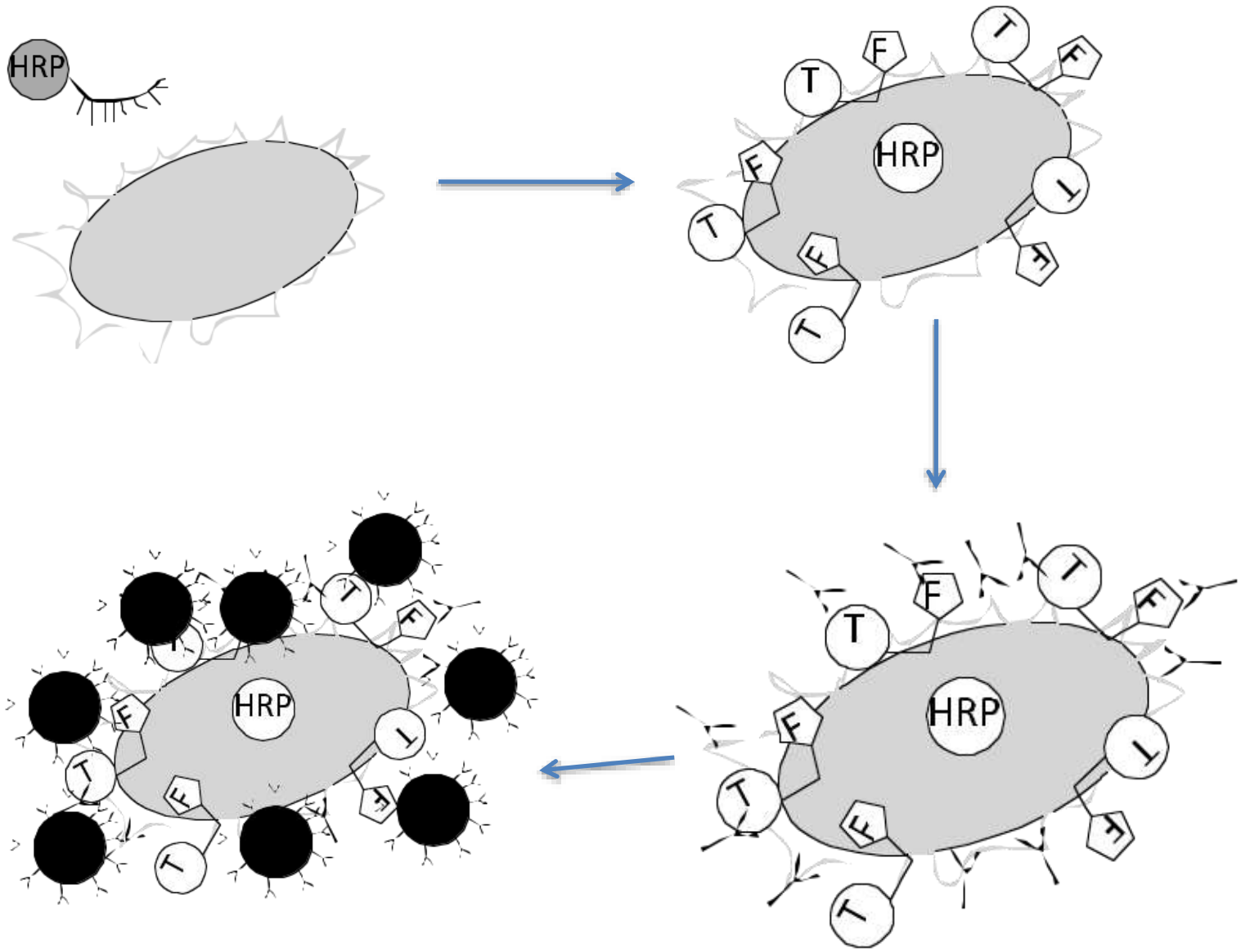


Magneto-FISH

- Capture and isolate desired organisms with antibody-labeled magnetic beads.

Magnetic Cell Capture

- Pernthaler *et al* describe the isolation of extremophilic syntrophies from deep sea methane vents [reference here] using magneto-FISH
- FISH→ fluorescent molecules bound to specific DNA probes tag desired genes or organisms.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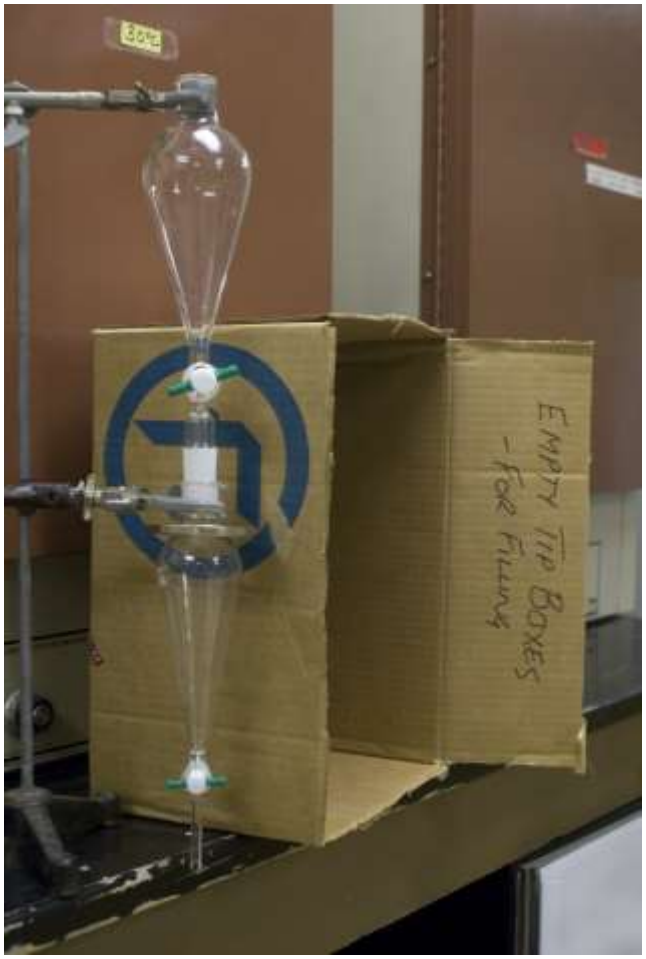

EMPTY TIP BOXES
- FOR FILLING


g
B
M
A

Experimental

- Yields of separatory funnel technique and glass tube w/ two diameters (~3/8” and ~1/4

Precipitation of paramagnetic microparticles

- The antibody coated magnetic beads sold for magnetic cell separation are expensive, sold in volumes too small for calibrating yields
- So . . .

  - $Fe(II)SO_4 + 2Fe(III)Cl_3 + 8OH^- \rightarrow Fe_3O_4 + 4H_20$

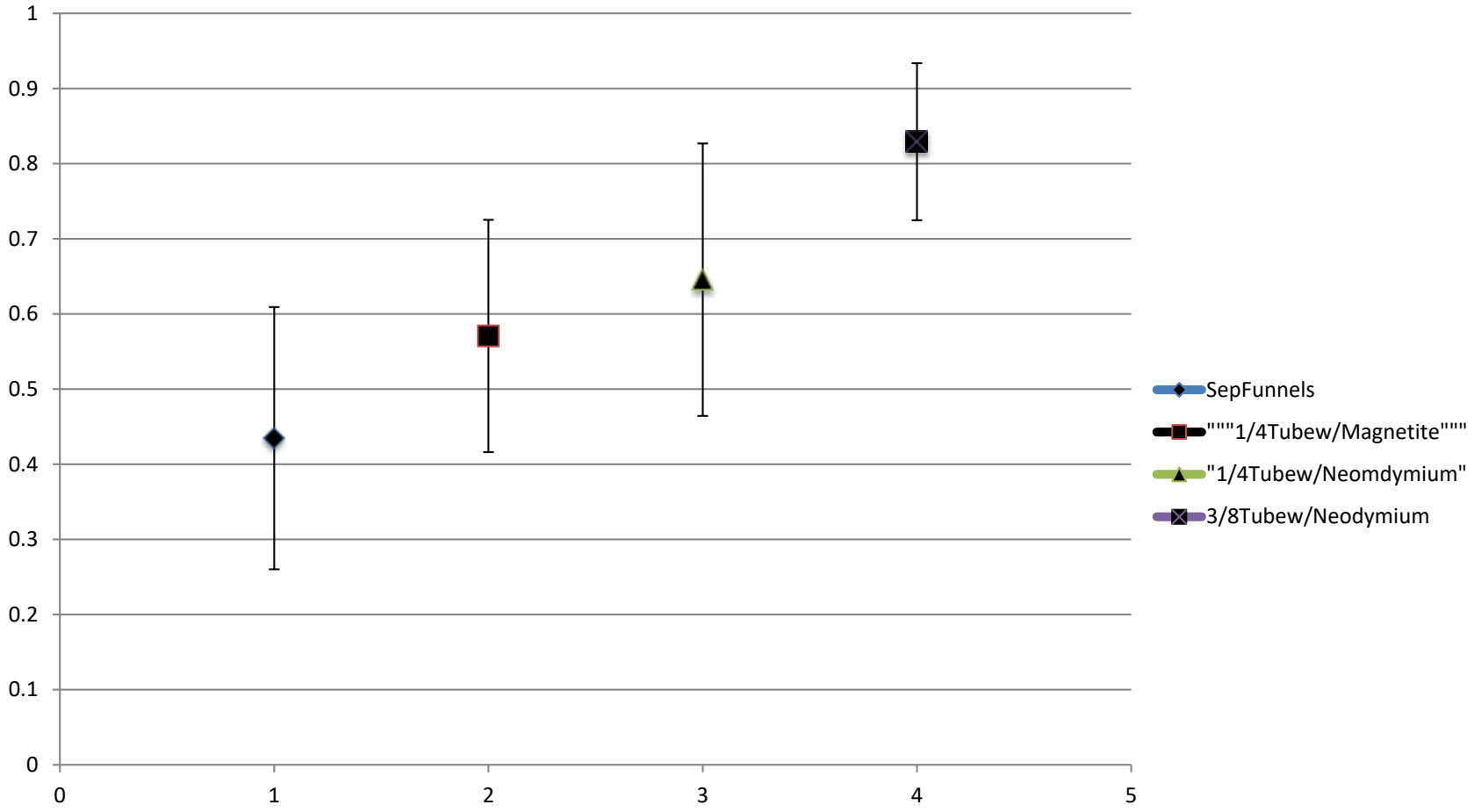
- Adopted iron oxide coprecipitation protocol from [2] for manufacture of paramagnetic microparticles.

Protocols

- Fill funnel A with buffer
- Place magnet on top step of A
- Add sample to A
- Place funnel B on top and fill with buffer
- Flow rate is controlled by bottom valve
- Magnetic-tagged sample pulled to side, wash with buffer in funnel B.
- Empty and rinse funnel A
- Add recovery buffer, tip funnel and collect sample
- Spin down and quantify recovered sample

From Pernthaler *et al* 2007

Iron Microparticle Recovery for Various Magnetic Capture Protocols



Results cont’d

	Mean (%)	Standard Deviation (%)	Sample Number (N)
Seperatory Funnels	43.5	17.5	12
¼” Tube	60.1	16.6	10
3/8” Tube	82.9	10.4	6

Conclusions

- By decreasing the diameter (to a point) of the vessel used for magnetic cell capture and creating a one-way flow, yields can be significantly improved. With potentially low cell counts and the expense of antibody-tagged magnetic beads, this creates significant advantages for cell capture protocol goals
- In order to further quantify efficiencies for different mag. Cell capture apparatuses, prevalence of false positives needs to be investigated.

Acknowledgements

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References

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