Cytotoxicity assays with the ciliate *Tetrahymena pyriformis*

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Toxin production by a new microbe, how can we know?

- Identify and test all metabolites
- Look for known toxins
- Test for production of other toxic metabolites that we do not yet know about?

*Means that we have to test the living microorganisms or homogenates*

Our object with *Tetrahymena* assays:

To design a method for early screening: Does the microbe of interest produce toxic compounds?
Why *Tetrahymena pyriformis*?

- Phagotrophic ciliate, also osmotrophic growth on dissolved media

- Previously used for toxicity testing of organics, metals etc. ‘Tetratox’

- Several possible end-points:
  - Growth rates
  - Viability: live/dead staining, morphometry
  - Grazing activity

- BACTOX assay, qualitative
  1. Resuspended bacteria mixed with ciliates
  2. Proportion live/dead estimated by visual inspection
Results BACTOX

- Higher response from spent media than washed bacteria, implies soluble compounds caused the effect

- Low resolution; strong or no effects

- Response not correlated with life-style; plant or mammalian pathogens
‘New’ approach *Tetrahymena*

- Determine effects of *dissolved* substances under controlled conditions

- Effects of known toxins *in extracts from microorganisms*?  
  Models: general cytotoxins

- Effects of known microbial toxins *in extracts from microorganisms*?  
  Models: mycotoxins

- Look at effects on growth rate: High through-put systems for absorbance
Results *Tetrahymena* growth rates

- Control
- Ethanol
- Cycloheximide 0.1 mg/L
- Cycloheximide 0.5 mg/L
- Cycloheximide 1 mg/L

- Plate reader, 24 wells
- Model toxin, cycloheximide
- Solvent: ethanol
- Clear effect on growth rate and final yield
Results *Tetrahymena* growth rates

- Final absorbance, i.e. growth yield
- Clear effect on yield

Next: look at effects of cycloheximide in extracts of *Fusarium* and *Aspergillus*
Questions to be resolved

• What types of microbial toxins can be detected?

• Susceptibility to problematic microbes, i.e. level of discrimination between ‘friendly’ and ‘dangerous’ organisms?

• Which are the most relevant extraction/homogenisation methods?

• Can one toxicity assay detect all relevant toxins?
Thanks!

The *Tetrahymena* studies are part of the safety related work in:

Research programme DOM - Domestication of microorganisms
http://www.mistra.org/dom

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