

Bioprospecting: Uniqueness of Antarctica and potential for commercial success

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Bioprospecting in Antarctica

- Definition Bioprospecting
- Uniquely Antarctic
- Current Work Worldwide
- Current Work University of Waikato & K021
- Potential Commercial Success

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Defining Bioprospecting

“The search for commercially valuable biochemical and genetic sources in plants, animals and microorganisms. These resources may be used in food production, pest control, the development of new pharmaceuticals and other biotechnological applications.”

Dean Peterson, ANZ

“Scientific research that looks for a useful application, process or product in nature.” US National Park Service

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Defining Bioprospecting

- Applied Science in Biodiversity
- Biodiversity is the abundant variation in the types of all living organisms taken together in any geophysical area

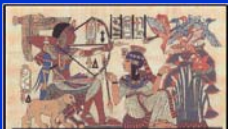
Smithsonian Institution Antarctic collection includes 97, 599 specimens of catalogued invertebrates, 74,996 uncatalogued specimens, 213, 492 specimens on loan to researchers!

- Consequences of biotechnology, bioinformatics revolution - the post-genome era

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Biocatalysis and Bioprospecting

BioSci 352 August 2002
David Saul



“You are the one who soaks the malt in a jar
The waves rise, the waves fall.
Ninkasi, you are the one who soaks the malt in a jar
The waves rise, the waves fall.”

You are the one who spreads the cooked
mash on large reed mats. Coolness overcomes.
Ninkasi, you are the one who spreads
the cooked mash on large reed mats,
Coolness overcomes.

You are the one who holds with both hands
the great sweet wort,
Brewing [it] with honey and wine
(You the sweet wort to the vessel) Ninkasi, (...)
(You the sweet wort to the vessel)

The filtering vat, which makes a pleasant sound,
You place appropriately on [top of] a large collector vat.
Ninkasi, the filtering vat, which makes a pleasant sound,
You place appropriately on [top of] a large collector vat.

When you pour out the filtered beer of the collector vat,
It is [like] the onrush of Tigris and Euphrates.
Ninkasi, you are the one who pours out the
filtered beer of the collector vat,
It is [like] the onrush of Tigris and Euphrates.”

The Hymn to Ninkasi, inscribed on a
nineteenth-century B.C. tablet - contains a
recipe for Sumerian beer

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Defining Bioprospecting

- Inherently, the perception exists of exploitation, pollution of the environment
- Sustainable, or removal with no replenishment
- Linkage to prospecting/farming/harvesting
 - “Go on exploring expedition, institute search, (esp. for gold)”

The Oxford Dictionary

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Who are Bioprospectors?

- Sometimes scientists, governments, commercial firms identify a need for a certain 'activity'/biochemical/substitute chemical and look for it in nature
- Just as often, a scientist on a strictly academic project suddenly notices a valuable use for what started as curiosity-driven research

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Commercial bioprospecting

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Bioprospecting: Discoveries changing the future.

"The potential for building new industries on the discoveries made from biological resources is huge... With 10-13 per cent of the world's biodiversity, Australia is one of the 12 most diverse regions in the world".



Geneva, 26 March -- A bio-prospecting deal between the US National Park Service and a private corporation, Diversa Corporation, enabling Diversa to "harvest", patent and commercialise micro-organisms and other resources of thermal springs in Yellowstone National Park has been "suspended" by a court order in Washington DC.

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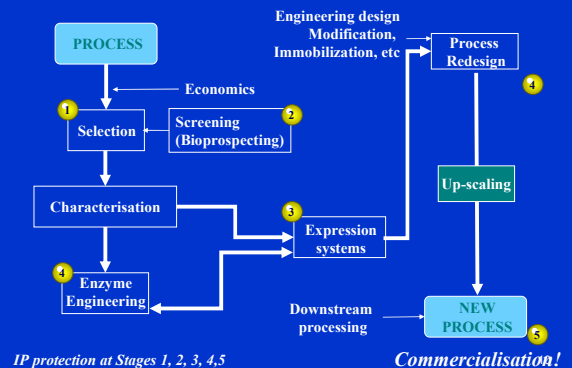
Key stages of Bioprospecting Dean Peterson

1. Discovery – collection, screening, describing
2. Protection of Intellectual Property
3. Product Development – isolation, purification, modification to improve efficacy, clinical testing
4. Manufacturing – production, shelf life stability
5. Marketing – registration, sales

COMMERCIAL RETURN ON INVESTMENT- RLF

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Searching for the ideal Molecule



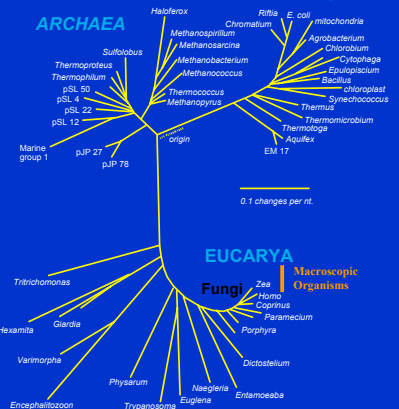
Scope of Bioprospecting

- Plants, Animals, Microorganisms
- Terrestrial, Marine
- Bioprospecting biomass
 - Genes without organisms
- Whole Organism - *in vivo* Biocatalysis
 - Brewing
 - Antibiotics
- Purified Enzyme and Bioactives - *in vitro* Biocatalysis
 - Food enzymes, Detergents
 - Pharmaceutical biotransformations

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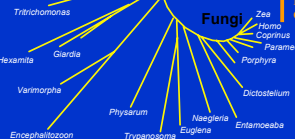
ARCHAEA BACTERIA

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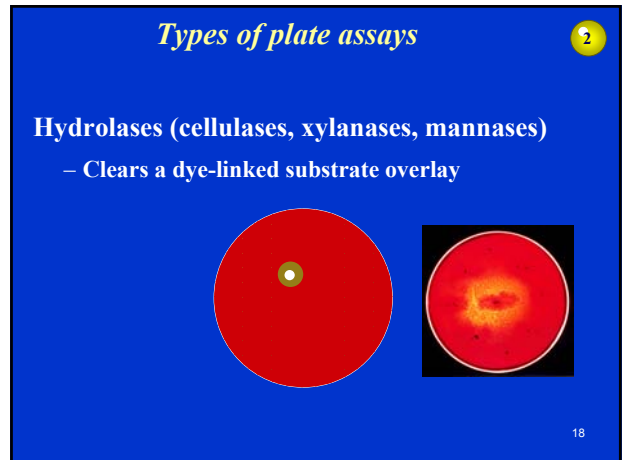
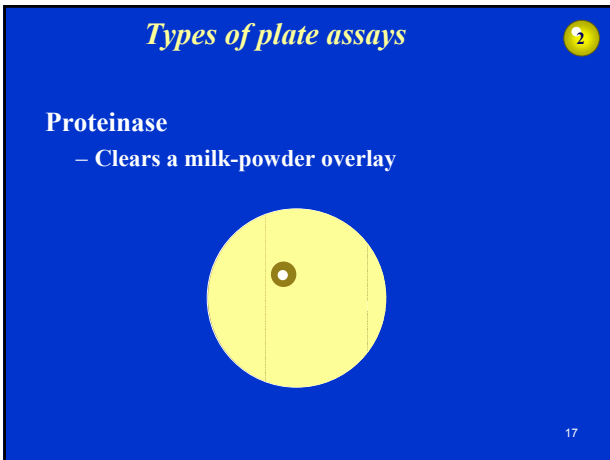
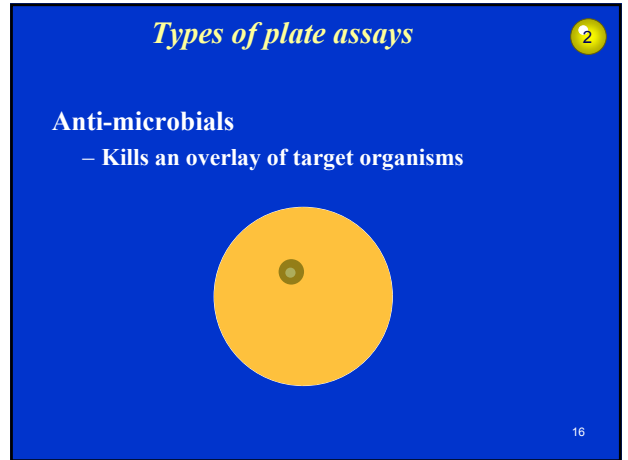
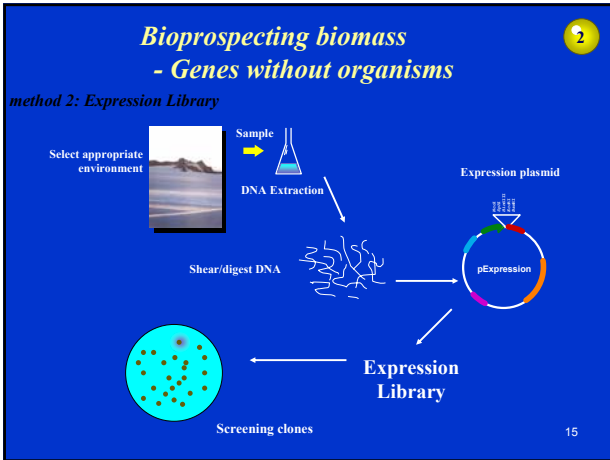
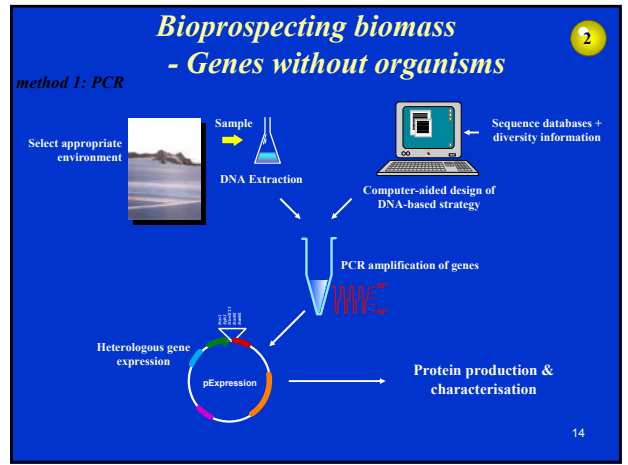
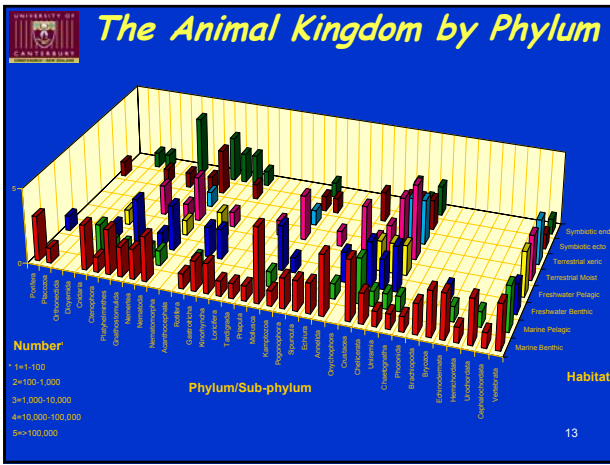


EUCARYA

Fungi Macroscopic Organisms



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Uniquely Antarctic

If it can be found elsewhere do it elsewhere!

"I could not say that we would not isolate the same organisms elsewhere - we have isolated new bugs, but I could not guarantee, or even comment on, whether or not they are unique to Antarctica. My impression is that true psychrophiles require permanently-cold environments in which to survive if they are not spore-formers. This is different from thermophiles, many (?) of which can survive outside their hot environments even without forming spores. This leads me to think that there are Antarctic-unique microbes, but the work required to do this has not been carried out, and would be very difficult to prove conclusively."
Michael Danson

Issues:

Antarctic Treaty, environment, logistics, economics

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Find a source of organisms

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Geothermal Spring



Cold Environment



Industrial Plant

Match environment with desired parameters...

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Scope of Bioprospecting Why Antarctica?

- Interest for biotechnology as biota are poorly known – potential novelty.
- Environmental extremes: cold, aridity, extremely salty, unusual maritime lakes etc

Habitat	Defining Condition	Bioprospecting Opportunities
Seawater, maritime lakes sea-ice	Low temperature	Cold active enzymes/biocatalysts; bioremediation; anti-freezes; polyunsaturated fatty acids, novel pigments
Southern Ocean seawater	Low nutrient concentration	High affinity catalysts & ligands
Hypersaline lakes	High salinity	Halotolerant enzymes; novel metabolites; novel pigments
Marine/lake sediment	Anaerobic/low temperature	Anaerobic biotransformations; Novel bioactives
Soil, lithic habitats	Cold, dry	Novel bioactives

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Antarctica a Global 'Hot Spot': Biodiversity and Biotechnology Dr John P Bowman

Fragility of Plants: Growth Rate

This patch was sampled in 1980

Virtually no regrowth in 20 years plus

Courtesy Prof. Allan Green, 2003



Henediella heimii,
Canada Glacier Flush

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Current Work Worldwide

- Antifreeze Glycoprotein "one of the leading success stories of Antarctic biology" John C. Montgomery
- AFGP arose from conversion of trypsinogen gene
- Protein evolution, organismal adaptation and environmental conditions are linked
- Applications – pharma, agricultural to cement

EVOLUTIONARY RADIATION of ANTARCTIC NOTOTHENOIDS

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Current Work Worldwide

- Antarctic larvae monitoring changes in climate
- Exopolysaccharide of Antarctic fungus *Phoma herbarum* (Selbmann and Onofri)

Patents:

- Highly specific lipase variants from *C. antarctica* (Novo Nordisk)
- Anti-Freeze peptides incorporated in frozen food products – from *Pseudomonas sp* and *Marinomonas protea* (Laybourne-Parry)
- Cosmetic Skin treatment – an extract from the green alga *Praireola crispa ssp. antarctica* (Henkel)
- Re-epithelialisation of wounds and treating skin, hair and nails – glycoprotein from *Pseudoalteromonas antarctica* (Casaroli Marano et al)

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Current Work Worldwide

Antarctic Microbiology Group University of Tasmania

- Polyunsaturated fatty acids & cold active enzymes produced by psychrophilic bacteria
- Pigments formed by sea-ice bacteria
- 1995 Antarctic CRC made commercial agreement - Cerylid Ltd.
- Bioprospecting for novel, pharmaceutically-active compounds for new medicines
- \$1 million industry funding for Antarctic microbiology and biotechnology since 1997.

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Current Work Worldwide

University of Canterbury Murray Munro

- Series of bioactive metabolites the variolins from the Antarctic sponge *Kirkpatrickia variolosa* - complex new heterocyclic skeleton.
- Derivatives patented by the Spanish pharmaceutical company PharmaMar - 100 analogues have now been synthesised and are currently being assessed in vivo.



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The Variolins

- Potent apoptosis-inducing agent
- Under development by PharmaMar SA
- Analogue syntheses completed (UC & PharmaMar)



Variolin B

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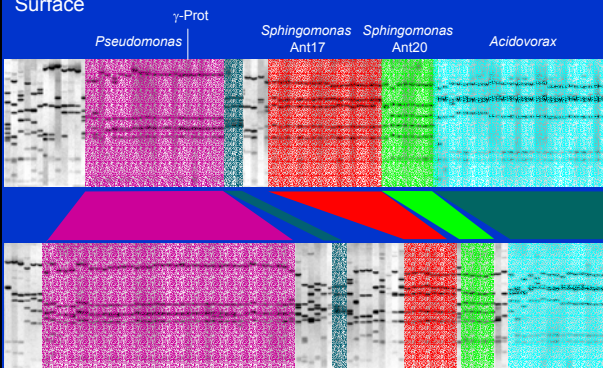
Microbial Diversity in Oil Contaminated and Pristine Antarctic Soils



David Saul,
Jackie Aislabie
Lisa Harris

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Surface



Sub-Surface

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Current Work University of Waikato

K023 Roy Daniel and Hugh Morgan



- A thermophilic *Bacillus* (strain EA1) isolated on first expedition to Erebus in 1980, part of a study of the worldwide distribution and characteristics of thermophilic bacteria



- As a result of studies on protease secretion by the Waikato Thermophile Unit in the early 1990s, EA1 was quite recently selected for an applications study, and its protease now patented (2002) for a forensic analysis

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Current Work University of Waikato

Don Cowan with Craig Cary courtesy Allan Green, Roy Daniel

Molecular phylogenetic studies prokaryotes in Dry Valleys
Terrestrial biotopes – field based
Cold-active enzymes

Dry Valleys not only possible option – need exposed gravels, preferably suitable for transect analysis
gradients of water content, salinity, C/N content



“We would like to extend this substantially, looking at the effects of various environmental variables on microbial diversity. Want to have a portable wannigan into the field and set up a proper molecular biology lab to focus much more on species-specific PCR (and even DGGE and BrdU labeling, in situ).”

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Transect Analysis

Sampling:

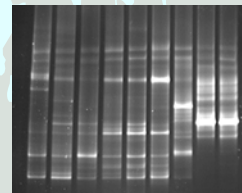
1. 9 STATIONS - 200M - 850M
2. Moisture content
3. ATP analysis
4. Molecular profiling

Results to date:

- Biomass decreases with elevation and moisture content
- Molecular communities remarkably diverse throughout transect
- Dominant phylotypes



1 2 3 4 5 6 7 8 9



Current Work University of Waikato

Michael Danson courtesy R Farrell, R Daniel

Collaborating Companies Reckitt-Benckiser, GlaxoWellcome Viridian.

Target psychrophiles and/or cold-active enzymes; organisms for bioremediation and gas recovery, cytolytic, bacteriolytic and fungolytic activities.



Bioreactor of inert and porous ceramic beads allow microbial colonisation and cultivation. Automated, rapid enzyme and bioproduct screening procedures developed to complement bioreactor facility.

Proteases now going for gene cloning and expression.
If successful, recombinant enzymes tested in cleaning applications.

From oil-contaminated soil at Cape Evans, isolated oil-degraders

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Current Work K021

Roberta Farrell & Robert Blanchette NSF BO-038-0

Identify causes of biological and non-biological deterioration in Historic Huts and artefacts

Test conservationally acceptable materials

Identify terrestrial micro-organisms historic sites, fuel spills and sites of ancient forests ...

Compare to global isolates, establish viability and biochemical responses of these organisms, bioprospect.



Spores Discovery Hut

Supply box – Cape Evans Hut

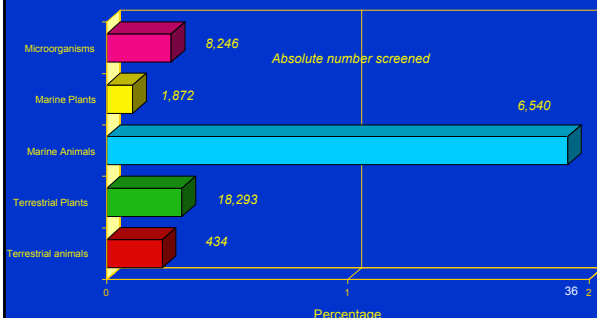
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Potential Commercial Success



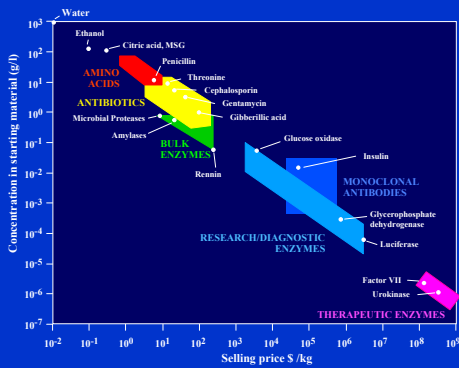
Probability of a "Hit"

"Hits" = Samples × "Biodiversity" × Assays



Concentration in starting material of various substances and their selling prices

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Microbial expression hosts

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ORGANISM	TYPE	TYPICAL PROTEIN	AMOUNT / LITRE
<i>Escherichia coli</i>	Bacterium	xylanase	50 – 150 mg
<i>Bacillus subtilis</i>	Bacterium	proteinase	200 – 300 mg
<i>Kluyveromyces lactis</i>	Yeast	xylanase	200 – 300 mg
<i>S. cerevisiae</i>	Yeast	carboxypeptidase Y	100 – 400 g
<i>Trichoderma reesei</i>	Fungus	collobiohydrolase Proteinase	up to 24 g 500 mg – 5 g
<i>Aspergillus niger</i>	Fungus	phytase Acid phosphatase	2 g 0.5 g

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Example of industrial process Pulp bleaching with Xylanase

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- 1 g of enzyme required to bleach 1 tonne of pulp
- An average mill process 8000 tonnes of pulp per day
8 kg enzyme per day
- Cost must not exceed US\$4.50 / tonne
- Enzymes must be produced at less than US\$2.25 per gram
- Yeast system can produce 0.3 g / L
26,400 L / day
- *Trichoderma* system can produce 5 g / L
160 L / day
- Cost of enzyme = 8,000 X \$4.50 = US\$36,000/day

Summary

- Bioprospecting activities in Antarctica are increasing
- Unculturable biomass offers a 100-fold increase in genetic resource. Bioprospecting by PCR or expression libraries can access this resource
- Funding for basic research is crucial to deliver research outcomes necessary for commercialisation
- Issues of access, ownership and benefits must be defined and hopefully standardised

Acknowledgements

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 Dr Dean Peterson and Shulamit Gordon, ANZ



Logistic Support by Antarctica New Zealand

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