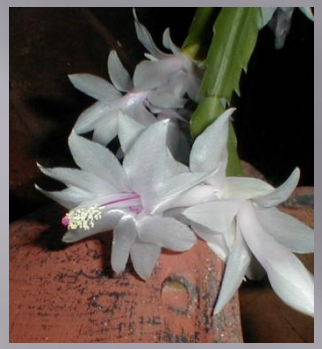
ACTINOGEN Ltd ANNUAL R&D REPORT 2009-2010

David Keast



INTRODUCTION

Actinogen isolates a group of gram positive bacteria mainly from the soils of Western Australia.

These bacteria are unique in that they have a wide ranging ability to mobilise metabolic pathways and to utilise many unusual sources of nutrients. They produce many bioactive molecules of use to man including antibiotics, anti fungal agents, anti cancer agents as well as isolates that are useful in agriculture, mining and bioremediation processes.

STAFF & FUNCTIONS

- 2009-2010
- **3 FULL TIME STAFF**

  3 PART TIME STAFF

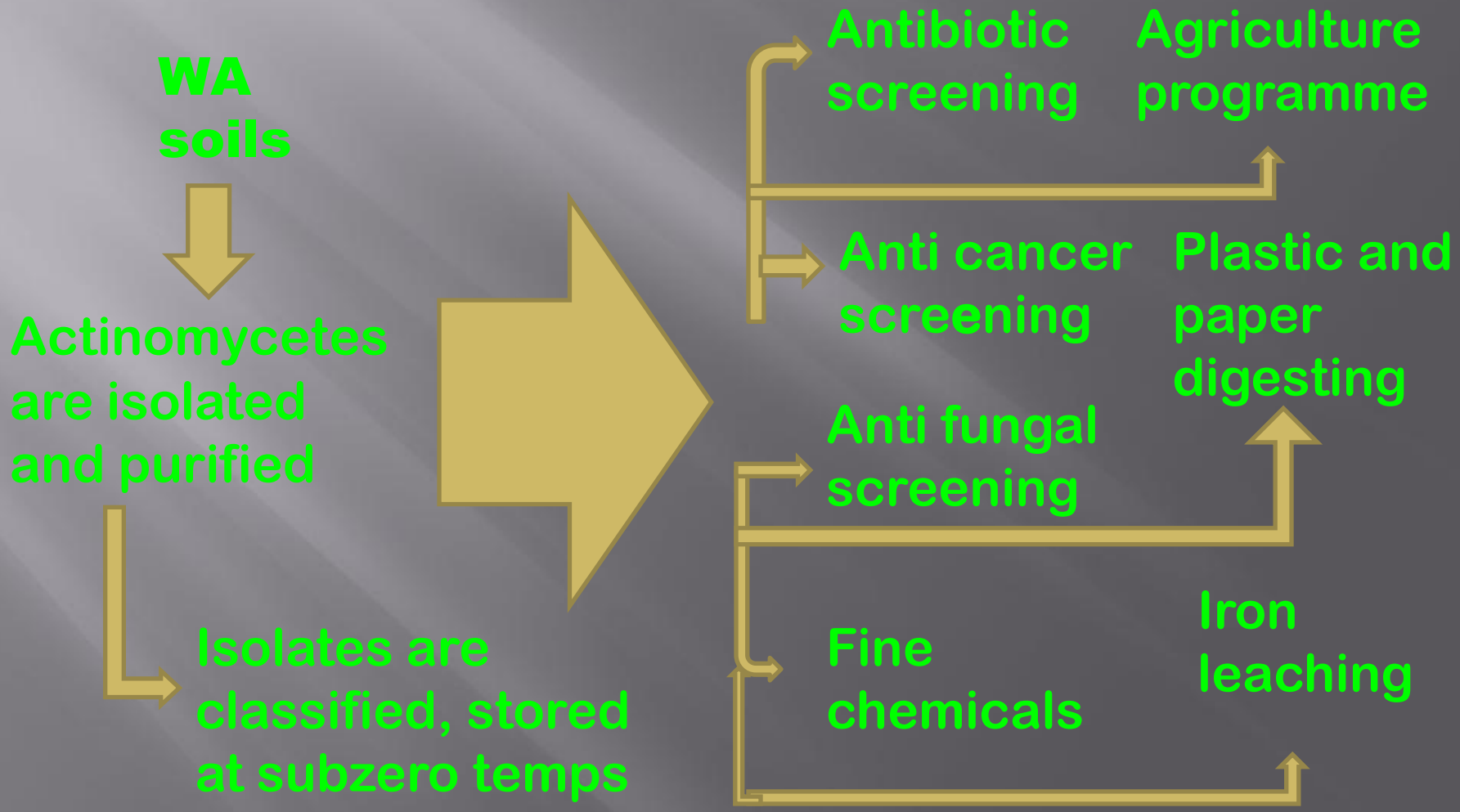
Weekly brainstorming meetings

Monthly report goes to all Board members

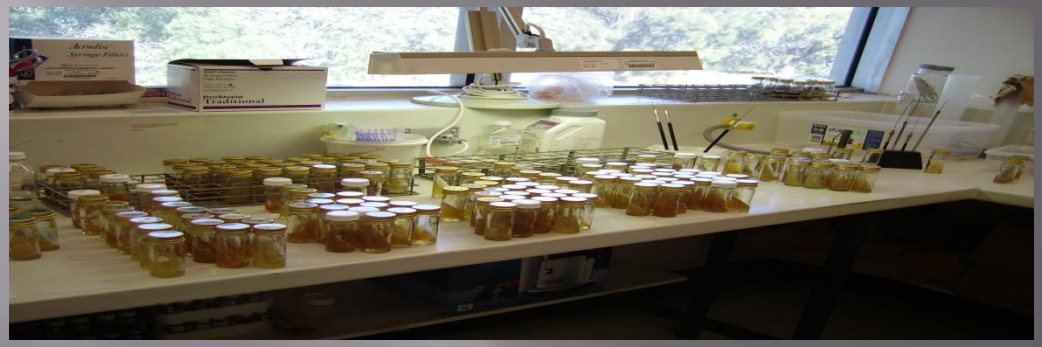
Quarterly; Half yearly and Annual Reports

All go to Board members to enable Board to assess progress and formulate policies

CURRENT R&D PROJECTS



HOUSEKEEPING



This section continues to be the area in which all new isolates are purified and classified. They are recorded along with a range of biochemical properties in an electronic data base.

The Company now has in the order of 14,000 isolates in two subzero libraries. These represent about 3600 actinomycetes.

The isolates are distributed to other R&D sections as required.

Housekeeping continued

- This year this section finalised the development of a specialised culture medium that both enhances the amount of antibiotic that an actinomycete can produce and also enhances the variety of antibiotics produced.

- A Provisional Patent has been lodged for the medium and Actinogen is currently seeking commercialisation of it, both in the UK and the USA.

ANTIBIOTIC SCREENING

1526 isolates representing 7630 cultures were passed through primary screening . Approx. 691 of these showed antibiotic activity and moved forward to secondary screening.

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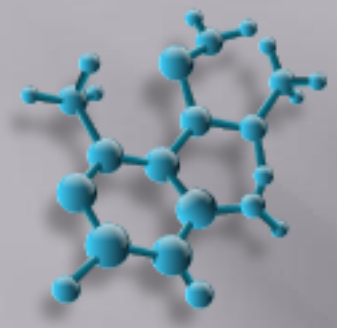
135 isolates showed antibiotic activity against the full panel of MRSA
64 isolates were active against the whole of Actinogen's *Candida* panel.
72 isolates were active against a Vancomycin resistant *Enterococcus*, while 6 were active against *Pseudomonas aeruginosa*
7 isolates exhibited quorum quenching properties.

Antibiotic screening continued

Clostridium difficile is an anaerobic bacterium that is emerging as a significant pathogen for man and farm animals. This bacterium is also becoming resistant to currently used antibiotics. Actinogen has introduced a screening programme directed against this organism.

19 of 182 isolates screened had significant activity against the panel.
Screens are being developed to ensure that normal gut flora both aerobic and anaerobic are not killed as well.

DEREPLICATION



Many bioactive molecules that we detect have been found before and these have to be excluded from further testing.
Liquid chromatography and UV spectra of the culture media are compared to libraries of known substances

Actinogen currently has a reference library containing some 880 known UV spectra. It is developing , in house, a highly specialised library for cancer chemotherapeutic agents and also has a large reference library developed from its own isolation studies. All actinomycetes producing antibiotics active in our bacterial screens are screened against these libraries.

Dereplication continued

Currently Actinogen has 60 isolates active against MRSA that are not identified by the reference libraries.

30 isolates active against the *Candida* panel cannot be identified

21 isolates active against VRE and 2 isolates active against *Pseudomonas aeruginosa* also cannot be identified

There is one isolate active in quorum quenching

Dereplication continued

Interesting UV peaks are present in the active isolates and it is from these that new antibiotic activity is most likely to be present.

However, our identification procedures are limited by the extent of our reference libraries

We continue to expand these libraries and we are also developing new technology to isolate peaks not in the library to confirm ones that have antibiotic properties. These will be sent for structural analyses. Currently we have a working agreement with the Government Chem. labs in this area.

ANTICANCER SCREENING IN VITRO

to L929 cells not exposed to actinomycetes supernatant. In this instance the cells did stain well with both Annexin V and 7AAD (Fig 8).

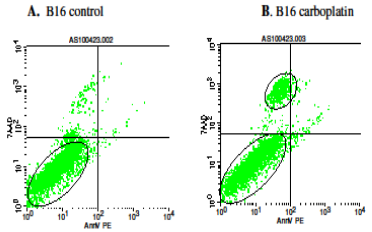


Figure 7. Figure A denotes cell scatter of B16 cells not exposed to actinomycetes supernatants. The Lower left quadrant indicates healthy cells with no positive stains of 7AAD (y-axis) or Annexin V (x-axis). Figure B denotes cell scatter of B16 exposed to the positive control Carboplatin. The second smaller population indicates a positive stain in 7AAD (upper left quadrant). However, there are very little cells in the upper right quadrant (positive for 7AAD and Annexin V stains), indicating dead cells. dead cells have only stained for 7AAD and not for Annexin V, which suggests that B16 does not stain well with Annexin V.

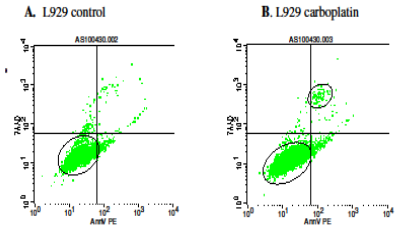


Figure 8. Figure A denotes cell scatter of L929 cells not exposed to actinomycetes supernatants. The lower left quadrant indicates healthy cells with no positive stains of 7AAD (y-axis) or Annexin V (x-axis). Figure B denotes cell scatter of L929 exposed to the positive control Carboplatin. The second smaller population indicates a positive stain in 7AAD and Annexin V (upper right quadrant). This indicates that L929 stains well with both 7AAD and Annexin V. The lower right quadrant indicates those cells undergoing apoptosis (negative for 7AAD and positive for Annexin V).

Two groups of actinomycete cultures are used:

[1] All cultures that have antibiotic activity which on dereplication do not have known anti cancer molecules present in our reference libraries.

[2] All cultures that have no antibiotic activity and no anti cancer molecule profiles by Actinogen's current dereplication libraries.

Anticancer screening continued

[1] Human adenocarcinoma of cervix: Hela
[2]Human epithelial carcinoma of the lung: A549
[3] Murine spindle cell melanoma of skin: B16
[4] Neoplastic subcutaneous fibroblast from mouse: L929

Primary screening:

455 actinomycetes have been tested over 41,888 individual cultures for both cytostatic and cytotoxic activity.

Anticancer screening continued

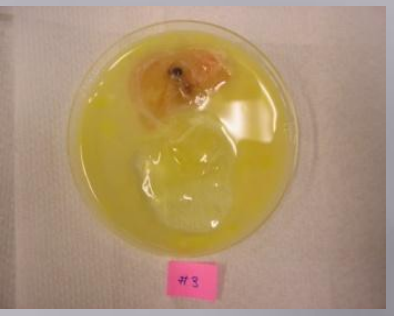
After primary screening 128 isolates were shown to have either significant cytostatic or cytotoxic activity.12 isolates had activity against all the cell lines.

Dereplication studies have detected a chemical known as MACUSOLIN in active cultures

While this has been reported to have antibiotic activity against bacteria and fungi, **it does not appear to have been reported to have potential anti cancer activity**.

Further testing of this chemical and its potential anti cancer properties is planned.

Anticancer screening continued



Tertiary screens are being developed to attempt to define the modes of action of active preparations from the actinomycetes.

These include testing for:

Apoptosis – lethal suicide induced in the target cell
Anti-angiogenesis- factors that inactivate a tumour's ability to develop, its own blood supply and hence gain access directly to increased nutrients.

ANTIFUNGAL SCREENING

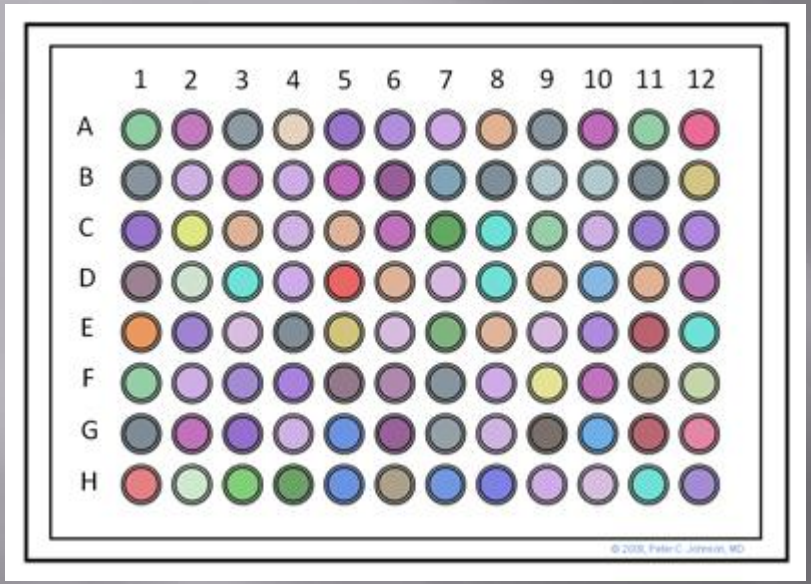
Candida albicans

Fungal yeast infections caused by *Candida*s are widespread in humans especially in immune suppressed subjects.

There are several species that cause serious disease in humans The majority of anti-*Candida* agents available are synthetic and toxic.

1526 new actinomycete isolates were screened for anti-*Candida* activity over a panel of 10 species and 64 were active against all the panel.

Antifungal screening continued



A 96 well tray assay system was shown to eliminate volatile anti *Candida* molecules, which are commonly found to be active against yeasts.

One isolate has been shown to remain active against the full panel of *Candidas* and one other was active against part of the panel.
The isolate active against the full panel is being investigated further.

FINE CHEMICAL

An anacardic acid

An actinomycete isolate has been discovered that synthesizes an anacardic acid. The structure has been determined by mass spectrometry to be identical to the structure above.

Fine chemical continued

The synthesis of this anacardic acid by an actinomycete appears to be a rare event

Actinogen has registered the actinomycete isolate under the terms of the Budapest Treaty which is a requirement for patenting procedures.

Actinogen has taken out a Provisional Patent on the methodology required for the synthesis of anacardic acid by the isolate.

Actinogen is seeking commercial partners to progress the production of anacardic acid by microbiological methods.

AGRICULTURAL SECTION

FOUR AREAS HAVE BEEN INVESTIGATED:

[1] Anti fungal activity for curly leaf

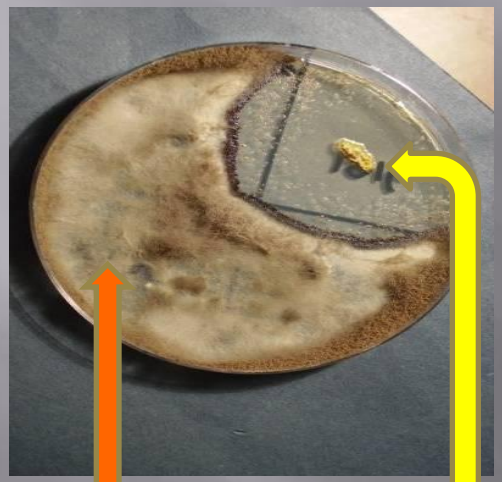
[2] Search for actinomycetes that can live in high salt conditions

[3] Discovery of two actinomycetes that can kill a brown rot fungus pathogenic to stone fruit

[4] Discovery of two actinomycetes that produce plant growth hormones.

Agriculture continued

Brown rot of stone fruit



The fungus

An actinomycete with strong anti fungal activity

Agriculture continued

One of the two isolates is producing an unidentifiable active molecule.

Further extensive tests using external library sources have so far not been able to identify the active molecule.

Actinogen is now progressing to attempts to determine a structure for the active molecule.

The isolate has been registered under the terms of the Budapest treaty .

Agriculture continued

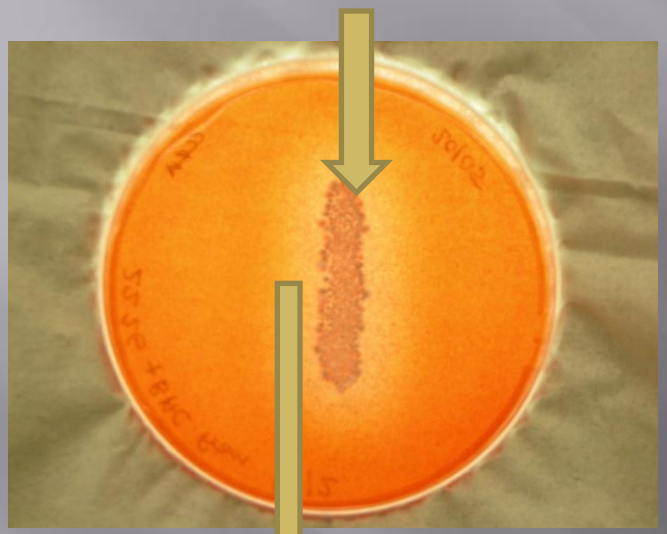
Dereplication located two actinomycetes that were producing plant growth hormones.

Growth trials in petri dishes using wheat seeds indicated that the seeds grow faster in the presence of media from the isolates

Out door pot trials using olive plantlets are in progress.

PAPER & PLASTIC DIGESTION

Actinomycete that digests cellulose



Clear zone indicates cellulase activity

Actinogen has isolated a number of actinomycetes that digest cellulose.

Actinogen has also discovered a bacterium which has been classified as *a Paenibacillus* species..

This bacterium in cooperation with the actinomycetes leads to the digestion of paper and "biodegradable' Plastics.

Paper & Plastic continued

Control

Test

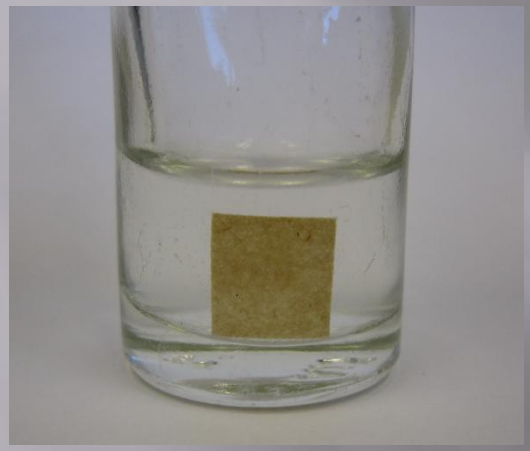
Three areas have been investigated:

[1] Digestion of cigarette paper

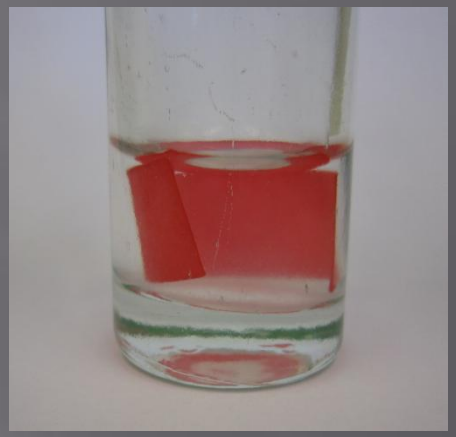
[2] Digestion of brown paper bags

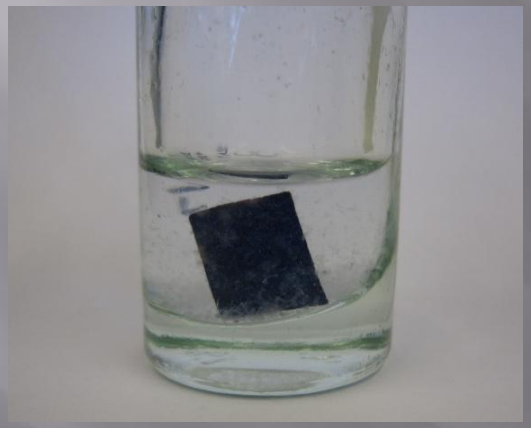
[3] Digestion of "biodegradable" plastic bags

Paper & Plastic continued

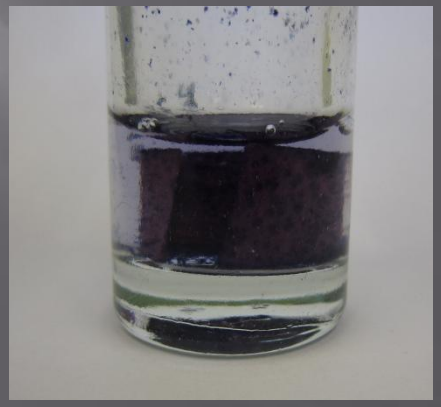


PAPER CONTROLS





TESTS



Bacterial growth and purple pigment production denotes utilization of paper as a nutrient source

Paper & Plastic continued

Control

Biodegradable plastic

Test

Bacterial growth and purple pigment indicate use of plastic as a nutrient source

Paper & Plastic continued

Actinogen has filed a Provisional Patent for methodologies of incorporating bacterial isolates into the preparation of both paper and plastic bags that will aid the digestion of the bags on their disposal.

Actinogen is currently seeking commercial interests to develop this are further.

BIOLEACHING OF IRON

Iron is an absolute requirement for life to exist.

Bacteria have developed methods that allow them to "search" for and recover iron from the environment.

One of the most powerful mechanisms is through a group of complexes known as the Siderophores.

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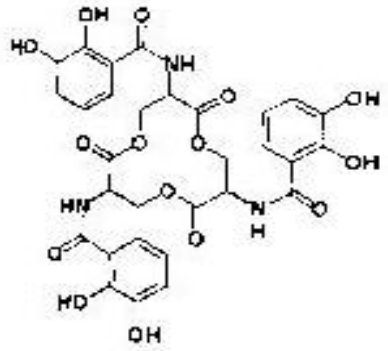
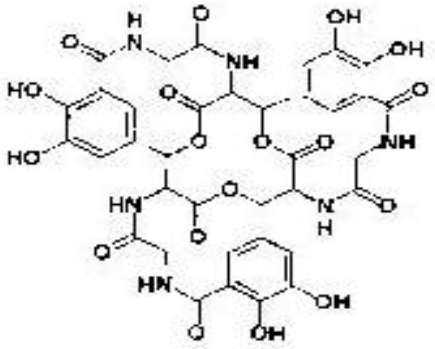
Bioleaching continued

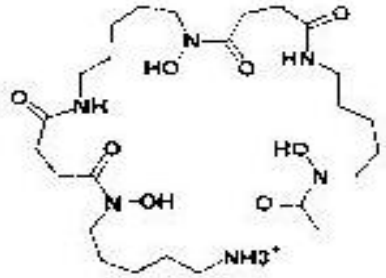
Examples of siderophores



Enterobactin



Corynebactin



Desferrioxamine B

Actinogen's dereplication section has shown that the actinomycetes contain a number of siderophores and we have explored these for their capacities to trap iron molecules

Bioleaching continued

Twenty two actinomycetes have been shown to be capable of strong siderophore production under iron starvation. However, siderophore activity is under strong iron ion control.

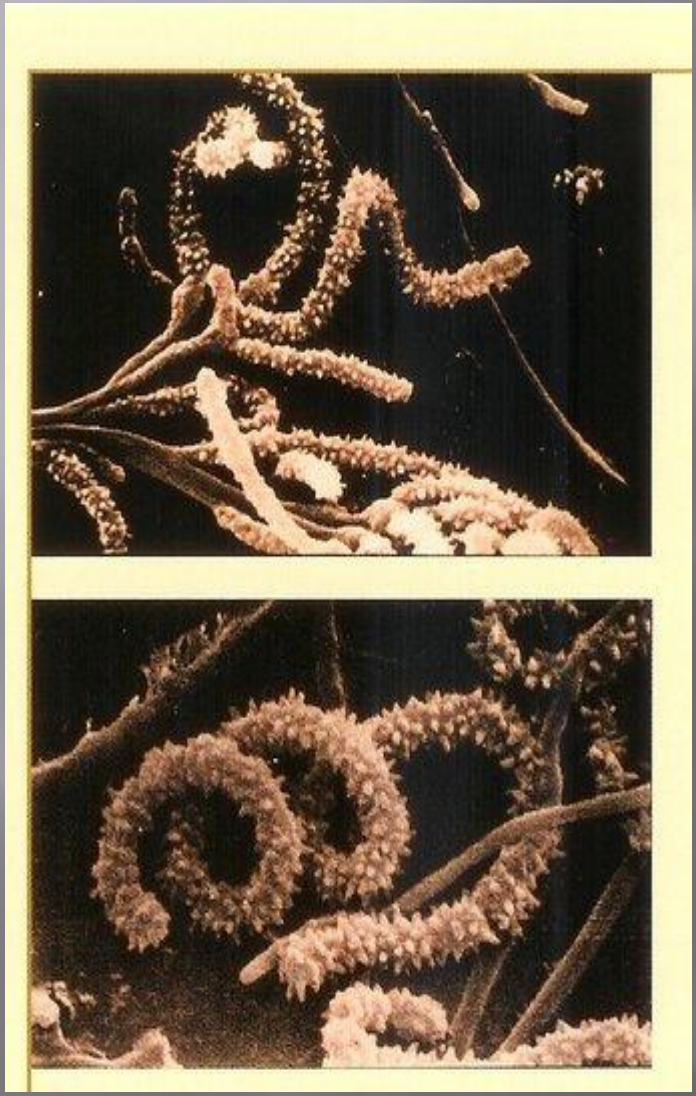
The majority of siderophores produced were either catechols or hydroxamates.

One isolate produced enterobactin which is unusual for the actinomycetes and there were a small number of siderophores that were unclassifisble with our current libraries.

The enterobactin producing isolate has been registered under the Budapest Treaty to provide Actinogen some protection prior to any Provisional Patent development.

A provisional Patent has been lodged for the use of siderophores in iron leaching.

SUMMARY & CONLUSIONS



I hope that I have indicated to you that Actinogen's R&D programme is extremely active with several of its projects now under Provisional Patent and Budapest Treaty protection.

Commercial interests are now being actively persued.

Field sampling has been reduced this year as our current projects have required detailed development aimed at interesting other parties.

THANK YOU