

ACTINOGEN Ltd Annual

R&D Report

2011-2012

CONFIDENTIAL



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INTRODUCTION



2011-2012 : A YEAR OF
CONSOLIDATION

CONSOLIDATION: General

Field sampling has been reduced as a result of some of Actinogen's R&D approaching the need for detailed trials in preparation for seeking commercialisation

CONSOLIDATION:

Areas

- [1] Cancer cell screening
- [2] Agriculture/horticulture
- [3] Cellulase & bioethanol
- [4] Antibiotics
- [5] Special project { **Shikimic acid** }

CONSOLIDATION:

[1]:Cancer cell screening

Actinogen acquired Celgenics in September 2011.

Celgenics was a company dedicated to the discovery and isolation of soil actinomycetes with bioactive molecules directed to a recently discovered set of cancer cells known as the Cancer Stem Cells (CSC).

CONSOLIDATION:

The CSC

The CSCs are a small number of cells (1-2%) now thought to exist in most cancers. They share some properties with embryonic stem cells.

They can be detected by characteristic markers on their cell surfaces especially one named the CD133 marker.

They are thought to be largely resistant to current chemotherapy and therefore continue to exist after most treatments.

It is also considered that these cells are responsible for the development of secondary cancers.

CSC : Continued

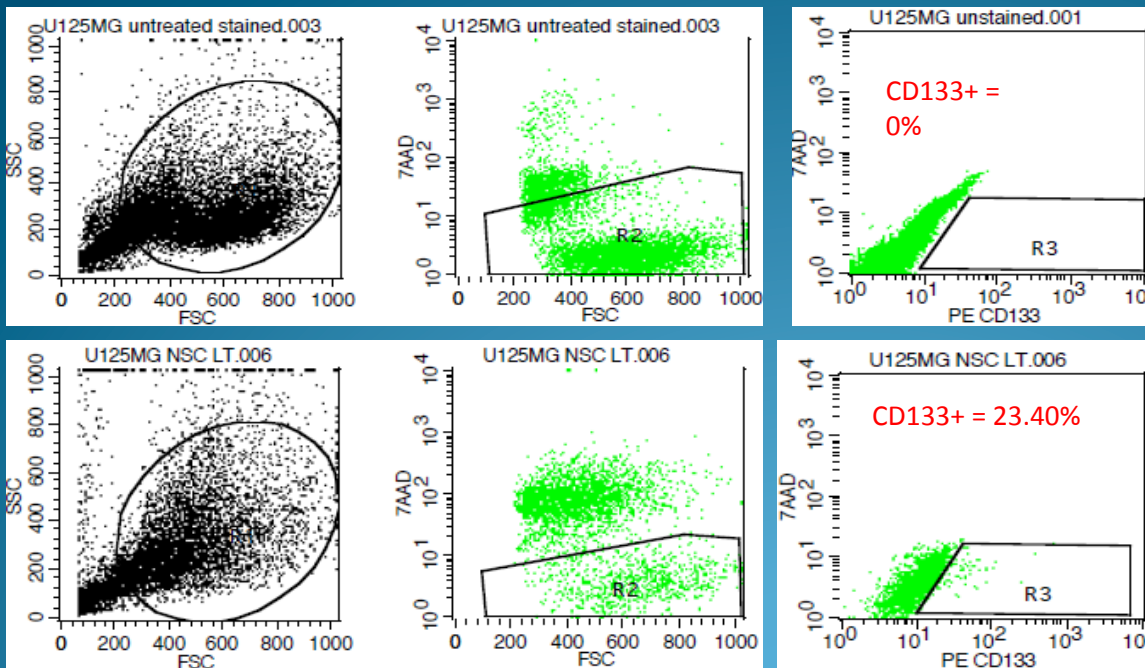
New agents are required to specifically target these cells

Actinogen is applying its expertise to the discovery of such agents from the actinomycetes.

New highly specialised equipment has recently been installed at the QE II Medical Centre that can aid in this area and Actinogen has full access to it

CSC: Continued

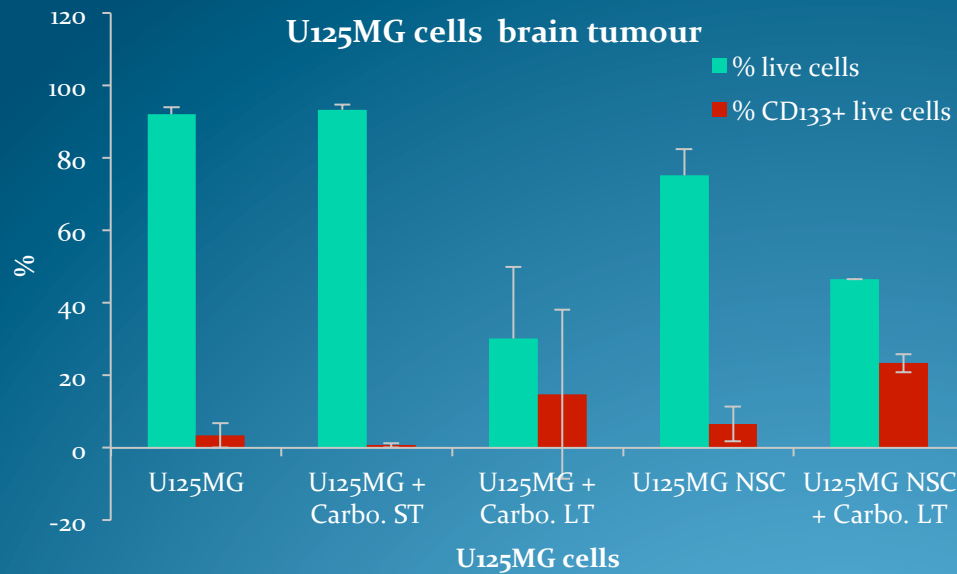
An example of results



Enrichment of
CSC using
Actinogen's
techniques

CSC: Continued

Illustration
of some results of
treatments overall to
enrich for CSCs



ST short term: LT long term
treatment

NSC neurospheres

CONSOLIDATION:

[2]: Agriculture/horticulture

{A}: Plant hormones

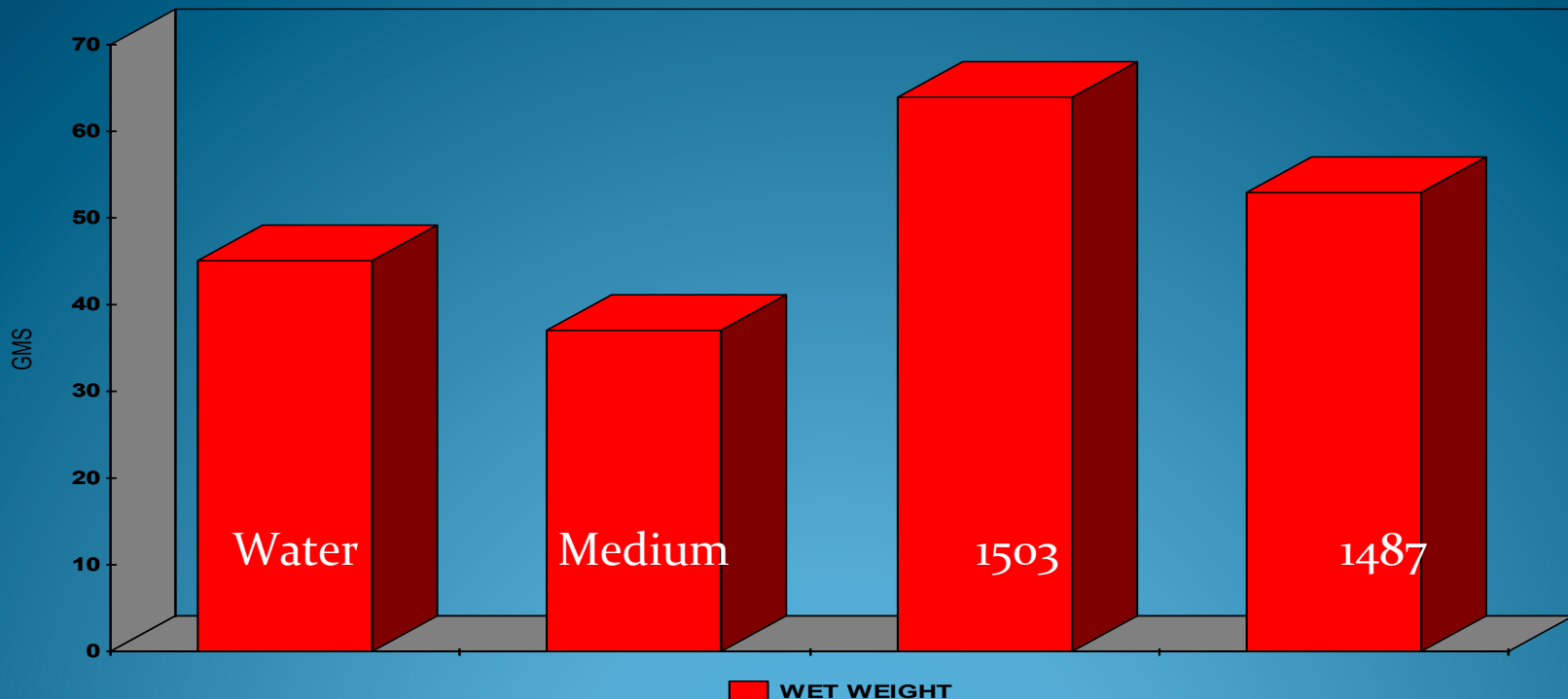
{B} Salt tolerant actinomycetes

{C} Biofumigation

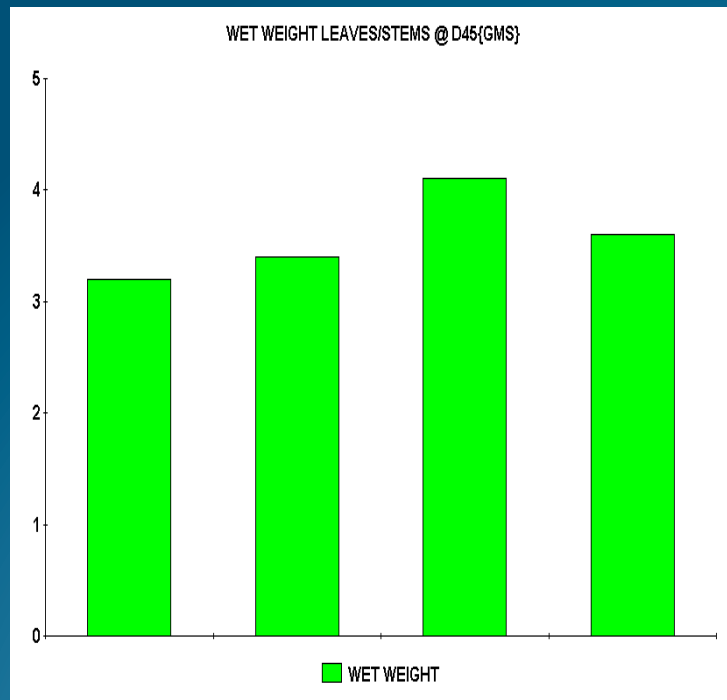
{A}: PLANT HORMONES

From 2010-2011 results

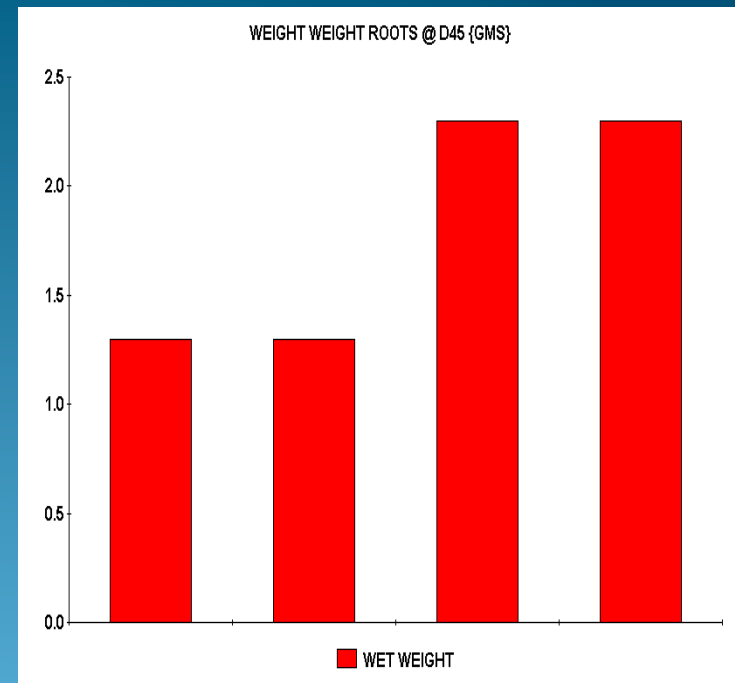
TOTAL BEAN WEIGHT {GMS @ D45}



Plant hormones



Water medium 1503 1487



Water Medium 1503 1487

Plant hormones

A further larger field trial is in progress using newly planted young commercial olive trees. Results should become available over 2012-2013.

Actinogen is currently awaiting government approvals needed before being eligible for commercialisation.

Once government approval has been received Actinogen intends to seek commercial interests in the agriculture/horticulture and nursery industries.

{B}: SALINE TOLERANT ACTINOMYCETES



OUTLINE

Worldwide problem involving millions of hectares.

Increasing economic problems throughout Australia through loss of broad acre farmlands

Important elements of soil fertility

HUMUS

The natural soil fertility cycle is dependent on the availability of natural nutrients largely from humus production

Humus supplied by the soil micro-flora and fauna. Although their biomass is only 1-5% it is their rapid turnover events that are critical

Humus is made up of **Bacteria**, fungi, protozoa worms etc. and their products

ACTINOGEN'S APPROACH **TO REHABILITATION**

In all soil rehabilitation programmes it is essential to rebuild a viable fertility cycle through the soil microflora.

A salt tolerant microflora is the first essential to begin to rebuild the humus and encourage three important elements:

ACTINOGEN'S CURRENT **SITUATION**

Actinogen now knows that some of the salt tolerant actinomycetes that it has selected produce cellulase while others produce anti fungal agents.

Both of these properties should aid in soil rehabilitation processes.

Now moving to field trials and marketing

{C} BIOFUMIGATION

The use of actinomycetes that produce volatile anti fungal agents

Actinogen's concept : Replace current chemical fungicides with spraying actinomycetes producing volatile anti fungal agents

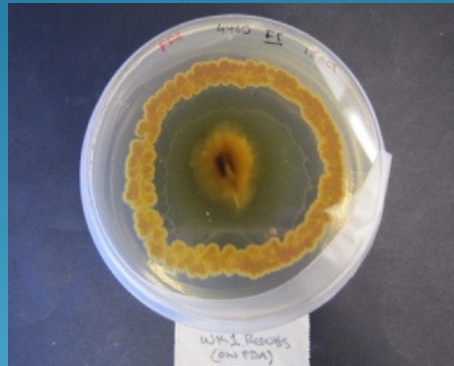
The bacteria filter through the upper biomass and establish underneath to allow their volatile agents to filter up through the biomass and attack pathogenic plant fungi

The initial screen test

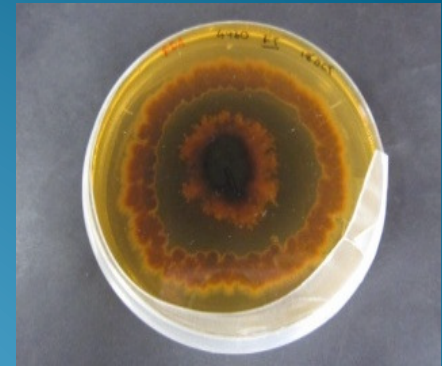
This is used against a panel of five common plant pathogenic fungi with isolates already indicating that volatile (polyenes) are being produced



No added
actino isolate



1week after actino
placed around fungi

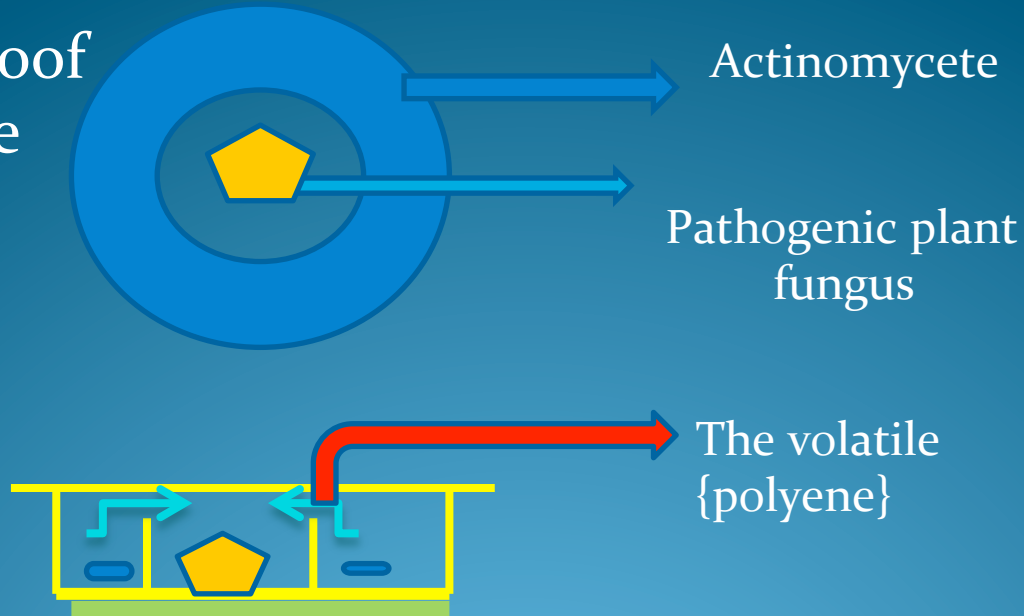


3weeks later

Biofumigation:

Current trials

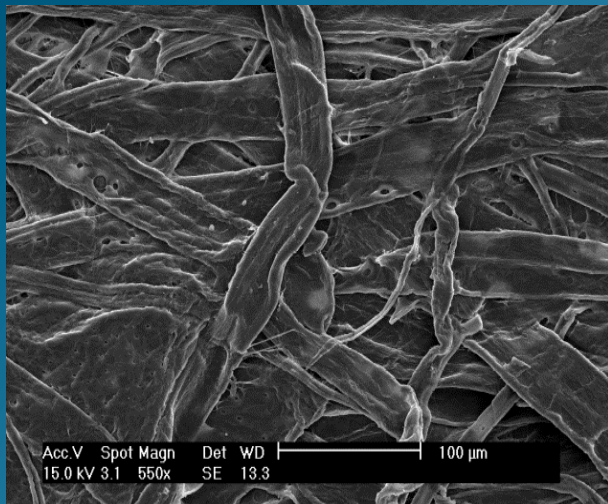
{1}: Final Proof
of volatile
product



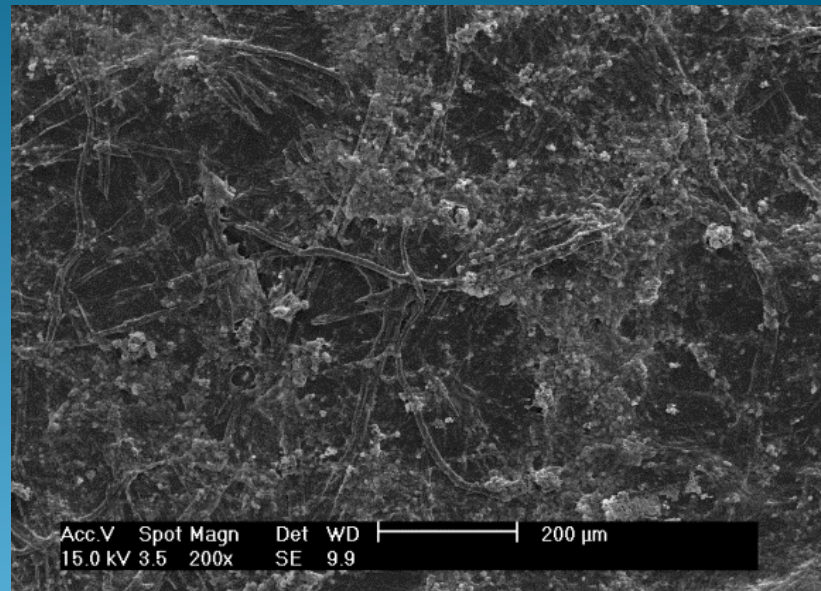
CONSOLIDATION:

[3]: Cellulase and Biofuel

Electron microscopy



Paper structure



Paper after actinomycetes digestion

Cellulase production

The CCRA screening assay

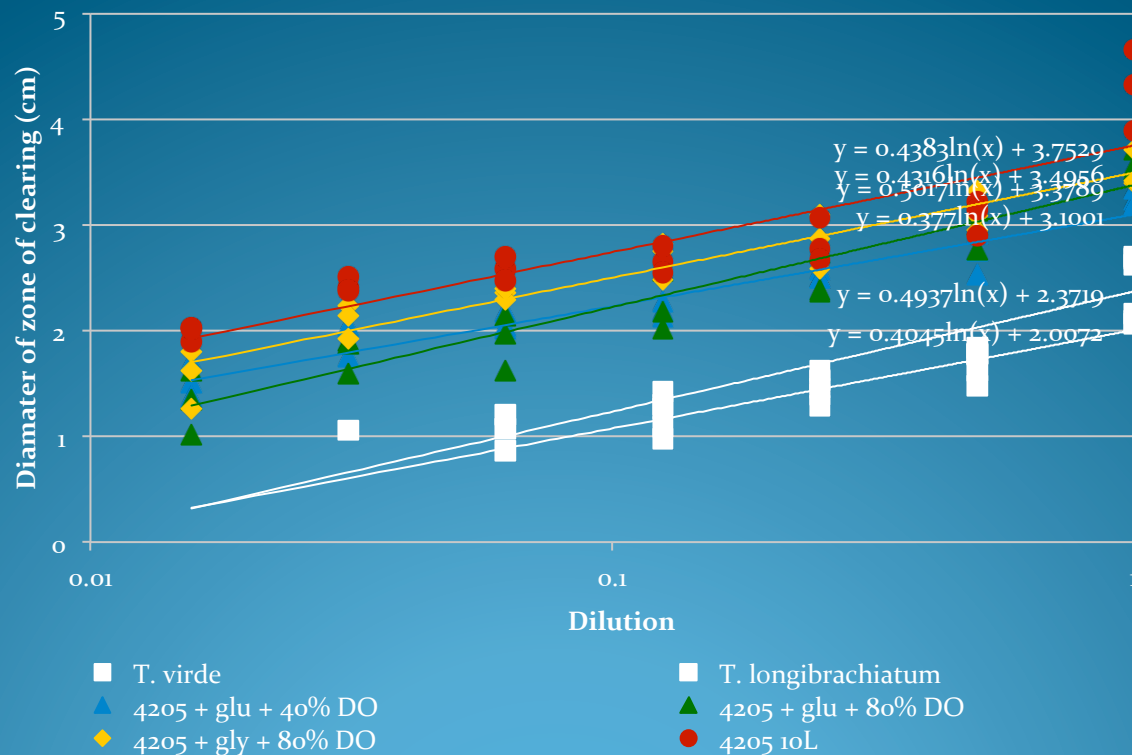


This is a simple diffusion screening assay

As cellulase diffuses through the coloured agar it digests the cellulose which leads to clear zones

Diffusion titration for Cellulase

Commercial cellulases were titrated against an
actinomycete ACN 4205



Cellulase:
Confirmation of results by
CSIRO

Actinogen's results were confirmed and cultural conditions optimised using larger fermentation vessels up to 10 litres.

Cellulase:

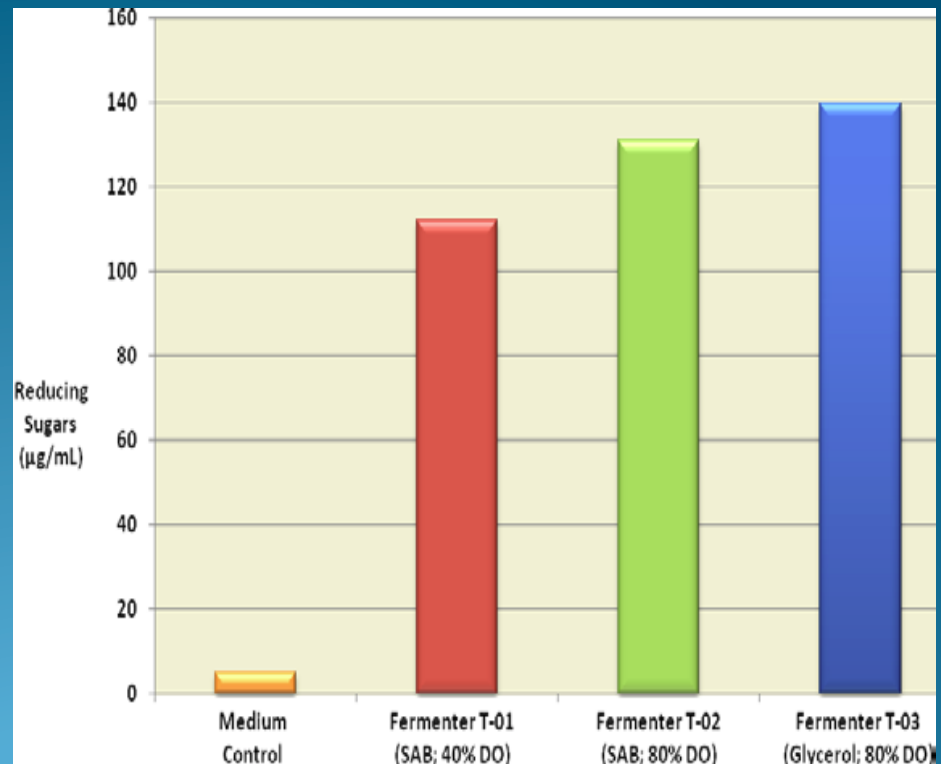
Cellulase Fermentations



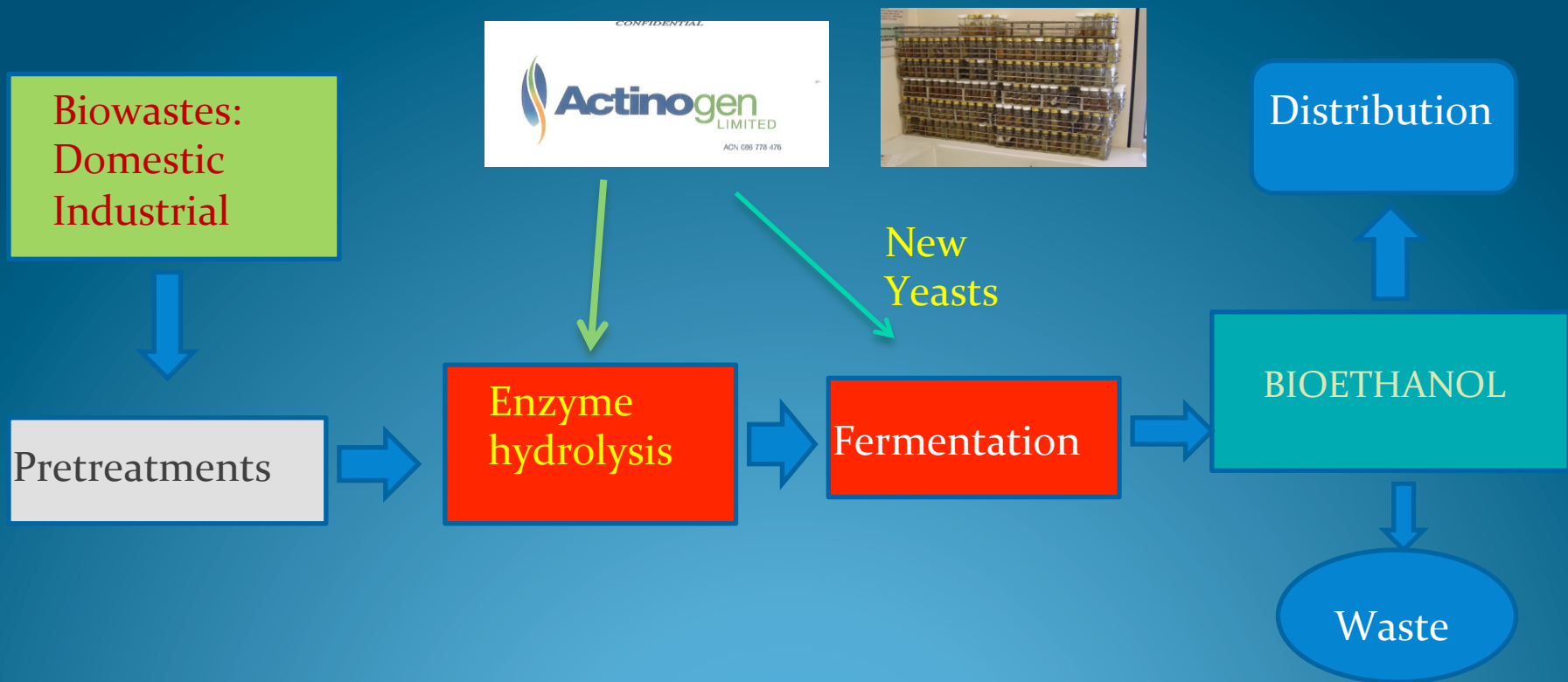
Cellulase:

Fermentation Results

Figure 2: Cellulase activities in the supernatants from the 2-litre fermentations. Yield is expressed as amount of reducing sugar produced in the cellulase assay, as determined by incubation with DNS reagent, measurement of absorbance at 540 nm, and plotting a standard curve from glucose standards.

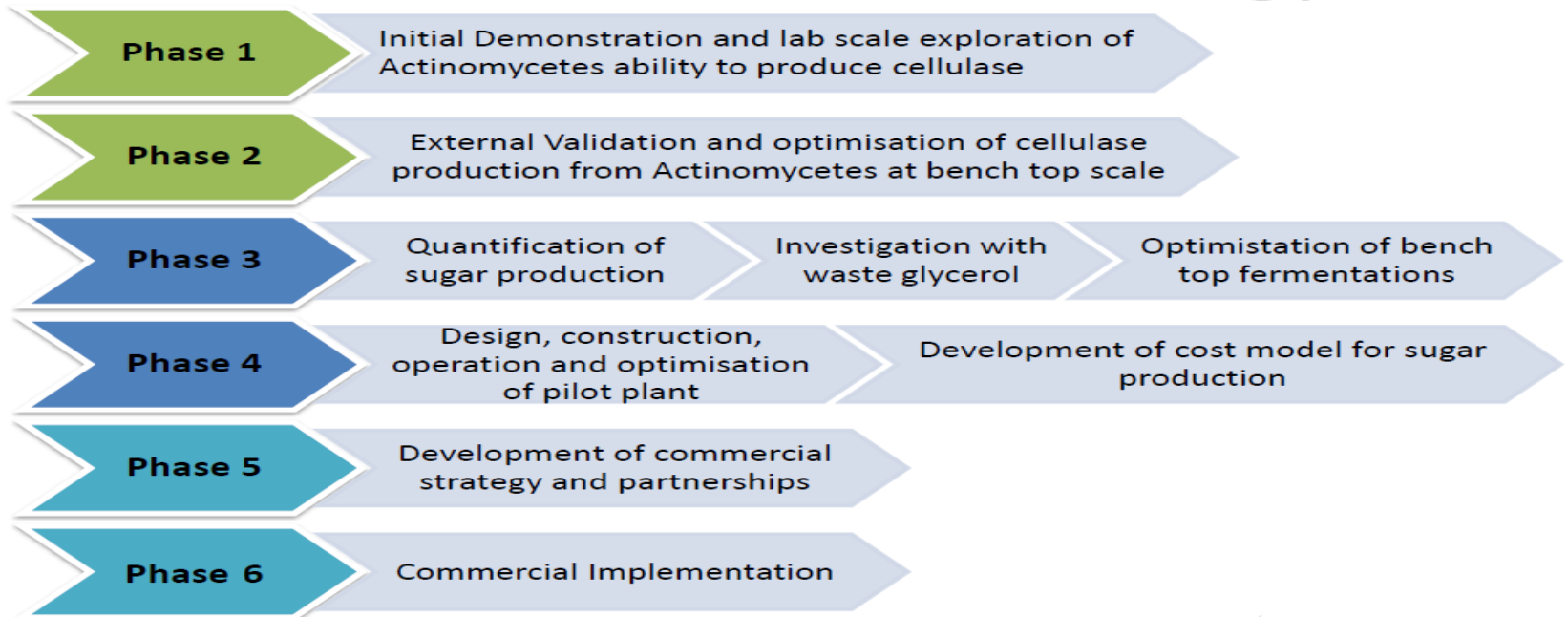


Second Generation BIOETHANOL Its production



Bioethanol : *Actinogen's programme*

Commercialisation Strategy



[4]: ANTIBIOTICS:

Currently three areas of interest



- {a}: MRSA: antibiotic resistant golden *Staphylococci*
- {b}: Anti *Clostridium difficile*
- {c} Anti *Candida* agents

Antibiotics:

{a}: MRSA

Actinogen currently has **19 actinomycetes** that are producing antibiotic molecules that are different from all antibiotics that we can screen for through our dereplication libraries. Shortly these active molecules will be prepared to go to larger library testing; any that are not excluded will go for structural analysis.

If this still shows the agents to be “new” then commercial strategies are next in line.

Antibiotics:

{b}: *Clostridium difficile*:

This is an anaerobic bacterium that is one of the newer so called emerging pathogens. It causes lethal diarrhoea, through toxins that it produces and is now significant for both man and many commercial farm animals.

It lives in the gut and current antibiotics used in its control also kill normal gut flora. This leads to complications and new antibiotics are needed.

Actinogen currently believes that it has an actinomycete which in bench trials, kills the *Clostridium* but does not kill normal gut flora.

These results are being confirmed and the active molecule(s) is/are being prepared for structural analysis. Commercialisation could follow these trials.

Antibiotics:

{c}: Anti *Candida* antibiotic:

Candida infections from several species are important when the immune system is depressed. This occurs following:- organ transplants, AIDS, with increasing age, and in conditions such as oral and vaginal thrush in children and in adults..

Actinogen has an actinomycete which is producing an anti *Candida* antibiotic that under our present tests appears to be new.

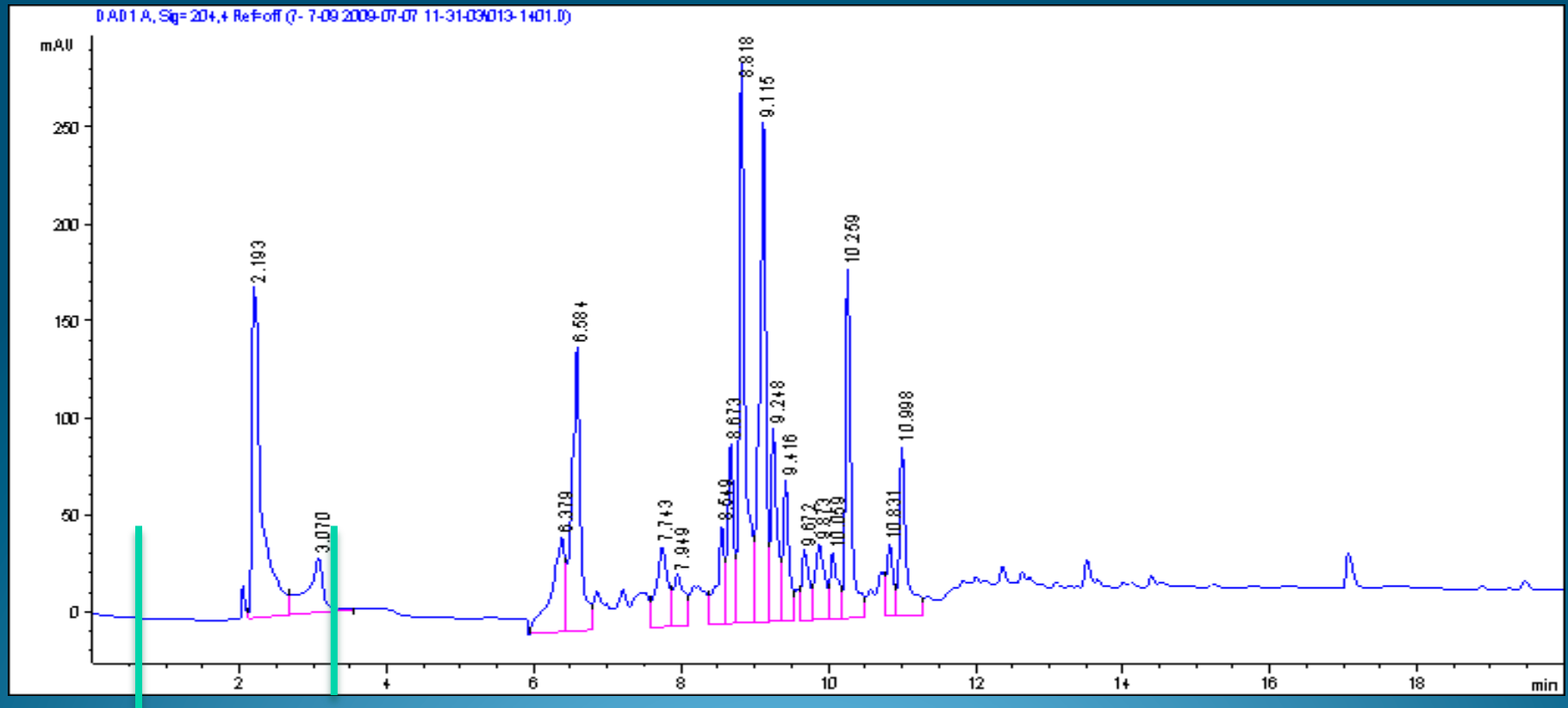
This is currently being purified for the determination of its structure

[5] SPECIAL PROJECT: Shikimic acid the basis of Tamiflu

Actinogen has discovered an actinomycete that synthesizes shikimic acid the organic acid which forms the basis for the construction of Tamiflu.

Current methods of obtaining this acid are extremely expensive and time consuming and there is always an under supply.

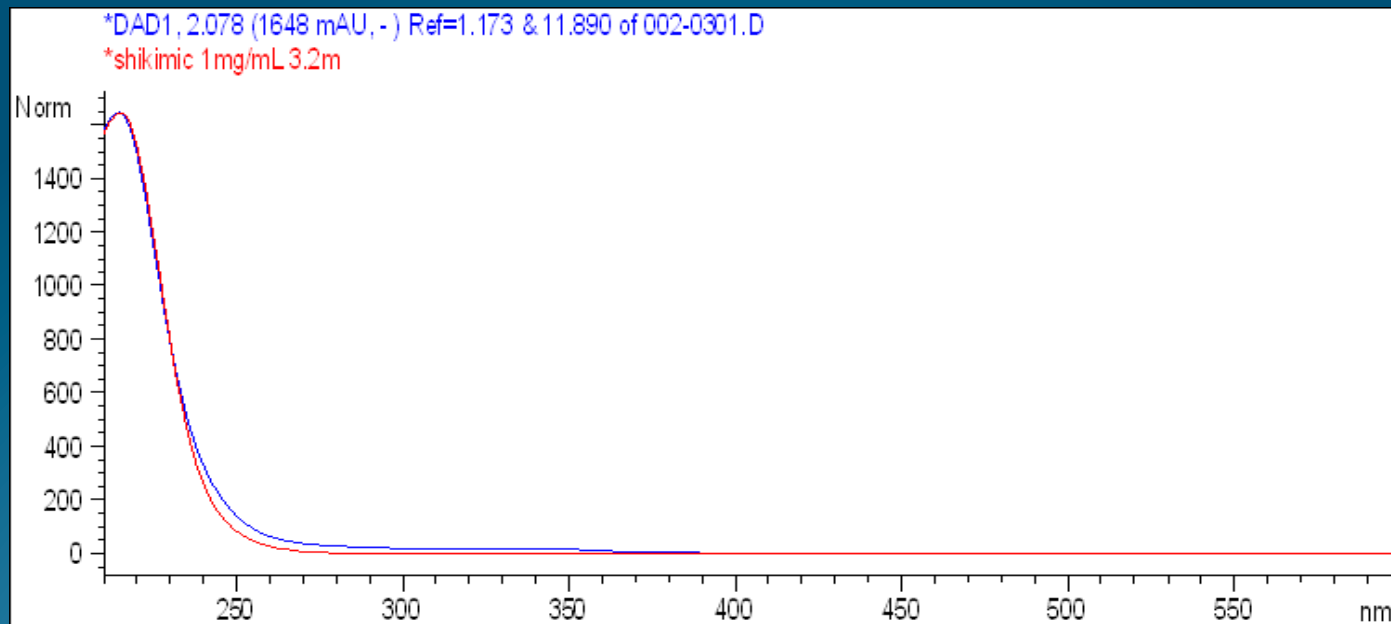
Shikimic acid



Shikimic acid

The culture medium profile for isolate
1488

Shikimic acid



The ultra violet profiles of pure shikimic acid(blue) and that from isolate 1488 (red).

Shikimic acid:

This is an exciting new development.

Trials are in place to:

- (i): obtain the shikimic acid as pure as possible to confirm its structure by Mass Spectrometry.**
- (ii): determine the amount of shikimic acid that is being produced. This will allow preliminary costings.**
- (iii): Purify the shikimic acid from the rest of the culture medium.**

SUMMARY:

Actinogen is now in an exciting phase of its development with a series of its projects close to or ready for commercialisation.

Thankyou