29 August 2013
ASX Release

Actinogen Limited (ASX Code: ACW)

Market update and clarification – August 2013

1. Background

On 31 July 2013 Actinogen Limited (**Actinogen** or **the Company**) announced that it had elected to withdraw the rights issue offer announced on 1 July 2013 (**Offer**). The purpose of withdrawing the Offer was to provide shareholders with a detailed summary of the Company's operations and future plans before shareholders make a decision whether or not to participate in any capital raising conducted by the Company.

This market update provides a detailed summary of Actinogen's operations and future plans. Actinogen recommends that shareholders read this market update in full, and in place of the prospectus dated 1 July 2013 in respect of the Offer (**Prospectus**). Actinogen recommends that shareholders disregard the contents of the Prospectus.

2. Clarification of previous statements

It has been brought to the attention of the Company that certain statements made in previous announcements by the Company may be construed differently to how they were intended. The Company wishes to clarify those statements as follows:

- References to the Company's projects being close to commercialisation were not intended to suggest that the Company's projects have been proven to be commercially viable, nor that any project is at a stage where it can be commercially exploited in the immediate future. Those statements should be disregarded. Each of the Company's projects is at an early stage and the results have only been achieved on a molecular level. In order to be commercialised, the Company will need to go through the trial process to prove up the technology and achieve the results on a commercial scale. There is no guarantee that any of the Company's projects will be successfully commercialised. The current status of the Company's projects is explained in more detail in the market update below.

- References to the Plant Growth Project rendering a "new discovery" for the Company were not intended to suggest that the hormones identified are new plant growth hormones. Rather, the Company has determined that it is able to produce (on a molecular level) growth hormones from certain actinomycetes, which growth hormones have been shown to cause an increase in the dry weight of pea tops in testing (observed in one test on pea tops). The growth hormones themselves are

not new, however Actinogen is not aware of any other person that has produced growth hormones from Western Australian actinomycetes. The Plant Growth Project is explained in detail in section 4 of the market update below.

3. Actinogen's operations

3.1 Overview

Actinogen's aim is to identify and isolate soil microorganisms, known as **actinomycetes**, which are capable of producing bioactive compounds (or exhibiting properties in their own right) of commercial value. Actinogen seeks to achieve this aim by sampling Western Australian soils and testing actinomycete isolates identified in those soils. The southwest of Western Australia is one of only a few places in the world known to have dry sclerophyll soils. They are also found in eastern and southern parts of Australia and in a small number of places elsewhere. They are often inhabited by specialised Sclerophyllous plants and as a result may also contain unique microflora.[1]

3.2 What are actinomycetes?

Actinomycetes are a group of gram positive bacteria that are distributed in the natural environment worldwide. They are metabolically diverse and produce bioactive molecules such as bacterial antibiotics, anti-viral agents, anti-tumour agents, antifungal agents and immunosuppressive agents that are used for humans, animals and in agriculture. They can also produce bioactive molecules effective in industry at large.

Actinomycete isolates are individual actinomycetes in pure form. Actinogen produces actinomycete isolates in liquid form to use for its testing.

3.3 Projects

Set out in the table below is a list of Actinogen's projects, including their current status and a reference to the relevant section of this market update. Each of these projects involves identifying and applying actinomycetes for a particular purpose.

Project	Aim	Status	See section
Plant Growth Project	Production of plant growth hormones using actinomycete isolates	Active	4
Antibiotic Research Project	Research to find bacteria killing actinomycetes which can be used to produce antibiotics	Active	5

[1] Ecology and silviculture of eucalypt forests, RG Florence, *CSIRO 2004: Tech and Engineering*

Project	Aim	Status	See section
Salt Tolerant Actinomycetes Research Project	Research to find salt tolerant actinomycetes which can be used to help farmers grow plants and crops in salt affected environments	Minor activity	6
Bioethanol Project	Production of bioethanol using cellulase produced by acinomycete isolates	On hold pending further funding	7
Shikimic Acid Project	Production of shikimic acid, a key component of influenza medication, from actinomycetes isolates	On hold pending further funding	8
Biofumigation Project	Production of polyenes, which inhibit or kill fungi, using actinomycete isolates	On hold pending further funding	9
Cancer Stem Cell Research Project	Research to find actinomycetes that kill cancer cells and cancer stem cells	On hold pending further funding	10

Each of the above projects requires significant further testing in order in order to establish its commercial viability. All of the Company's results to date have been on a molecular level, and must be repeated on a much larger scale in order to be commercially viable. There is no guarantee that any of these projects will ultimately be commercially viable.

Actinogen will continue to look for commercial partners and joint venture opportunities for each project as it develops.

3.4 Key personnel

Actinogen's operations are headed by its Scientific Director, Dr David Keast, who has a scientific background spanning over 40 years. Over that time he has developed research programs in immunology, bacteriology and cancer research and has published over 225 scientific papers, review articles and book chapters. During the 1980s Dr Keast developed and ran a large actinomycetes isolation, screening and research and development program at the University of Western Australia and financed by the multinational pharmaceutical company, Merck Sharp and Dohme.

Until November 2012, Actinogen employed six research scientists, with Dr Keast as director and research coordinator. Three of the scientists had PhDs (two employed full time and one part time) and three were science graduates (one full time and two part time). In November 2012, Actinogen's directors resolved to reduce the number of research staff as a cost cutting measure. Currently Actinogen employs one full time scientist and one part time scientist who work under Dr Keast's instructions. Dr Keast oversees all of Actinogen's research and development programs. Actinogen liases with other scientists as required.

David Zohar and Zhukov Pervan are both executive directors. They meet frequently with Dr Keast to discuss the direction of the research and development programs and to plan the development of the programs and any fundraising, which may be needed. Alan Morton and Simon England are both non-executive directors. All directors except for Dr Keast are not receiving any remuneration until Actinogen raises sufficient funds. All funds raised since April 2012 have been used solely for research and development and to remunerate Dr Keast (on a reduced salary) and the two research staff.

3.5 Actinogen's laboratory and processes

Actinogen's operations are run from laboratory space at the Queen Elizabeth II Medical Centre in Nedlands (**Actinogen Laboratory**) under the supervision of Dr Keast. Actinogen rents the Actinogen Laboratory on an annual basis.

Actinogen stores actinomycetes isolates at minus 80^{o}C at the Actinogen Laboratory (which Actinogen refers to as its "library").

Actinogen actively collects new actinomycetes from the environment in Western Australia by gathering and testing soil samples. The samples are then assessed by Actinogen and catalogued by the properties that they have. For example, if an actinomycete shows an effect against bacteria such as MRSA, it is catalogued as a possible antibiotic and entered into the Actinogen library.

On occasion, Actinogen uses independent facilities to verify or expand on its research. Actinogen supplies the actinomycetes and outlines the experiment and it is performed by the independent body. Currently, independent testing is done by an independent university in Western Australia.

Actinogen uses specialised in house rapid screening techniques, High Pressure Liquid Chromatography (**HPLC**) and other specialised equipment to identify compounds that are produced by actinomycetes. Specific details of Actinogen's processes have been withheld because they are confidential and, depending on the success of those processes, may be the subject of patent applications by Actinogen in the future.

3.6 Protecting Actinogen's research

Where Actinogen's research and testing yields potential commercially valuable compounds produced from its discovered actinomycetes isolates, Actinogen will seek to protect those compounds by:

- registering a provisional patent; and

- sending the relevant actinomycete isolates to be stored at the Australian Deposit Centre at the National Measurement Institute in Melbourne under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure (**Budapest Treaty**). This lodgement effectively protects against others using the actinomycete isolates discovered by Actinogen.

Actinogen lodges a provisional patent when the Company believes it has potentially found something new and novel. A provisional patent only lasts for 12 months, after which the Company must decide whether to proceed to a full patent, which is expensive and requires a substantial level of detail as to the process used. If Actinogen is not ready to lodge a full patent after 12 months (either because the project is not developed enough to justify the cost of lodging a full patent or the project is not be ready to be described in enough detail for a full patent), Actinogen will allow the provisional patent to lapse. Currently Actinogen has no active provisional patents.

In order to protect actinomycetes where there is no patent protection, Actinogen lodges the actinomycetes under the Budapest Treaty. This protects the actinomycetes and prevents anyone else from using the same actinomycetes without Actinogen's consent for a minimum of 30 years from the date of lodgement.

Actinogen has lodged a series of actinomycetes under the terms of the Budapest Treaty. These are ACN 33 (produces antibacterial and anti-fungal antibiotics on a molecular level), ACN 141 (produces bacterial antibiotics on a molecular level), ACN 1034 (produces antibiotics and anacardic acid on a molecular level), ACN 1487 and ACN 1503 (produce antibiotics and plant hormones on a molecular level), ACN 1488 (produces shikimic acid on a molecular level), ACN 4205 (produces cellulases on a molecular level) and BACN 001 (a bacterium that aids cellulose digestion).

3.7 Actinogen's publications

Actinogen has released 4 publications on findings on scientific interest arising in the course of its research on actinomycetes, namely:

- "Growth in glass and plastic cultureware of Actinomycetes isolated from WA soils – a comparison" (2012);

- "Siderophore production by actinomycetes isolates from two soil sites in Western Australia" (2012);

- "Siderophore production by Actinomycetes isolates from soil in Western Australia" (2010); and

- "Isolation of Actinomycetes from West Australian Water Samples" (2009).

These publications do not relate to the Company's projects, but rather matters of scientific interest that have arisen in the course of the Company's research. Copies of

these publications can be accessed from the Company's website at www.actinogen.com.au.

3.8 Actinogen's future plans

Actinogen's short term future plan is to:

- focus on the Plant Growth Project, with the aim of producing commercially valuable plant growth hormones that have been independently verified to increase growth rates in plants;
- continue to test actinomycetes for antibacterial properties; and
- seek commercial partners to assist in developing Actinogen's projects.

In the longer term, and subject to Actinogen raising additional funds, Actinogen intends to pursue each of its other projects in order to bring them to commercialisation.

4. PLANT GROWTH PROJECT

4.1 Background

Actinogen's research has identified certain actinomycete isolates that produce plant growth hormones. The most effective actinomycete isolates discovered by Actinogen, being ACN 1487 and ACN 1503, were isolated and deposited at the National Measurement Institute in Melbourne under the Budapest Treaty.

While the plant growth hormones are not unique in themselves and are used worldwide, Actinogen is not aware of any other person that produces plant growth hormones from Western Australian actinomycetes.

4.2 What are plant growth hormones?

All living organisms begin life as a single cell which then divides and multiplies. The multiplied cells go through a differentiation process, following which different cells have different roles in the developing organism. Hormones are a group of chemicals which (together with other chemicals) drive that differentiation process.

In plants, genetic information directs the synthesis of these hormones and transportation of the hormones throughout the plant as required. The hormones are biologically active at low concentrations and interact with specific target tissue to cause significant physiological responses at whole plant level, such as growth of roots, stems and leaves, the development of flowering and subsequently fruit.

Five major groups of plant hormones are currently recognised. These are:

	Hormone	Role
1.	Auxins	These hormones are involved in cell elongation and both apical and root growth, and can stimulate root development in plant and leaf cuttings.
2.	Gibberellins	Gibberellins primarily affect the elongation of plants, although they do have other properties such as breaking dormancy and promoting uniform germination. Gibberillins have commercial uses such as increasing the production of sugar in the malting process in the beer industry.
3.	Ethylene	Ethylene is responsible for the ripening of fruits.
4.	Cytokinins	Cytokinins promote cell division and bud development in flowering plants.
5.	Absicsic acid	Absicsic acid inhibits the action of other hormones and is active in stomata closure to conserve water loss in plants.

There are other growth regulators that are not hormones but either function in a similar way to hormones or influence the activity of hormones. An example is shikimic acid (see section 8 of this market update), which is important in the aromatic biosynthetic pathways in plants that enhance plant growth.

4.3 The method Actinogen uses to identify plant growth hormones

Initially Actinogen uses an HPLC dereplication process using a large reference library containing a wide array of bioactive substances to check for known plant growth hormones that are already in the public domain. The final confirmation of the active substances is by the use of mass spectrometry. The following method of identifying plant growth hormones is used for the Plant Growth Project:

- An Agilent 1200 series DAD-HPLC with a Zorbax Eclipse Plus C-18 solvent saver plus column (3 × 4.6 mm, 5 µ) fitted with a guard column (12.5 × 4.6 mm, 5µ) is used for all HPLC analyses.

- All culture broths are sterile filtered through a 0.2µm pore size syringe filter, 10µl volumes are injected into the HPLC set to a flow rate of 0.4 ml/min.

- The mobile phase employed consisted of:

- Solvent A: 0.1% orthophosphoric acid in Milli-Q water; and
- Solvent B: HPLC grade acetonitrile.

- The mobile phase is run as a linear gradient from 0% - 100% B over 20 minutes, with a 5 minute hold at 100% B and a 5 minute post-time at initial conditions.

- Ultra violet detection peaks are recorded at a data sampling rate of 500msec using a slit width of 2 nm over the range 200-600 nm.

- Chromatograms are constructed and ultra violet spectra at 204 nm were compared by dereplication with three UV-spectral libraries containing spectra for bioactive molecules. The spectra are matched to Actinogen's in-house reference libraries.

Using this method, Actinogen has identified, on a molecular level, the production of hormones from actinomycetes. The exact breakdown of what substances the actinomycetes are producing (including which group of hormones those substances belong to) is confidential as it may be used in patent applications in the future.

4.4 First Actinogen wheat seed experiments

Dr Keast first ran experiments in the Actinogen Laboratory in 2011 on Wyalkatchem wheat to determine whether putative plant hormone containing preparations could be detected using a simple screening assay.

The process for the experiments was as follows:

- The wheat seeds were placed on filter papers in glass petri dishes at room temperature, enclosed in a lid-fitted container for the first 4–6 days then exposed to sunlight.

- The wheat seeds were sterilised (in an effort to avoid fungal contamination) in accordance with the following protocol to maintain consistency:
  - immersion in 70% ethanol for 10 minutes;
  - quick rinse in sterile distilled water (**SDW**);
  - immersion in household bleach for 10 minutes; and
  - a final thorough rinse in SDW.

  While this is the best method so far devised to retain viability in the seeds, this does not totally control naturally occurring fungi in all seeds.

- The 2 actinomycete isolates ACN 1487 and ACN 1503 that have been shown by Actinogen to produce plant hormones were added to Wyalkatchem plantlets to observe their effect on wheat growth.

- The sterile, sandy soil (heated on a tray in an 180^{o}C oven for 3 hours then autoclaved in Schott bottles) was weighed into individual yellow-top, sterile culture tubes.

- The two isolates were grown up in a liquid culture medium, filter-sterilised then added to separate soil/tubes. The tubes were capped and left on the bench for approximately 1 week to allow for isolate establishment within the soil environment. Control soil/tubes were not inoculated.

- 6-day old germinated wheat plants were then transplanted from the petri dish into the soil/tubes of each of the 3 treatment types (control, or filtrates of actinomycete isolates ACN 1487 or ACN 1503), then moistened with 3 to 4 ml of SDW.

- For each treatment type, the wheat was planted in replicates to yield 3 plants for weighing across 3 weeks, a total of 6 plants per treatment type. The plants were capped with the hole-drilled-lid used in trial 3, positioned on the window sill for sunlight and moistened with SDW when needed.

All test plants treated with the filtrates of ACN 1487 or ACN 1503 grew faster than the control plants. By day 19 of the experiment all of the treated plants had grown between 1 and 4 more leaves than the control plants.

Both the control and test plants remained healthy until day 13 when fungal contamination appeared in a proportion of the control and ACN 1487 plants. All seeds contain fungal spores which can lead to fungal contamination. The seeds used in Actinogen's trials were sterilised in an effort to minimise fungal contamination, however it is not possible to eliminate all fungal contamination given some fungal spores are airborne and can attach to seeds after the sterilisation process has been completed. The fungal contamination occurred for a portion of both the control and ACN 1487 plants, so cannot be attributed to any issue with ACN 1487.

TABLE 1: The average weights (mg) of the leaf, stem and root portions of the Wyalkatchem wheat seeds after treatment in accordance with the above process.

	Leaves		**Stem**		**Roots**	
	Week 1	**Week 3**	**Week 1**	**Week 3**	**Week 1**	**Week 3**
SDW	0.41	1.15	0.29	0.35	0.58	0.52
1487	0.62	1.60	0.39	0.41	0.58	1.30
1503	0.55	0.81	0.35	0.40	0.79	1.30

Conclusion: The plants treated with the isolate filtrates stimulated Wyalkatchem wheat growth significantly over the trial period. The filtrates were known to contain plant growth hormones.

4.5 Second round of wheat seed experiments

A second trial, using ten replicas per treatment was performed in order to substantiate the first trial results. In this instance three replicas were taken at each sample time and the results were graphed (see section 3.6 below) as means and standard deviation at weekly intervals.

By the end of the first week (day 8) since transplant of seedlings of Wyalkatchem wheat:

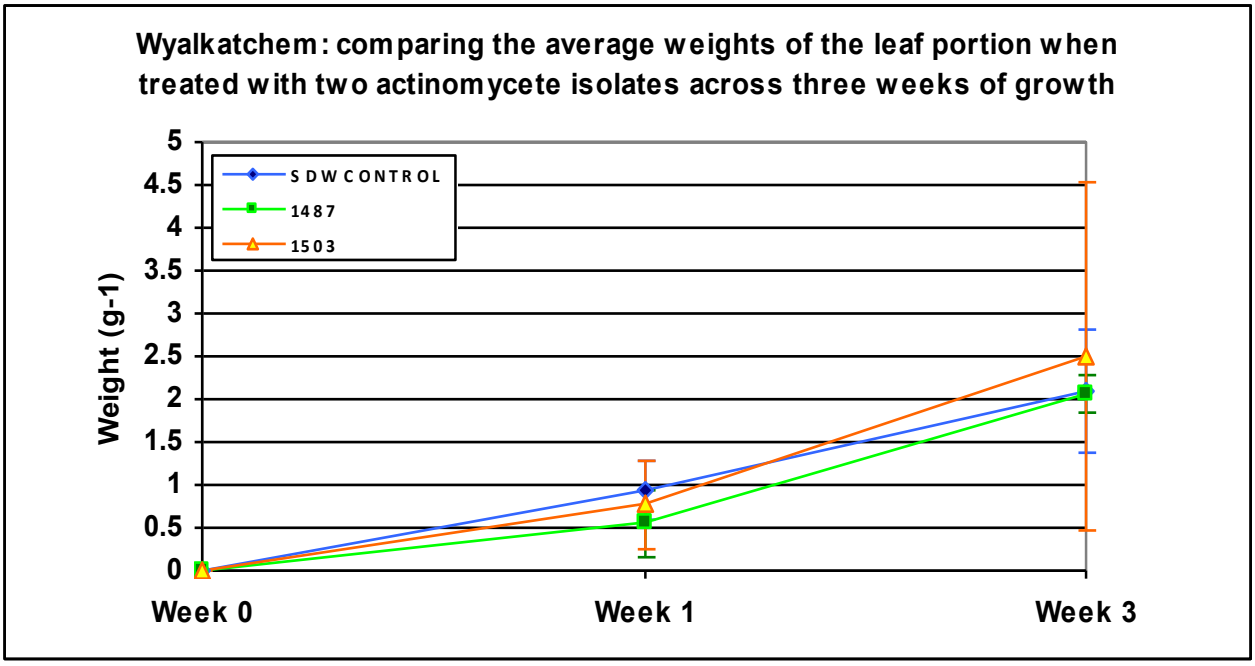
- all (10) control plants grew well and produced up to 3 leaves;
- 7 of the ACN 1487 test plants produced up to 2 leaves; and
- 8 of the ACN 1503 test plants produced up to 3 leaves, with 1 tube contaminated with fungal growth.

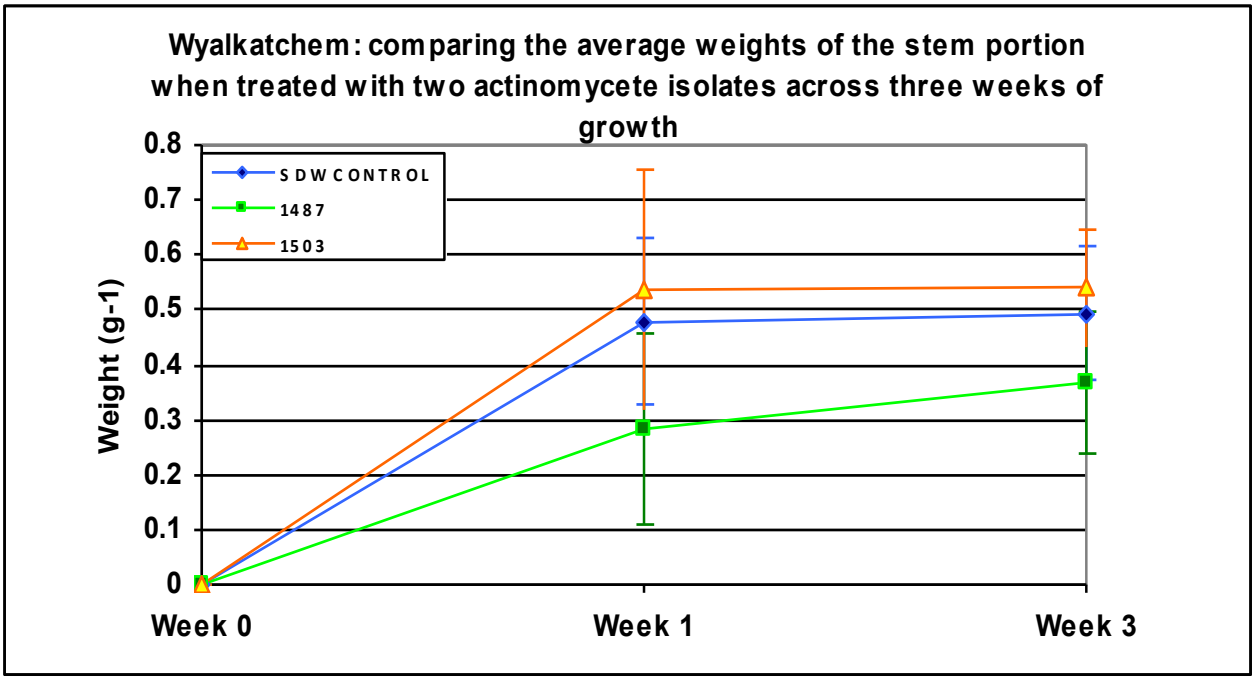
By the end of the third week (day 22) since transplant the remaining plants (5 plants per plant type) showed continued healthy development among:

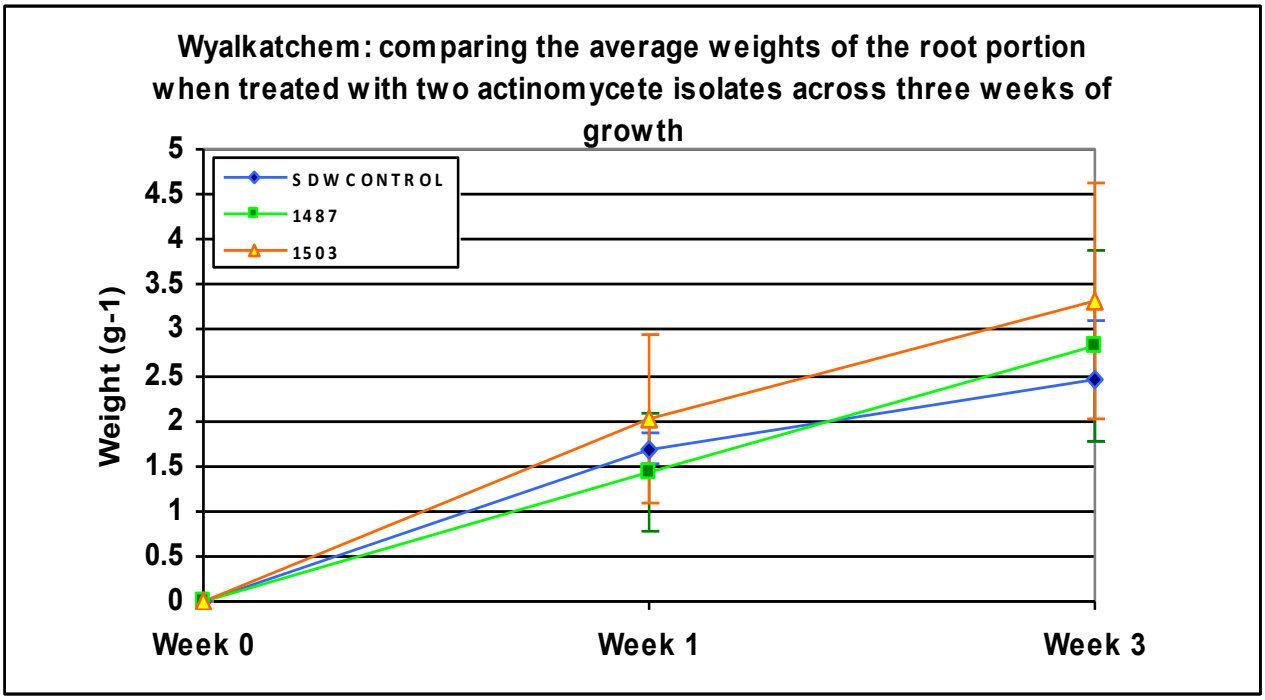
- all control plants, which produced up to 4 leaves;
- 3 of the 5 ACN 1487 plants, which produced up to 4 leaves, with 2 tubes showing nil growth; and
- 3 of the 5 ACN 1503 plants, which produced up to 3 leaves, with 2 tubes contaminated with fungi with nil plant growth.

The monitoring of plant biomass production across three weeks of growth showed that the highest weights recorded for the leaf, stem and root portions were mostly from ACN 1503, despite less number of leaves turned and 2 shoots failed to mature relative to control (see section 3.6 below). The average weight values of the leaf and root portions of the 7 surviving ACN 1487 plants were lower than control by the end of the first week, however higher if not the same as control by the end of the third week.

4.6 The growth patterns of Wyalkatchem wheat seedlings with respect to leaf biomass, stem biomass and root biomass







4.7 Broad bean pot trials

After the wheat seed trials were conducted at the Actinogen Laboratory, Dr Keast performed pot trials on broad beans at a field laboratory he had set up at a private property in Kalamunda. This trial was carried out in small pots under semi field conditions with a total of 40 replicas and as such could not be carried out within the confines of Actinogen Laboratory. This property is available to Actinogen on a continuing basis.

The process for the pot trials was as follows:

- The two actinomycetes were grown in liquid SAB medium for 3 weeks and then diluted to contain at pot inoculation approximately 3 X 10^{12} viable bacteria/spores per trial pot.
- Bacteria and culture media were added to ten replica pots per test in 30 mL volumes. Water and sterile medium control series were also established.
- The seedling pots were filled with an indoor/outdoor potting mix.
- Each pot then received one broad bean which had been dipped in one of the treatment materials.
- All pots were treated every second day with four sprays of water from a standard hand spray.

- All treatment groups were observed daily and once germinated, above ground growth was recorded every two days (on a centimetres of growth basis).

- On day 45 of the trial, all plants were harvested and the wet weights of the total plant, the leaves and stem and the roots were recorded.

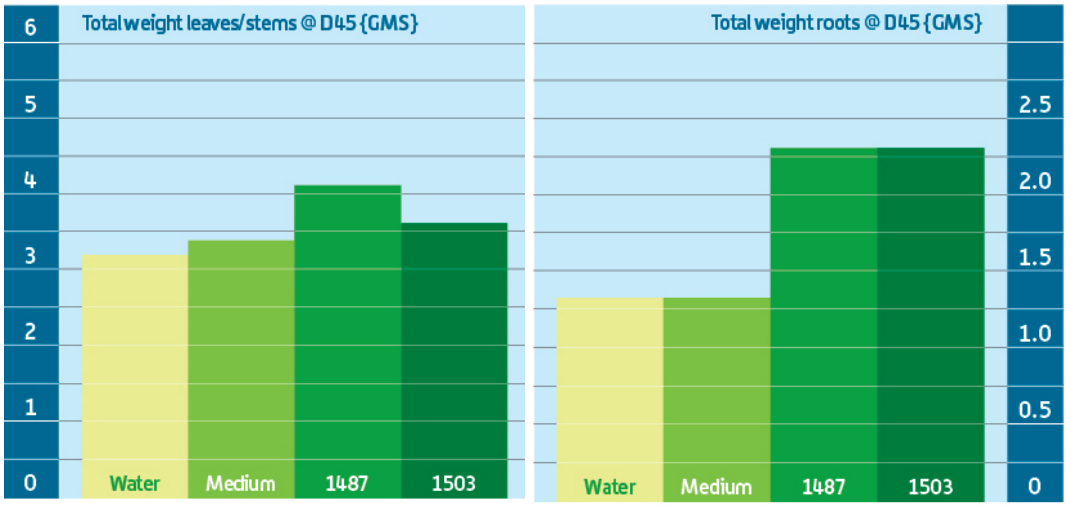


Figure 3.7 The results illustrated as histograms for the green plant and the root production following the protocols described above.

The results (Figure 3.7) indicate that the trials using the actinomycete isolates ACN 1503 and ACN 1487 stimulate both green wet weight and root wet weight over and above controls. The major contribution to the growth of these plants is by the stimulation of added root growth.

4.8 Pot trials on peas and wheat in March 2013 (first independent trials)

Independent greenhouse trials were then conducted at an independent university to confirm (or otherwise) Actinogen's in house trials. The independent university's trials included (in addition to ACN 1487 and ACN 1503) a new actinomycete, ACN 4432, which was found by Actinogen after it had conducted its in-house trials and showed the potential to produce plant growth hormones.

The process used for the independent trial was as follows:

- 100 mL cultures of actinomycete isolates ACN 1487, ACN 1503 and ACN 4432 were inoculated and incubated for up to 2 weeks.

- The cultures were then spun down, pooled and resuspended in sterile distilled water with a final volume of 250mL.

- The viability of the actinomycetes used were determined as colony forming units/mL (cfu/mL) of each isolate in the final suspension are shown in Table 2 below.

Table 2: the colony forming units per mL used in the first independent trial.

Isolate	Average (cfu/mL)	Standard deviation
ACN 1487	2.11×10^5	7.07×10^3
ACN 1503	1.39×10^5	3.82×10^4
ACN 4432	1.47×10^5	2.12×10^4

- The actinomycetes had to be cultured in the Actinogen Laboratory and checked by HPLC before being sent to the greenhouse for application.

- Actinogen took no further part in the design or running of the trial or its monitoring.

- The actinomycete isolates were then applied to the pots in the same liquid form as the previous experiments. There were 30 pots containing wheat seeds (5 seeds per pot) and 30 pots containing pea seeds (5 seeds per pot).

- The first harvest was performed at 5 weeks. Four pots of wheat and four pots of peas were harvested and measured.

- The second harvest was performed at 7.5 weeks. 17 pots of wheat and 21 pots of peas were harvested and measured.

4.9 Wheat results for the first independent trials

Leaf, stem and root results

The results for the growth of the leaf, stem and root portions of the wheat plants compared to the control plants were not reported because:

- The results at 5 weeks contained a mathematical recording error. Actinogen could not verify the results, so the results were not reported.

- The results at 7.5 weeks were not conclusive because the time span to harvest the plants had proved to be too long and the plants had become 'root-bound'. The plants should have been harvested at 6 weeks and another trial was planned for harvest at 6 weeks.

For these reasons Actinogen decided not to report these results. Given these limitations, Actinogen decided to perform a second round on independent testing for the leaf, stem and root portions as described in more detail in section 4.11 below.

Tillers (shoots) results

Despite the above limitations for the leaf, stem and root results, the results for the growth in tillers (shoots) for the wheat were clear. The tillers from the wheat plants in the 17 pots harvested at 7.5 weeks were measured. The control plants were compared to the plants that received the actinomycetes. There was an increased amount of tillers on the plants that received the actinomycetes as compared to the control plants.

Control plants had on average produced 15 tillers per pot while plants grown in the presence of the actinomycetes produced on average 18 tillers per plot.

4.10 Pea results for the first independent trials

At 5 weeks the pea plants that received the actinomycetes were not growing better than the control plants. At 7.5 weeks, the pea plants that received the actinomycetes showed an average increase in growth for the pea tops of 10.9%. The pea roots were not measured because they were too brittle.

There were 10 pots of peas harvested at 7.5 weeks that had received the actinomycetes and 11 pots of plants harvested that had not received the actinomycetes. There were 27 plants measured that received the actinomycetes and 33 control plants measured.

4.11 Results from the second independent wheat trial

Given the limitations from the first independent wheat trials, Actinogen instructed the independent university to conduct a second round of wheat trials.

The trials were conducted in a non-sterile soil, with all nutrients and water supplied in a non-limiting amount. There was no root disease evident, and the plants were healthy. There were 21 control plants measured and 24 plants that received actinomycetes measured. The plants were all harvested at 6 weeks.

Actinogen received the results from the second round of trials on 27 August 2013. The results showed that the plants that received actinomycetes had very slightly increased tillering, but very slightly decreased root and shoot growth.

In summary, the results showed that there was no real effect on the wheat from the application of the actinomycetes.

4.12 Summary of results

Actinogen's testing has shown:

- an increase in the leaf, stem and root weights of wheat plants treated with ACN 1503 compared to the control plants;

- an increase in the root weight, a decrease in the stem weight and no effect on the leaf weight for wheat plants treated with ACN 1487 compared to the control plants; and

- an increase in the leaves, stems and root weights for broad bean plants treated with ACN 1487 and ACN 1503 when compared to the control plants.

The independent testing completed at the independent university showed an increase in the wheat tillers from the plants that received actinomycetes compared to the control plants and a 10.9% increase in growth for pea tops that received actinomycetes compared to the control plants.

As mentioned in section 4.9 above, the initial independent testing results for the wheat leaf, stem and roots were compromised and the independent university repeated that trial in order to obtain reliable results. The results of that trial were sent to Actinogen on 27 August 2013 and showed no real difference between the control plants and the plants that received actinomycetes.

The Company's results from the Plant Growth Project to date have been on a molecular level, and must be repeated on a much larger scale in order to be commercially viable. There is no guarantee that the Plant Growth Project will ultimately be commercially viable.

4.13 IP Protection

Actinogen has lodged a provisional patent in the past, which has now lapsed because Actinogen determined that the project was not developed enough (and did not warrant incurring the significant application costs) to proceed to a full patent application. Actinogen is not ready to proceed to a complete patent application, however the actinomycetes are protected under the Budapest Treaty.

4.14 Current status of the Plant Growth Project

As mentioned in section 4.11 above, the results in the second independent wheat trial showed no real effect on the growth of wheat from the application of the actinomycetes. Actinogen is currently considering performing another trial on peas and then on broad beans to see whether the actinomycetes have a greater effect on peas and broad beans than on other types of plants. Actinogen is also considering what further trials it should perform with wheat, since there were significant results obtained in the first independent trials regarding the wheat tillers. Actinogen will keep shareholders updated on further trials in the Plant Growth Project.

4.15 Project timeline

Actinogen is continually looking for more funding and partners to continue the progression of the plant growth project. Actinogen has developed an estimated timeline for the Plant Growth Project:

Stage	Milestone	Estimated completion
1.	Actinogen Laboratory trials on wheat seeds	Completed in 2011
2.	Pot trials on broad beans	Completed in 2011
3.	Independent pot trials on wheat and peas	Completed in August 2013
4.	Pot trials on peas	Planned for September/October 2013
5.	Independent pot trials on broad beans	Planned for March 2014
6.	Larger scale pot trials on wheat, peas and broad beans	Planned for 2014
7.	Field trials on wheat, peas and broad beans	Planned for Spring 2014/Spring 2015 (depending on the results of the trials above)

Actinogen is currently up to stage 4 of the timeline. Actinogen should be finished with the trials by the end of 2015. However, this timeline is just an estimate based on all the current results in the Plant Growth Project. With each trial's results, the goals and aims in the Plant Growth Project may change and the estimated timeline may be altered.

Actinogen has previously made reference to awaiting government approvals for the Plant Growth Project. That reference was to approval from the Australian Pesticides and Veterinary Medicine Authority (**APVMA**) for registration of a product. Actinogen has since determined that registration with APVMA is not required.

5. ANTIBIOTIC RESEARCH PROJECT

5.1 Background

Actinogen continues to run its isolation, screening and research and development program in order to find actinomycetes that produce substances which have an effect against bacteria, including bacteria which has become antibiotic resistant.

5.2 Actinogen's method

Actinogen collects soil samples which it then screens for new actinomycetes. If new actinomycetes are found, they are added to Actinogen's private existing database of over 6000 actinomycetes.

The new actinomycetes are then tested against the MRSA panel, *Candida*, VRE, *P. aeruginosa* and the anaerobic pathogen Clostridium difficile, to determine whether they have activity against the bacteria. These testing panels consist of clinical isolates of microorganisms that have developed serious antibiotic resistance patterns and can therefore be used to increase the likelihood of finding new antibiotics.

Actinogen employs a series of screening tests which become more stringent. Primary screening is a rapid test to detect the production on solid agar of an isolate producing an antibiotic directed to one or more of the test organisms outlined above.

Secondary screening is then carried out on known antibiotic producing isolates, in liquid culture. Tertiary screening is then used to determine the chemical nature of the antibiotic.

Once an actinomycete produces a substance in the Actinogen Laboratory that shows resistance to bacteria such as MRSA, Actinogen tries to identify the substance from the public literature and databases. If the substance cannot be matched to an existing substance, it is sent to an independent laboratory to obtain a molecular structure of the substance.

Actinogen has lodged a small series of isolates under the Budapest treaty (See section 3.6 above).

5.3 Actinogen's results

The following results are the results from the Antibiotic Research Program.

MRSA panel, Candida, VRE and P. aeruginosa screening

After primary screening:

- 294 isolates have activity against the entire MRSA panel and 74 have activity against some of the panel.
- 113 isolates have activity against the entire panel ± one strain of *Candida* and 96 have activity against part of the panel.
- 167 isolates have activity against VRE.

- 6 isolates have activity against *P. aeruginosa* after secondary testing.
- 7 isolates have quorum quenching ability.

After tertiary screening:

- 69 isolates have activity against the entire MRSA panel.
- 11 isolates have activity against the entire Candida sp. panel.
- 58 isolates have activity against VRE.

Clostridium difficile screening

405 isolates have been tested; 23 have activity against *C. difficile* and after primary screening:

- 17 isolates have activity against all 3 C. difficile strains tested.
- 1 has activity against PCR ribotype O27 and WA25.
- 1 has activity against O27 and R1 (VPI10463).
- 3 have activity against R1 and WA25.
- 1 only has activity against O27.

Conclusion

The results show that Actinogen has found substances that have activity against the MRSA panel and Clostridium difficile. Each substance with activity against the MRSA panel and Clostridium difficile has the potential to become a new antibiotic, however significant further testing is required in order for this to be established. There is no guarantee that any of these substances will be successfully established as a new antibiotic.

5.4 Current status of the Antibiotic Research Project

If the substance cannot be matched to an existing substance, it is sent to an independent laboratory to obtain a molecular structure of the substance. This testing is expensive and Actinogen has had to suspend sending actinomycetes samples to the independent laboratory for the present time until further funds are raised. However, the collection of new samples, the screening program and bench level testing are ongoing in the Actinogen Laboratory.

The Company's results from the Antibiotic Research Project to date have been on a molecular level, and must be repeated on a much larger scale in order to be commercially viable. There is no guarantee that the Antibiotic Research Project will ultimately be commercially viable.

6. SALT TOLERANT ACTINOMYCETES PROJECT

6.1 Background

Actinogen is currently running a research program on salt tolerant actinomycetes. Actinogen screens actinomycetes from its existing database and tests them to see if they have any ability to survive in salty environments. The aim of this research is to develop a product that will help farmers and other plant producers grow plants and crops in salt affected environments.

6.2 Current work and status of the Salt Tolerant Actinomycetes Project

Actinogen has screened 100 actinomycetes to test for salt tolerance. The screening process was as follows:

- The isolates were inoculated onto an ISP2 agar with NaCl concentrations of: 0%, 2.5%, 10%, 15%, and 20% weight/volume.

- The isolates were then incubated for 14 days at 26°C, following which the growth of the isolates was recorded.

The results of Actinogen's screening are summarised in the table below.

	0% NaCl	2.5% NaCl	10% NaCl	15% NaCl	20% NaCl
High growth	64	32	-	-	-
Medium growth	36	45	-	-	-
Low growth	-	13	4	-	-
Nil growth	-	10	96	100	100

The level of colony development is a visual observation taken by experienced staff.

Conclusion

The more salt tolerance an actinomycete exhibits, the more likely it is that it will establish and survive in soils containing high levels of salt. The four isolates that can tolerate 10% saline have the potential to survive in high salt environments and continue to lead to the production of humus to aid in the re-establishment of salt tolerant plants and the rehabilitation of salt affected soils.

There is a wide range of soil salinity in nature, with the saturation level in solution for pure salt being around 23%. Thereafter salt is deposited as crystals. It is known that in WA salinity of soils can vary over a wide range up to and including levels where salt is deposited as crystals where rehabilitation of these soils would be unlikely to occur using microorganisms in the first instance.

Actinogen's next phase it to move to field trials with soils of various levels of salinity, however those field trials are on hold until Actinogen raised additional funding.

The Company's results from the Salt Tolerant Actinomycetes Project to date have been on a molecular level, and must be repeated on a much larger scale in order to be commercially viable. There is no guarantee that the Salt Tolerant Actinomycetes Project will ultimately be commercially viable.

7. BIOETHANOL PROJECT

7.1 Background

On 9 November 2011, the Company announced that it had discovered actinomycete isolates that produce cellulose, a key component in the production of bioethanol. The most effective actinomycete isolate discovered by Actinogen, being ACN 4205, has been deposited at the National Measurement Institute in Melbourne under the Budapest Treaty.

7.2 What is bioethanol and cellulase?

Bioethanol is a source of renewable energy made from fermenting sugars. It has 2 types:

- first generation bioethanol, which uses sugars from foodstuffs such as corn and sugar cane in the fermentation process; and

- second generation bioethanol, which uses cellulase to break down biomass (eg green waste and newspaper) to produce sugars for the fermentation process.

Second generation bioethanol production is preferable to first generation because it uses green waste rather than crops and food which could be used for human consumption.

Cellulase is one of the key components of making second generation bioethanol as it is used to break down the biomass and releases cellulose, which is then converted into sugars and waste. The sugars are then used in a fermentation process to produce bioethanol.

7.3 Actinogen's method

Actinogen screens for cellulase production from actinomycetes using an agar well diffusion assay on cellulose Congo red agar (CCRA) plates, with larger zones of clearing indicative of greater cellulase activity.

Actinogen's hypothesis was that defining the mathematical relationship between cellulase concentration and zone size by generating standard curves would allow more accurate assessment of cellulase activity from different isolates and cellulase sources.

Actinogen tested that hypothesis for 2 commercial cellulases (*Trichoderma virde* and *Trichoderma longibrachiatum*) and for cellulose produced from actinomycete ACN 4205, with the results shown in section 7.4 below.

7.4 Results

Testing for commercial cellulases

For the most part, the repeat well diffusion experiments of each cellulase were in concordance and standard deviations were generally low. However, the well diffusion results differed between the two cellulases tested and the *T. virde* cellulase typically produced a larger zone of clearing than the *T. longibrachiatum* cellulase at a given dilution.

Despite claims of similar levels of activity for both commercial cellulases, the results from this experiment indicate that the *T. virde* cellulase is more potent than the *T. longibrachiatum* cellulase as assessed by the diameter of the zone of clearing on CCRA.

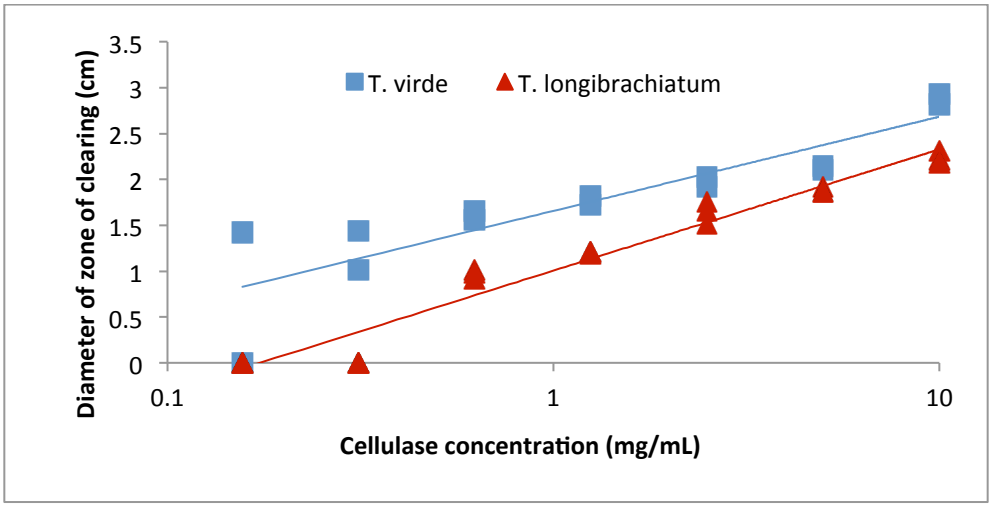


Figure 4.4.1. Standard curves of the relationship between cellulase concentration and diameter of the zone of clearing on CCRA.

Graphical representation of the zone of clearing data resulted in distinct standard curves for each cellulase standard (Figure 4.4.1). This suggested that standard curves of cellulase activity are specific to particular cellulases.

Comparison of ACN 4205-produced cellulase against and commercial cellulases

The specificity of the cellulase standard curves means that cellulase production of isolate ACN 4205 cannot be truly quantified without an appropriate standard; the identity of the cellulase enzymes produced by ACN 4205 are currently unknown. However, it is possible to generate standard curves for cellulase produced by filtrates of ACN 4205 cultured under different conditions and to compare these against *T. virde* and *T.*

longibrachiatum cellulase standard curves to gauge the relative strengths of the various cellulases.

Zone sizes were measured at 2 days and 4 days and zone diameters increased from day 2 to day 4 as the cellulase diffused further through the agar. Zone sizes were noticeably bigger for all the ACN 4205 filtrates compared to the *T. virde* and *T. longibrachiatum* commercial cellulases.

The well diffusion data measured at 4 days incubation was used to generate a standard curve for each cellulase (Figure 4.3.2). As expected, each cellulase produced a distinct standard curve. The standard curves for the ACN 4205 filtrates were also positioned higher on the y-axis, suggesting that they produce greater cellulase activity compared to an equivalent dilution of the *T. virde* and *T. longibrachiatum* cellulases.

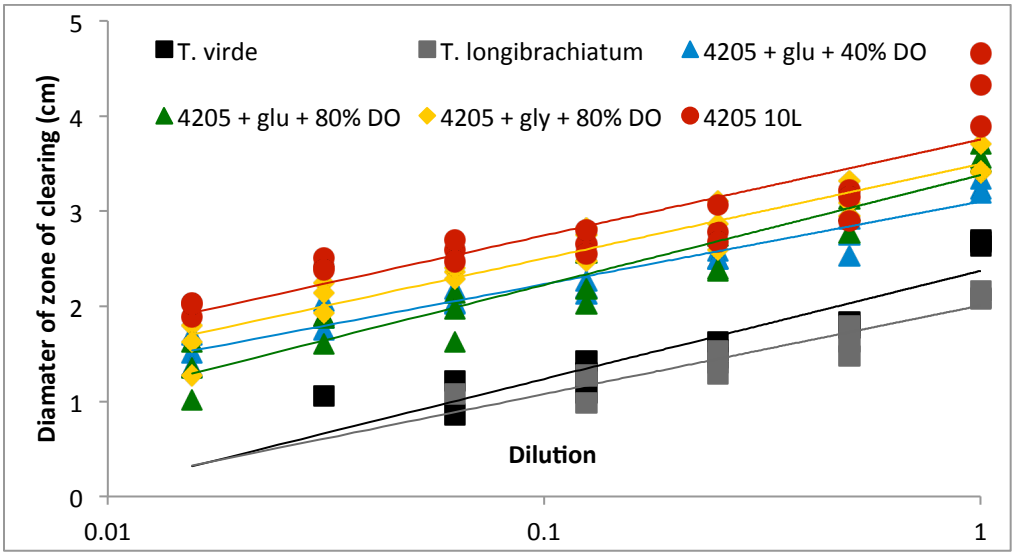


Figure 4.4.2. Standard curves of the relationship between cellulase concentration and diameter of the zone of clearing on CCRA.

A function describing the trendline for each cellulase dilution series was also calculated. Using the logarithmic functions for the *T. virde* and *T. longibrachiatum* standard curves, the equivalent dilution strength (x) could be calculated for each of the 4205 filtrate cellulases using the average measured zone diameters for a neat sample (y). As the *T. virde* and *T. longibrachiatum* standards are of known concentration, the equivalent concentration of each of these cellulase standards can also be calculated. This data is summarised in Table 4.4.3 below. Note that these estimates are predicated on the assumption that the functions can be used to extrapolate beyond the range of the experimentally collected readings.

Table 4.3.3. Comparison of ACN 4205 filtrate cellulase activity to standard commercial cellulases from *T. virde* and *T. longibrachiatum*.

ACN 4205 filtrate	Zone (cm)	Strength compared to commercial cellulases		Equivalent concentration of commercial cellulases (g/mL)	
		T. virde	*T. longibrachiatum*	*T. virde*	*T. longibrachiatum*
Glucose + 40% DO	3.25	6.00	21.95	0.06	0.21
Glucose + 80% DO	3.59	11.95	50.87	0.11	0.50
Glycerol + 80% DO	3.51	10.02	41.06	0.10	0.41
10L	4.29	49.00	284.78	0.49	2.84

Results indicate that ACN 4205 grown in SAB with glycerol and 80% DO in a 10L culture volume has the greatest cellulase activity of the 4 different growth conditions trialed for isolate ACN 4205. Its activity is 49-fold higher than cellulase from *T. virde* and 284-fold higher than cellulase from *T. longibrachiatum* (Table 4.3.1) with activity equivalent to 0.49 g/mL of *T. virde* cellulase and 2.84 g/mL *T. longibrachiatum* cellulase.

Although these results cannot objectively quantify the activity or concentration of cellulase produced by isolate ACN 4205, they can provide a general estimate of its strength relative to other cellulases.

Conclusion

The use of standard curves is an easy method for comparing the relative cellulase strength of different samples. Although this method did not quantify the cellulase activity of the samples investigated, it was able to estimate equivalent strength of cellulase samples when commercial cellulases of known concentration and activity were included in the comparison.

Results indicate that ACN 4205 grown in SAB with glycerol and 80% DO in a 10L culture volume has the greatest cellulase activity of the 4 different growth conditions trialed for isolate 4205. Its activity is 49-fold higher than cellulase from *T. virde* and 284-fold higher than cellulase from *T. longibrachiatum*.

These studies were independently confirmed by an independent scientific organisation, which in addition indicated that low levels of reducing sugars were also generated.

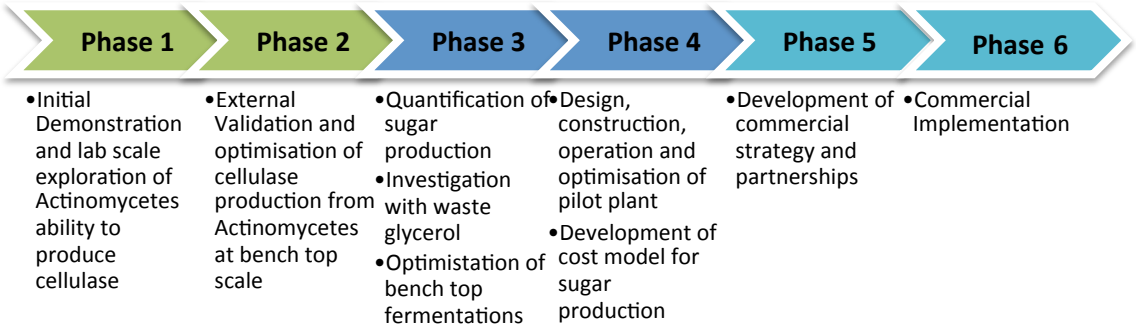
Actinogen subsequently procured an independent report on its work and the project from Dr Karne De Boer, managing director of Regenerate Industries Pty Ltd. Dr De Boer's report concluded that:

"Actinogen has made an exciting breakthrough in the area of second generation biofuels that could lead to reducing the cost of converting any biomass – trees, grass or paper into a green renewable fuel. The research work already conducted by Actinogen provides a solid foundation for future growth. The report has outlined a suggested pathway for Actinogen to bring about commercial results from this breakthrough."

A full copy of the independent report and details of the history of the Bioethanol Project are contained in the Actinogen rights issue prospectus dated 26 March 2012.

7.5 Development strategy

Actinogen followed the recommendations of Dr De Boer and adopted a proposed development strategy as set out below:



7.6 Current status of the Bioethanol Project

The Bioethanol Project is currently in phase 3 of the above development strategy, and temporarily on hold due to lack of funds.

The Company has performed further work on the Bioethanol Project in 2012 and 2013 by investigating the use of a range of yeasts in the fermentation process to convert the cellulase end products to bioethanol.

The next phase of the commercialisation strategy is to build a pilot plant, however the directors of the Company have elected to put the pilot plant on hold until further funding is raised and the results demonstrate that sufficient sugars are being produced by Actinogen's cellulose to commercially produce bioethanol.

The Company's results from the Bioethanol Project to date have been on a molecular level, and must be repeated on a much larger scale in order to be commercially viable. There is no guarantee that the Bioethanol Project will ultimately be commercially viable.

8. SHIKIMIC ACID PROJECT

8.1 Background

In July 2012, Actinogen discovered that it can produce shikimic acid from certain actinomycetes. This shikimic acid has been produced on a molecular level only, and not yet on a scale sufficient to commercialise this project.

Shikimic acid is the main component (and one of the most expensive components) used to produce the influenza medication Tamiflu.

One of the main manufacturers of Tamiflu (Roche) produces shikimic acid in 2 ways:

- harvesting it from a specific type of star anise grown in four mountain provinces in the south west of China (Guanxi, Sichuan, Yunnan und Guizhou) which provides a much higher purity and yield than the one grown elsewhere; and

- using a fermentation process where a special e-coli bacteria is overfed glucose, producing shikimic acid. The bacteria need to be multiplied and grown, and are transferred from small to large and larger fermenters.

Today the majority of shikimic acid is derived from the fermentation process.

Information in this section regarding Roche's production of shikimic acid is taken from the Roche fact sheet for Tamiflu, 17 November 2006 (available at http://www.roche.com/med_mbtamiflu05e.pdf).

Actinogen's method for the production of shikimic aid is different from, and potentially cheaper than, the current processes of producing shikimic acid because the shikimic acid has the potential to be produced in large scale fermenters in the open air at ambient temperature.

8.2 Actinogen's method

Actinogen has been able to produce shikimic acid using actinomycetes (and test that production) using the following method:

- Shikimic acid producing actinomycete isolates ACN 1488 or ACN 1512 are inoculated into liquid culture.

- The culture is incubated at 26°C with shaking at 110 rpm for 5 to 7 days.

- The presence of shikimic acid in the culture supernatant is confirmed by HPLC.

- Chromatograms were constructed and ultra violet spectra at 204 nm were compared by dereplication with three UV-spectral libraries containing spectra for bioactive molecules.

8.3 Results

The following chromatograms are from HPLC and fraction collection of ACN 1488 and 1512 (Figure 5.3.1 & 5.3.2 below). When the fraction collection method is used (injection volume 100 µl and flow rate 1 mL/min), the retention time for shikimic acid is 2.2 minutes. In the normal HPLC method (injection volume 10 µl, and flow rate 0.4 mL/min.), the retention time of the shikimic acid peak is 3.3 minutes.

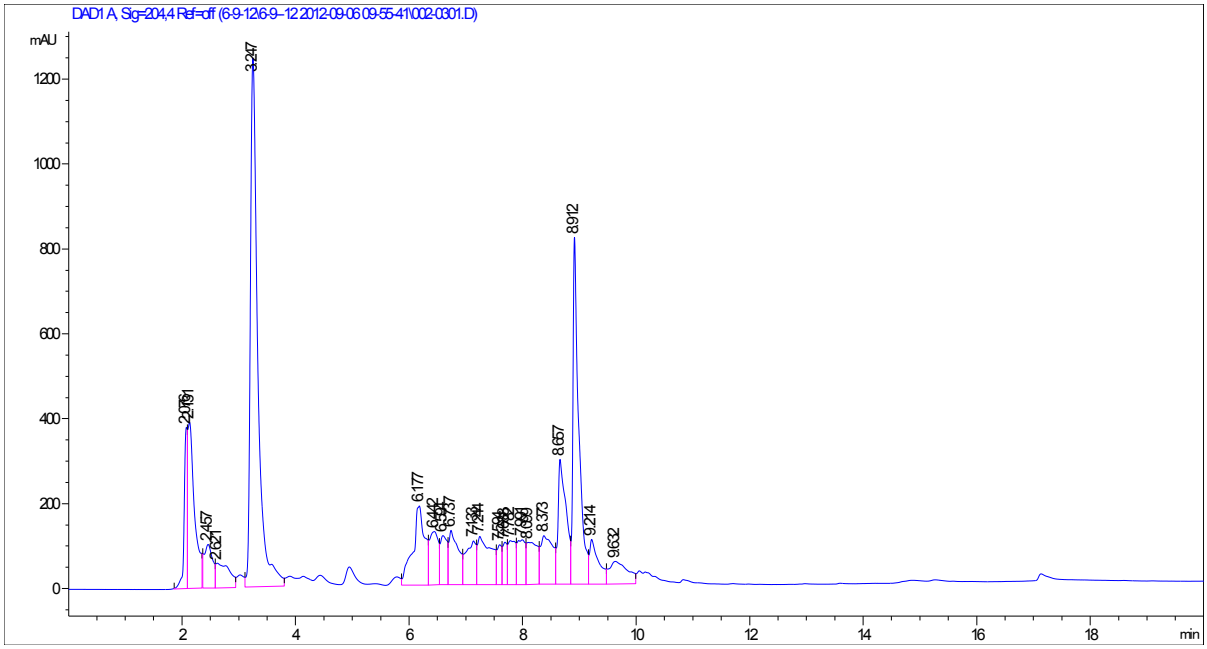


Figure 5.3.1: The HPLC chromatogram of the culture medium from ACN 1488 incubated at 26°C with shaking for 6 days. The shikimic acid peak at retention time 3.2 minutes.

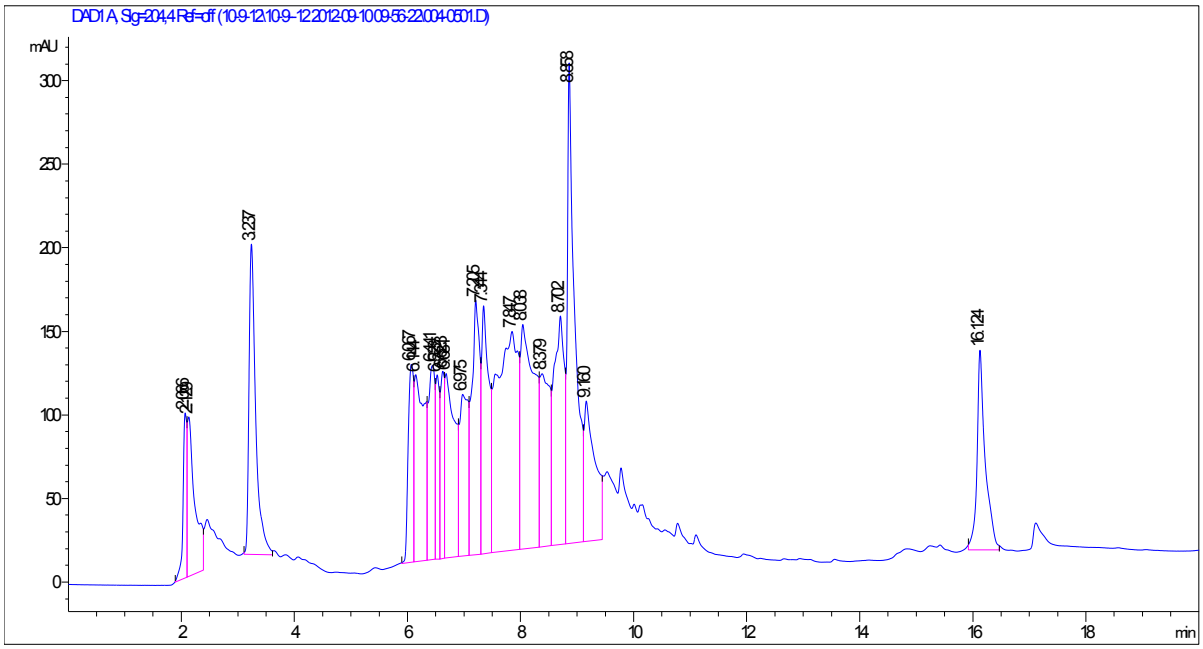


Figure 5.3.2: The HPLC chromatogram of the culture medium from ACN 1512 incubated at 26°C with shaking for 7 days. The shikimic acid peak at retention time 3.2 minutes.

When the HPLC fractions of both ACN 1488 and ACN 1512 were extracted and collected from the HPLC fractions and re run through the HPLC the major peaks remained at the recovery times of 2- 3 minutes and were again shown to be shikimic acid (Figure 5.3.3).

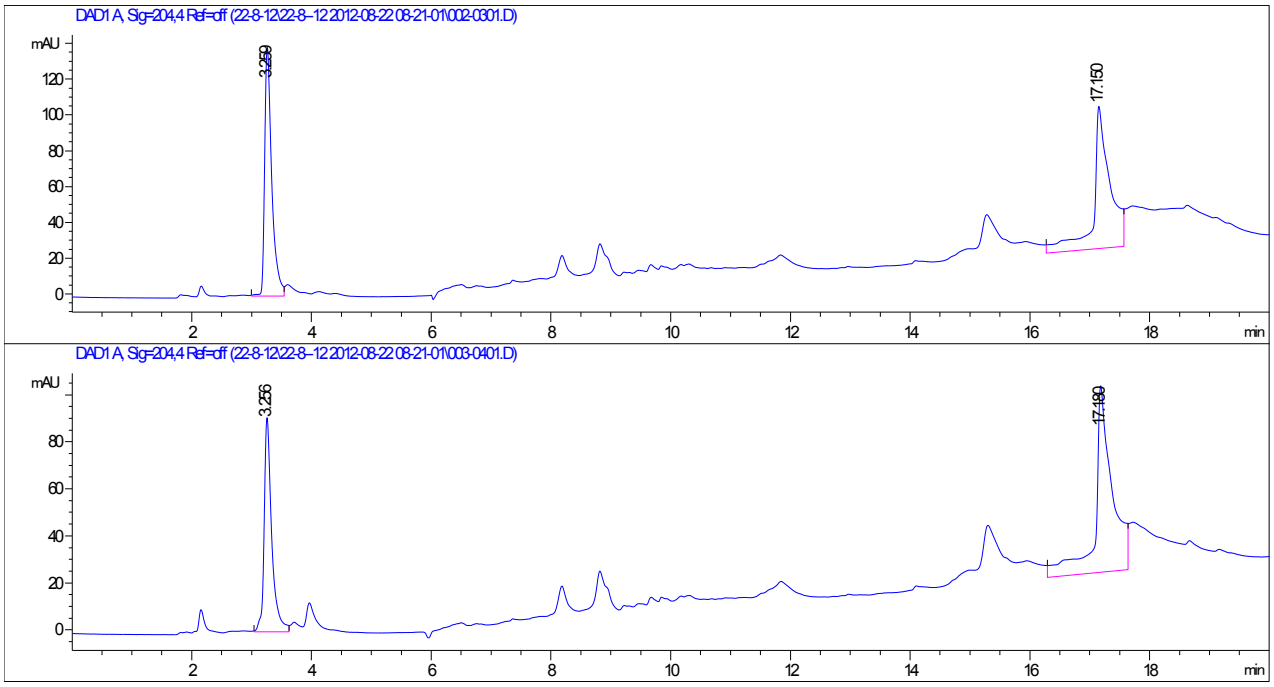


Figure 5.3.3: The HPLC profiles of extracts of the original chromatographic peaks detected at 2-3 minutes. Top: Fraction (collected between 2-3 minutes) of 1488 put through the HPLC, shikimic acid peak at 3.3m. Bottom: Fraction (collected between 2-3 minutes) of 1512 put through the HPLC, shikimic acid peak at 3.3m

Figure 5.3.4 below represents the ultra violet profile of the 2-3 minute peak (blue) from ACN 1488 compared to the ultra violet profile of pure shikimic acid (red). The profiles match to a degree that indicate that the ACN 1488 profile represents shikimic acid. Final confirmation will come from mass spectrometry, which is currently on hold.

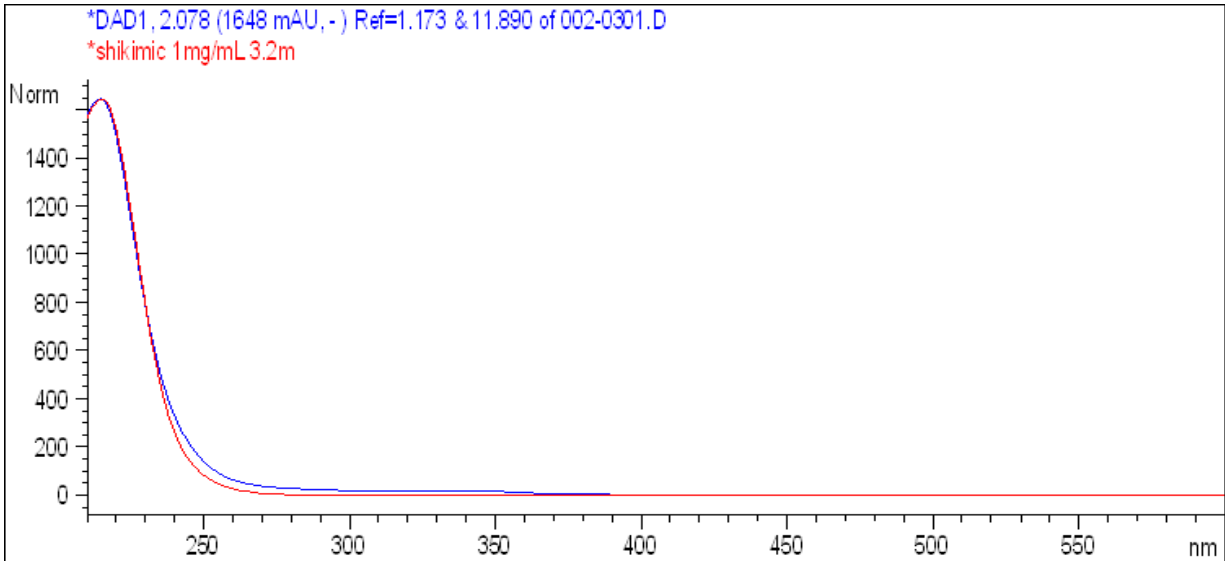


Figure 5.3.4 Comparison of the ultra violet profiles of the 2-3 minute HPLC chromatograph for ACN 1488 (blue) and pure shikimic acid, (red).

8.4 IP Protection

Actinogen has registered only the ACN 1488 actinomycete that produce shikimic acid under the Budapest Treaty. ACN 1512 was found to not produce shikimic acid when cheap potato starch replaced the pure starch used in the developmental trials and so has not been registered to date.

8.5 Current status of the Shikimic Acid Project

Actinogen has put the Shikimic Acid Project on hold to conserve cash for the development of the Plant Growth Project. Actinogen intends to pursue the Shikimic Acid Project once further funding is raised.

The Company's results from the Shikimic Acid Project to date have been on a molecular level, and must be repeated on a much larger scale in order to be commercially viable. There is no guarantee that the Shikimic Acid Project will ultimately be commercially viable.

9. BIOFUMIGATION PROJECT

9.1 Background

In early 2012 Actinogen found actinomycetes that synthesize volatile bioactive molecules (polyenes) which may inhibit and/or kill pathogenic fungi. Actinogen is investigating whether these polyenes can be used for biofumigation.

9.2 Actinogen's method and results

Actinogen ran experiments in the Actinogen Laboratory which showed that the actinomycetes producing polyenes did have an effect on certain plant pathogenic fungi.

The methodology developed by Actinogen remains confidential at this time as it may have patent implications.

9.3 IP Protection

Actinogen made an application for a provisional patent to protect the process. The provisional patent has lapsed but the relevant actinomycete has been registered under the Budapest Treaty.

9.4 Current status of the Biofumigation Project

Actinogen has put the Biofumigation Project on hold to conserve cash for the development of the Plant Growth Project. Actinogen intends to pursue the Biofumigaton Project once further funding is raised.

The Company's results from the Biofumigation Project to date have been on a molecular level, and must be repeated on a much larger scale in order to be commercially viable. There is no guarantee that the Biofumigation Project will ultimately be commercially viable.

10. CANCER STEM CELL RESEARCH PROJECT

10.1 Background

Actinogen acquired Celgenics Limited (**Celgenics**) in 2011, including all of Celgenics' equipment, new actinomycetes database and access to two human cancer cell lines for testing. Actinogen has continued Celgenics' research, with particular focus on the cancer cell lines which have expressed cancer stem cell antigens.

10.2 What are cancer stem cells?

Cancer stem cells (**CSCs**) are defined as those cells within a tumour that can self-renew and drive tumorigenesis. Rare cancer stem cells have been isolated from a number of human tumours, including haematopoietic, brain, colon and breast cancers.

Such cells are proposed to persist in tumours as a distinct population and cause relapse and metastasis by giving rise to new tumours. Therefore, development of specific therapies targeted at CSCs holds hope for improvement of survival and quality of life of cancer patients, especially for sufferers of metastatic disease.

10.3 Work done by Actinogen

The 2 cell lines obtained from Celgenics that have been known to produce cancer stem cells are U87MG and U125MG.

Actinogen tested these cell lines as follows:

- Neurospheres of U87MG and U125MG were isolated by a series of media manipulations, including:
    - no Foetal Calf Serum (a serum prepared from foetal calf blood and is a routine component of most tissue culture media);
    - DMEM/F12 as a base media instead of minimal essential media;
    - growth supplements (including human recombinant epidermal growth factor, human fibroblast growth factor B and insulin growth factor); and
    - antibiotics (gentamicin sulphate and amphotericin B).
- Adherent cells were incubated in half neurospheres isolation media and half growth media for four days, after which cells were passaged and grown in neurospheres media.

- After 4 days, neurospheres began to grow. These initially grew in large clumps so 10% Accutase was added to the neurosphere isolation media to dissociate the neurospheres into single cells as preparation for flow cytometry.

- To prepare cells for flow cytometry, neurospheres were treated with 10-20% Carboplatin (concentration dependant on cell lines used for testing) and/or actinomycetes supernatants (at various concentrations) for 24 hours. The actinomycete supernatents are prepared from the culture media of actinomycetes isolated by Actinogen and are being screened for the presence of potential anti cancer agents.

- After this, cells were collected by centrifugation, stained with CD133-PE (to stain for CD133+ surface marker) and 7AAD (viability stain), and then analysed by FACS Calibur Flow cytometer.

- Optimisation was conducted to determine the optimal concentration of Carboplatin to be used against the different cells and the optimum time of treatment.

10.4 Results

A total of 11 actinomycetes supernatants have been tested against U87MG and U125MG neurospheres. These were previously tested with 20% Carboplatin to assess whether:

- treatment of Carboplatin removed all CD133- cells; and

- actinomycetes isolates were toxic to CD133+ remaining cells.

Results were varied and depended on the specific isolates tested. For most, after treatment of Carboplatin, viability was approximately 30-50%, and these consisted of a high number of CD133+ cells. After treatment of these cells with actinomycetes supernatants, two isolates killed the whole cell population (ACN 5059 and ACN 5086) whereas the remaining samples with other isolate numbers remained viable at 60 – 80%, with a high percentage of CD133+ cells in the live population (2% vs 40-96%).

If those cells which had died due to supernatant treatment had a high percentage of CD133+ cells, actinomycete isolates ACN 5059 and ACN 5086 can be assumed to target CD133+ cells, which are thought to be the cancer stem cells. However, further testing needs to be done to confirm this.

10.5 Current status of the Cancer Stem Cell Research Project

Actinogen has put its cancer stem cell research on hold until additional funds are raised. The last research was performed in 2012.

The Company's results from the Cancer Stem Cell Research Project to date have been on a molecular level, and must be repeated on a much larger scale in order to be commercially viable. There is no guarantee that the Cancer Stem Cell Research Project will ultimately be commercially viable.

11. Consents

Regenerate Industries Pty Ltd and Dr Karne De Boer have each consented and have not, before the date of this announcement, withdrawn their consent to be named in this announcement in the form and context in which they are named.

More information

If shareholders are interested to know more about any of the projects set out below they can contact Actinogen at its registered office at level 7, 231 Adelaide Terrace on 9225 4815 or at info@actinogen.com.au. Some information cannot be publically announced due to confidentiality and future patent application issues.