Appendix 4E

Preliminary Financial Report

Name of entity

ACTINOGEN LIMITED

ABN

14 086 778 476

Financial year ended 30 June 2010
(Previous corresponding period: Year ended 30 June 2009)

Results for announcement to the market

Revenues from continuing operations	Down	27.5%	to	92,801
Comprehensive Loss from operations after tax	Down	23.3%	to	733,971
Comprehensive Loss for the period attributable to members	Down	23.3%	to	733,971

No dividends have been paid or declared during the year ended 30 June 2010, in the previous year ended 30 June 2009 or in the period from year end to reporting date. The company does not propose to pay dividends.

Actinogen Limited has not engaged in the acquisition of subsidiary companies in this financial year.

NTA Backing	30 June 2010 $	30 June 2009 $
Net Tangible asset backing per ordinary share	.03	.05

BRIEF EXPLANATION OF THE ABOVE FIGURES

The company listed on the ASX during the previous financial year. The year ended 30 June 2010 was the company's second full year of operations.

The reduction in Net Tangible asset backing per share reflects the loss from operations during the year.

CHAIRMAN'S COMMENTS AND OPERATIONAL REVIEW

ACN 1: Overview

During 2008-2009, Actinogen Ltd appointed three new positions; (1) a full time Research Assistant position to take over the actinomycete isolation and screening duties of Dr Kerry Carson who had gone to 0.5 time; (2) 0.6 time Research Assistant to assist DK with new project exploration and development; and (3) full time Research Scientist position on the siderophore project.

New protocols were developed for (1) secondary screening for quorum quenching compounds; (2) screening for activity against *C. difficile*; (3) secondary screening for antibiotic activity using 96-well trays; and (4) primary screening for cellulose producers using CCRA.

A new descriptive key for 'in house' classification of the actinomycetes was introduced which was more discriminatory than the previous system.

ACN 2: Antibiotic & Antifungals from Soil and Water Isolates

Project 1 - Isolation and 'in house' grouping of actinomycetes

The total number of isolates tested by primary screening during the period of July 2009 to June 2010 was 1526 (7630 cultures). Secondary testing for MRSA, and/or VRE, and/or *Candida*, and/or *P. aeruginosa*, and/or quorum quenching activity was carried out on 44% of all isolates. Testing for activity against *C. difficile* was also done on 182 isolates.

After secondary screening there are currently

- 135 isolates that have activity against the entire MRSA panel and 41 that have activity against some of the panel
- 64 isolates that have activity against the entire panel ± one strain of *Candida* panel and 78 that have activity against part of the panel
- 72 isolates that have activity against VRE
- 6 isolates that have activity against *Pseudomonas aeruginosa* after secondary testing
- 7 isolates that have quorum quenching ability
- 19 out of 182 isolates tested have activity against *C. difficile* after primary screening and of these isolates
    - 17 isolates have activity against VPI10463 (R1)
    - 18 isolates have activity against PCR ribotype 027 (epidemic strain)
    - 17 isolates have activity against WA25

The 'in house' grouping system medium was changed to ISP4 which allowed for greater differentiation between isolates.

Project 2 - Secondary assay for quorum quenching using biofilm formation

A biofilm screening assay for quorum quenching compounds from both Gram positive and Gram negative bacteria was designed. It proved to be very labour intensive and was discontinued. Using this methodology, isolate 643 appeared to produce quorum quenching compound(s) that interfere with pigment production in *Chromobacterium violaceum* and biofilm production in *Escherichia coli* NCTC 10418.

Project 3 - Primary screening for antibiotic activity against *C. difficile*

A primary screening technique for activity against *Clostridium difficile* was designed and implemented. This method readily identifies those isolates with activity against *C. difficile* and has proceeded into regular use by Actinogen. The method has now identified 19 isolates of interest in this area that will now proceed to dereplication.

Project 4 - Comparison of 96 well tray and zone sizes

The 96 well tray method was very useful in identifying isolates that produce the most active antibiotics. This method does not suffer from the inconsistencies seen with agar diffusion that relate to the water solubility, size and/or charge of the molecule. Volatile antibiotics can also be identified as these have little or no activity when this method is used.

By this method only one of 123 isolates (isolate 3293) had activity against the entire *Candida* panel, and will be investigated further to assess whether a novel antibiotic was produced.

Project 5 - Primary screening for cellulose producers

A rapid method for screening for cellulose producers has been established and a library of good cellulase producers was created. 1349 isolates were tested and 125 of these have good cellulase production. A method for secondary testing will be developed in the future in order to find the most efficient cellulase producers for use in the development of biodegradable paper bags.

Project 6 - Further characterisation of isolates 1488, 1822, 2226 and 2256 and cellulose producing bacillus

Isolates 1488 and 1822 have been characterized for the Budapest Treaty but have not yet been sent. Isolates 2226 and 2256 require 16S RNA sequencing. Isolate 1488 was identified as a *Streptomyces* species and until better methods of identifying individual species within this genus are available it cannot be given a species name.

Isolate 1822 belongs in the genus *Amycolatopsis* and t appeared to have more than one sequence for 16S rRNA and was thought to be a hybrid strain. The isolate cannot be identified further unless full genome sequencing is done. It does differ from *A. australiensis* the only species that has been previously identified in Australia.

The bacillus belonged in the genus *Paenibacillus* and was identified as either *P. ploymyxa* by biochemical techniques, and as *P. tundra* by 16S RNA sequencing. This may be a completely new species. Until further biochemical testing using unusual carbon sources has been completed the isolate cannot be further identified.

Project 7 - Optimisation of SAB medium

The optimised formulation of SAB appeared to allow good growth and antibiotic production in a wide variety of actinomycetes isolates.

Growth and pigment production was generally best for all isolates in the optimised formulation. The removal of the minerals usually resulted in no pigment and in some instances the loss of differentiated or normal mycelial pigment. Spore production was also greatest in SAB and generally least when no Mg^{2+} was present.

Anti-MRSA activity was generally dependent upon at least one of the minerals being present, though the essential mineral varied with isolate.

The carbon source present in the medium affected the growth, time of sporing and antibiotic production of individual actinomycetes isolates. In some instances only starch was required and in others dextrose, and others either carbon source resulted in good growth and antibiotic production.

Project 8 - Primary cytotoxicity screening

After primary screening there were 128 isolates that have either cytotoxic or cytostatic activity. Twelve isolates have activity against the four cell lines.

Initially, primary cytotoxicity screening was conducted only on isolates which produced antibiotics. The screening then expanded to include testing isolates which were negative for antibiotic production. All isolates that were negative for antibiotic production, but had activity against the cell line produced a compound known as cyclo(prolyltyrosyl). Cyclo (prolyltyrosyl), known as maculosin, was found previously in *Streptomyces*, and reported to possess phytotoxic and antibacterial properties only. No cytotoxicity has been reported. Further testing will be done to confirm this finding.

Project 9 - Secondary cytotoxicity screening: apoptosis vs. necrosis

Results so far have revealed that supernatant from actinomycetes isolate 1883 mediates cell death by apoptosis in L929 cells. Supernatants from other actinomycetes may not mediate cell death by apoptosis in HeLa and B16 cells. Other secondary screening methods to identify ways in which actinomycetes cause cytotoxicity (tumour angiogenesis, tumour invasion, tumour metastasis and cell cycle control) are being investigated.

One of the proposed methods is tumour metastasis and Becton Dickinson have an established method which involves an artificially made basement membrane and fluorescent labelling. This methodology is being further research and will be tested and optimised in the next financial year.

Project 10 - Cytotoxicity screening: CAM assay for angiogenesis

A screening method for angiogenesis using the CAM of egg embryos was designed but has not yet been completed due to inconsistencies in blood vessel development and a suitable quantification method. Once the response to the B16 medium, or other inducer or inhibitor agents, is confidently established with the use of an accurate quantification method it is anticipated that this method will be used to screen actinomycete isolates for pro- or anti-angiogenic activity.

ACN 3: Curly Leaf

A local strain of *Taphrina deformans* has not yet been isolated from infected peach leaves and whole peaches, however attempts will continue according to the availability of infected samples this spring.

The 18 actinomycete isolates that tested positive against both *T. deformans* and Brown Rot *in vitro* represent possible candidates for the development of an effective dual fungicide against both of these pathogens.

ACN 4: Salt Tolerant Work

Three native saltbushes, Oldman saltbush, Bladder saltbush and Salt Lake Pea, seeds or seedlings had no tolerance to the salt stress experiments conducted *in vitro*. Bladder saltbush seedlings failed to germinate and mature into plants. No further work will be done on these seeds.

It was suggested that Wyalkatchem, similar to the *Atriplex* species, was salt tolerant only when mature. This was confirmed when plants continued to grow in the presence of 5% and 10% saline, provided the seedlings were initially germinated within a saline-free environment.

The plant hormones/quorum sensing compounds produced by the two actinomycete isolates, 1487 and 1503, produced relatively larger plants than the control when inoculated onto Wyalkatchem seedlings. The presence of these compounds may help alleviate salt stress and further salt-stress experiments in the presence of select non/salt-tolerant actinomycete isolates are anticipated.

ACN 5: Plastics Work

Actiniomycete isolates 2226 and 2256 have been shown to digest cigarette paper in the presence of a Gram positive bacillus belonging to the genus *Paenibacillus*. Neither the bacillus nor the actinomycetes are capable of this digestion alone.

Actinomycete isolates continue to be being tested for degradation of cigarette paper, filter paper, brown paper and biodegradable plastic bags in an effort to find more actinomycetes capable of paper digestion. Isolates will first be screened on Congo Red plates to determine cellulolytic activity and only the isolates that produce large amounts of cellulose will go into paper/plastic digestion experiments.

ACN 6: Water Samples (Chitinase)

Chitinase producing bacteria and fungi are readily found in natural waters. The company now has a small collection of bacteria and actinomycetes that are capable of utilising chitin from either crab shells or mushrooms. These are stored at -80°C for future use in projects that incorporate the breakdown of chitin into glucosamine. No further work was done this year.

ACN 7: Dereplication

Project 1 Standard dereplication

To date there are

- 60 MRSA positive isolates
- 30 *Candida albicans*positive isolates
- 37 anticancer positive isolates
- 21 VRE positives
- 2 *P. aeruginosa* positives
- one quorum quenching positive isolate
- one antifungal isolate against Brown Rot

that have HPLC peaks that have not been identified by the Actinogen libraries. These peaks need to be further explored with more extensive libraries.

The new "in house" library has spectra of 23 anticancer drugs, 5 siderophores, 2 media, 2 antifungal compounds, 1 plant growth hormone and 3 others. All of these spectra may be of use in identifying 'unknown peaks' from HPLC analysis.

Project 2 Brown rot and isolate 1822

The filtrate from 1822 grown in SAB had *in vitro* activity against Brown Rot fungus. The ethyl acetate extract used for HPLC did not have any antifungal copmounds present. When the filtrate was tested no known antifungal compounds were identified by HPLC but there were unknown peaks.

Isolate 1822 was characterised for lodgement with the Budapest Treaty and further chemical analysis of the filtrate will be done to determine if there was a new antifungal agent produced by this isolate.

Project 3 Anacardic acid and isolate 1034

The optimum conditions required by1034 for anacardic acid production are:

- pH 7, temperature of 26°C
- starch concentration of 10%
- absence of nitrogen
- constant aeration.

When the volume was increased to 300 mL the growth time for maximal production was 4 weeks.

ACN 8: Siderophores

Many siderophore-producing isolates were identified, including 22 isolates that are capable of strong siderophore production under conditions of iron starvation and are of great interest. HPLC analysis successfully identified known siderophores, including enterobactin and heterobactin, as well as unknown analytes potentially representing novel

siderophores. Ongoing screening to identify isolates capable of producing siderophore in the presence of iron is being conducted.

Protocols for genetic screening were developed and screening for the carriage of an enterobactin synthesis gene is underway. Preliminary results indicate that carriage of this gene is relatively widespread among actinomycetes. Many actinomycetes may be capable of producing enterobactin under certain conditions, and data from continuing PCR genetic screening will further clarify this hypothesis.

Isolate 1481 was an isolate of particular interest as it was a strong siderophore producer and also shown to excrete enterobactin which is the strongest iron-binding siderophore known currently. This siderophore or its breakdown products and precursors were detected in 3 isolates (1481, 2342 and 2349). To date, production of enterobactin by actinomycetes has only been reported once in the scientific literature; that report described the production of membrane-bound enterobactin by two *Streptomyces* isolates. Thus, our data is the first report of the production of extracellularly excreted enterobactin by actinomycetes and is being readied for publication.

Detailed characterisation of 1481 was undertaken in preparation for its registration under the Budapest Treaty.

PRELIMINARY STATEMENT OF COMPREHENSIVE INCOME
For the year ended 30 June 2010

	2010	2009
	$	$
Revenue from continuing operations	92,801	128,126
Other Income	63,360	-
Administration expenses	(166,702)	(171,992)
Laboratory expenses	(404,666)	(219,199)
Employee Benefits expense	(464,886)	(504,746)
Finance Costs	(805)	(57)
Depreciation	(17,372)	(13,825)
Impairment losses – financial assets	(41,211)	(297,000)
Write back of impairment loss	22,000	-
(Loss) before income tax	**(917,481)**	**(1,078,693)**
Income tax benefit / (expense)	180,010	151,751
(Loss) for year	**(737,471)**	**(926,942)**
Changes in the fair value of available for sale financial assets (net of tax)	3,500	(30,000)
Total comprehensive income for the year	**(733,971)**	**(956,942)**
Basic and diluted loss per share (cents per share)	(1.85)	(2.32)

The above preliminary Statement of Comprehensive Income should be read in conjunction with accompanying notes.

PRELIMINARY STATEMENT OF FINANCIAL POSITION **As at 30 June 2010**	**2010** **$**	**2009** **$**
CURRENT ASSETS		
Cash and cash equivalents	1,085,386	1,849,668
Trade and other receivables	33,172	10,967
TOTAL CURRENT ASSETS	1,118,558	1,860,635
NON CURRENT ASSETS		
Property, plant and equipment	147,530	105,985
Available for sale financial assets	59,720	25,000
Intangible assets	16,029	16,029
TOTAL NON CURRENT ASSETS	223,279	147,014
TOTAL ASSETS	1,341,837	2,007,649
CURRENT LIABILITIES		
Trade and other payables	169,686	102,786
Provisions	3,892	2,633
TOTAL CURRENT LIABILITIES	173,578	105,419
NET ASSETS	1,168,259	1,902,230
EQUITY		
Contributed equity	4,322,640	4,322,640
Reserves	4,792,123	4,788,623
Accumulated losses	(7,946,504)	(7,209,033)
TOTAL EQUITY	1,168,259	1,902,230

The above preliminary Statement of Financial Position should be read in conjunction with the accompanying notes

PRELIMINARY STATEMENT OF CHANGES IN EQUITY
For the year ended 30 June 2009

	Contributed Equity	Accumulated (Losses)	Reserves	Total
	$	$	$	$
2009				
Balance 1 July 2008	**4,322,640**	**(6,282,091)**	**4,818,623**	**2,859,172**
Changes in the fair value of available for sale financial assets	-	-	(30,000)	(30,000)
(Loss) for Year	-	(926,942)	-	(926,942)
Total comprehensive income for the year	**-**	**(926,942)**	**(30,000)**	**(956,942)**
Balance at 30 June 2009	**4,322,640**	**(7,209,033)**	**4,788,623**	**1,902,230**

The above statement of changes in equity should be read in conjunction with the accompanying notes.

PRELIMINARY STATEMENT OF CHANGES IN EQUITY (Continued)
For the year ended 30 June 2010

	Contributed Equity	Accumulated (Losses)	Reserves	Total
	$	$	$	$
2010				
Balance 1 July 2009	**4,322,640**	**(7,209,033)**	**4,788,623**	**1,902,230**
Total comprehensive income for the year	-	(737,471)	-	(737,471)
Changes in the fair value of available for sale financial assets	-	-	3,500	3,500
Total comprehensive income for the year		**(737,471)**	**3,500**	**(733,971)**
Balance at 30 June 2010	**4,322,640**	**(7,946,504)**	**4,792,123**	**1,168,259**

The above statement of changes in equity should be read in conjunction with the accompanying notes.

PRELIMINARY CASH FLOW STATEMENT
For the year ended 30 June 2010

	2010	2009
	$	$
Cash flow from operating activities		
Interest received	44,390	102,537
Receipts from customers	29,240	39,148
Payments to suppliers	(981,005)	(840,310)
Research and development tax offset	180,010	151,751
Net cash (outflow) from operating activities	(727,365)	(546,874)
Cash flow from investing activities		
Payment for property, plant & equipment	(58,917)	(106,589)
Payment for intangible asset	-	(16,028)
Loans to related parties	-	(22,000)
Repayment of loans from related parties	22,000	-
Net cash (outflow) from investing activities	(36,917)	(144,617)
Net increase/(decrease) in cash and cash equivalents	(764,282)	(619,491)
Cash and cash equivalents at beginning of year	1,849,668	2,541,159
Cash and cash equivalents at end of year	1,085,386	1,849,668

The above cash flow statement should be read in conjunction with the accompanying notes.

1. ACCOUNTING POLICIES

The principal accounting policies adopted in the preparation of the financial report are set out below. These policies have been consistently applied to all the years presented, unless otherwise stated.

(a) Basis of preparation

This general purpose financial report has been prepared in accordance with Australian Accounting Standards, other authoritative pronouncements of the Australian Accounting Standards Board, Australian Accounting Interpretations and the *Corporations Act 2001.* The financial statements have been prepared on a going concern basis.

(b) Compliance with IFRS

The financial report of Actinogen Limited also complies with International Financial Reporting Standards (IFRS) as issued by the International Accounting Standards Board (IASB).

2. DIVIDENDS PAID

No dividends have been paid for the year ended 30 June 2010, in the previous year ended 30 June 2009 or in the period from year end to reporting date.

No dividend reinvestment plans are in place.

3. ACCUMULATED LOSSES

	2010	2009
	$	$
Accumulated losses at the beginning of the financial year	(7,209,033)	(6,282,091)
Net loss attributable to members	(737,471)	(926,942)
Dividends and other equity distributions paid	-	-
Accumulated losses at the end of the financial year	**(7,946,504)**	**(7,209,033)**

4. CONTROLLED ENTITIES & ASSOCIATES

Actinogen Limited does not have a controlling interest in any companies and no changes have occurred during the year ended 30 June 2010, nor have there been any joint ventures with other entities for the purposes of profitable activities.

5. INCOME TAX	2010	2009
	$	$
Operating loss before income tax	(917,481)	(1,078,693)
Prima facie tax benefit at 30% (2009: 30%)	(275,244)	(323,608)
Tax effect of:		
Provisions and accruals	(2,286)	8,881
Non deductible expenses	-	-
Impairment expenses	9,642	89,100
Capital raising costs	(45,328)	(45,328)
Research and development tax offset	180,010	151,751
Future income tax benefit not brought to account	313,216	270,955
Income tax benefit / (expense)	180,010	151,751

6. RECONCILIATION OF CASH AND CASH EQUIVALENTS		
Cash at bank and in hand	1,084,121	1,849,668
Amounts held in trust	1,265	-
	1,085,386	1,849,668

For the purposes of the Cash Flow Statement, cash includes cash on hand and in banks and investments in money market instruments, net of outstanding bank overdrafts.

7. CASH FLOW INFORMATION	2010	2009
	$	$
RECONCILIATION OF THE OPERATING (LOSS) AFTER TAX TO THE NET CASHFLOWS FROM OPERATING ACTIVITIES		
Loss for the year	(737,471)	(926,942)
Non cash items:		
Depreciation	17,372	13,825
Impairment expenses	10,141	297,000
Profit on sale of available for sale financial assets	(63,360)	-
Change in assets and liabilities		
Increase/(Decrease) in trade creditors and accruals	66,900	24,132
Increase/(Decrease) in provisions	1,258	(1,047)
(Increase)/Decrease in receivables	(22,205)	46,158
Net cash outflow from operating activities	**(727,365)**	**(546,874)**

This report is based on accounts which have been audited.

Dr Zhukov Pervan
Chairman
31 August 2010