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The cancer chemopreventive potential of novel *Streptomyces* strains derived from mangrove forest

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Core Member of Project: Ser Hooi-Leng & Goh Bey-Hing

2nd Applied Microbiology and Beneficial Microbes,

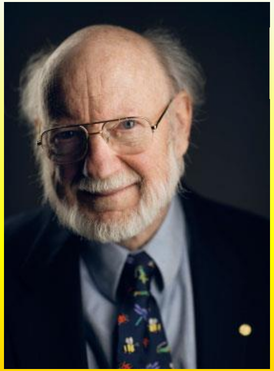
October 23-25, Osaka, Japan

GROUP OF EIGHT AUSTRALIA

Background – Genus *Streptomyces*

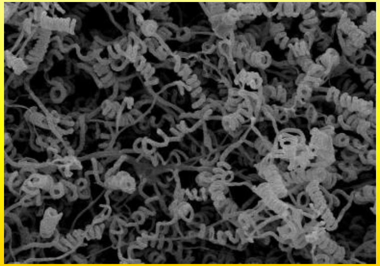
Streptomyces

High GC %
Large genome
Complex life cycle
Prolific producers



Prof. William C. Campbell

Streptomyces avermitilis



Prof. Satoshi Ōmura

The Nobel Prize in Physiology or Medicine 2015

Avermectin & Ivermectin (synthetic)
Effective against river blindness and elephantiasis

(Subramani and Aalbersberg, 2012; Kumar et al., 2014; Mannivasagan et al., 2014a; Mannivasagan et al., 2014b; Xu et al., 2014)

Background – Mangrove forest

Tidal current
Salinity
Oxygen
Temperature

Metabolic adaptations

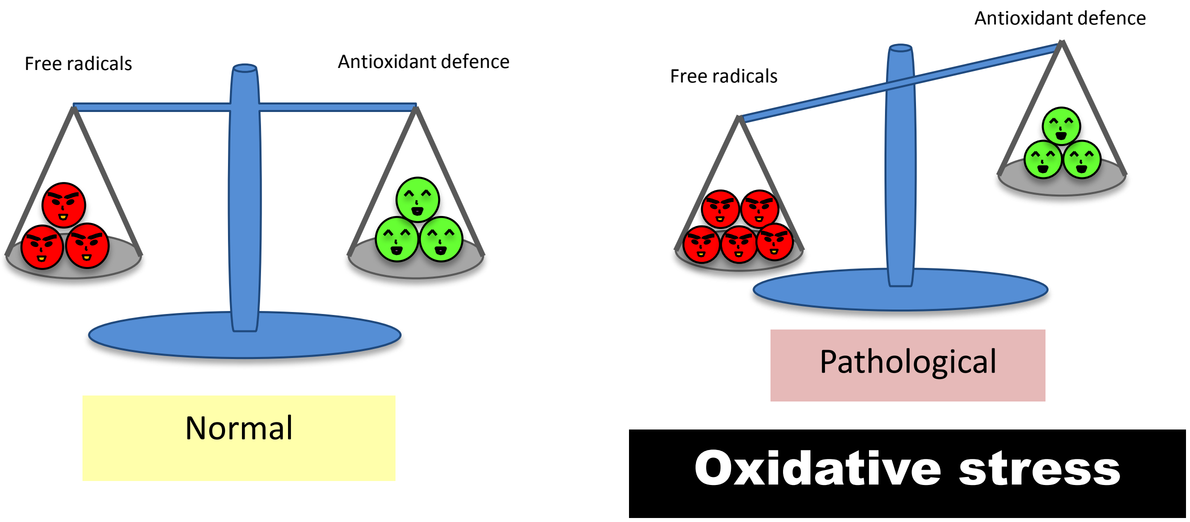
Valuable useful metabolites

(Alongi, 2002; Giri et al., 2010)

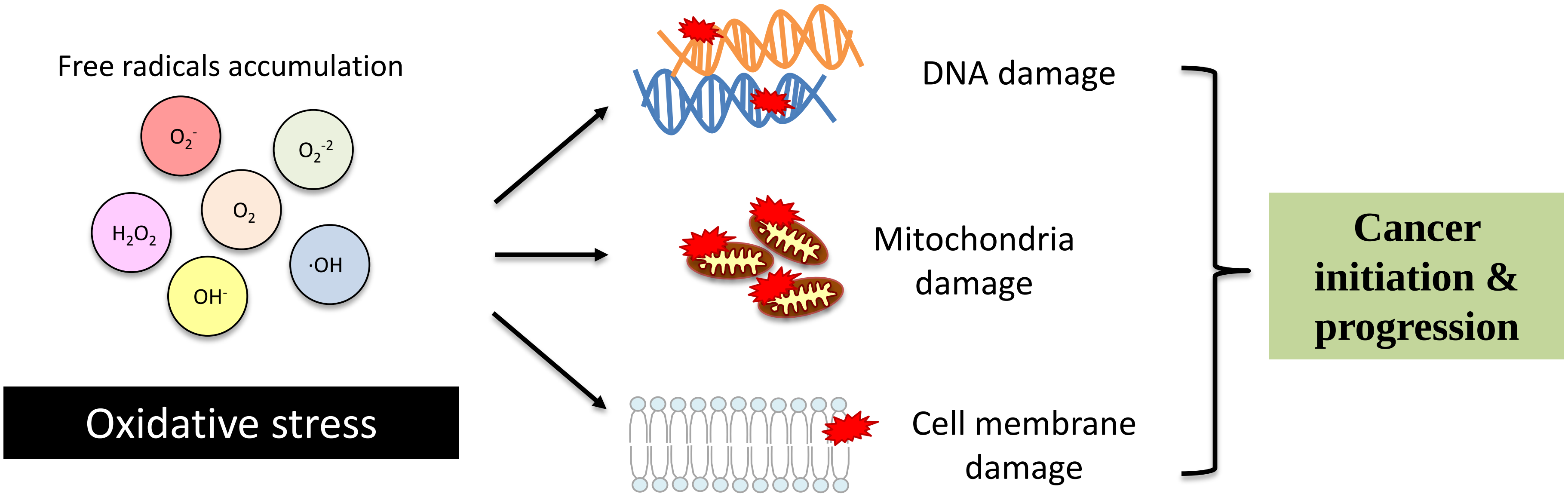
Region	Area (km^2)	Global total %
East Africa	7,917	5.2
Middle East	624	0.4
South Asia	10,344	6.8
Southeast Asia	51,049	33.5
East Asia	215	0.1
Australasia	10,171	6.7
Pacific Ocean	5,717	3.8
North and Central America	22,402	14.7
South America	23,882	15.7
West and Central Africa	20,040	13.2
Total	**152,361**	

Table 1: Summary of world mangrove areas (ITTO, 2014).

Background – Free radicals and cancer initiation



Background – Free radicals and cancer initiation

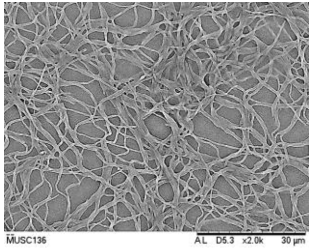


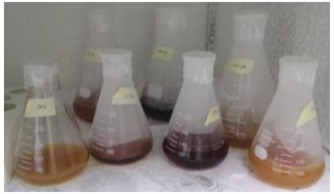
Aim of the study

87 Actinobacteria

52 *Streptomyces*

4 novel strains

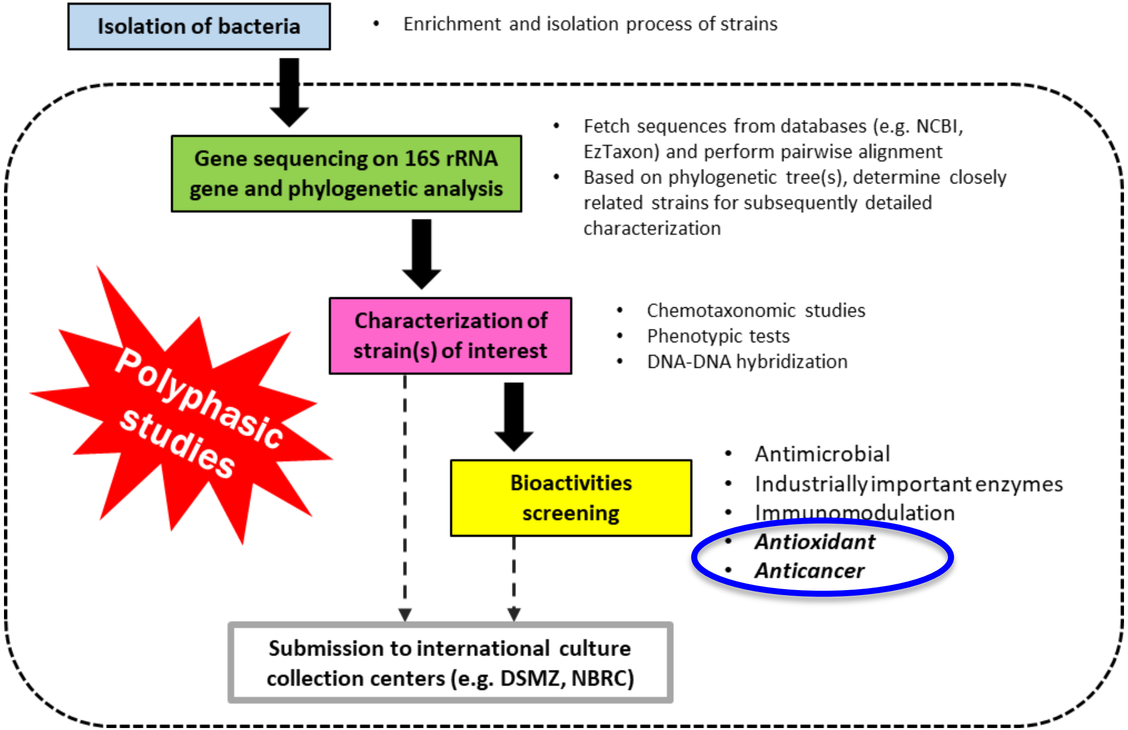




To examine **novel** strains via polyphasic approach

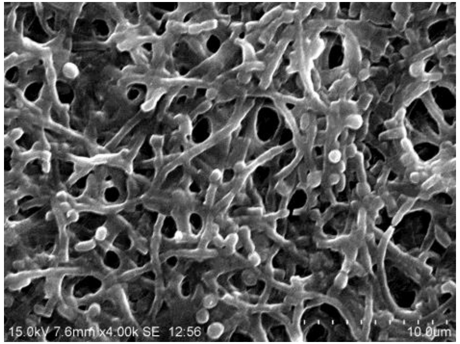
To investigate **bioactive potential** of their extracts

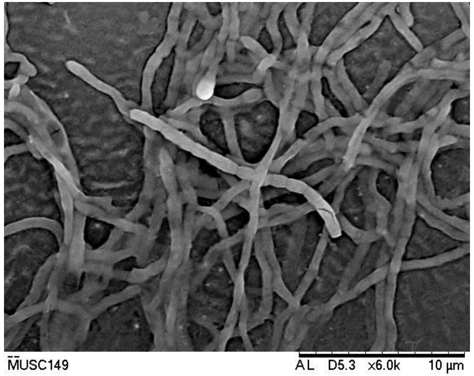
General workflow: The basis of polyphasic approach



Results – Morphology of four novel strains

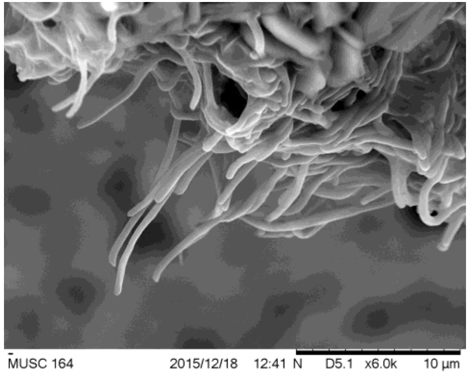
Streptomyces gilvigriseus MUSC 26^T

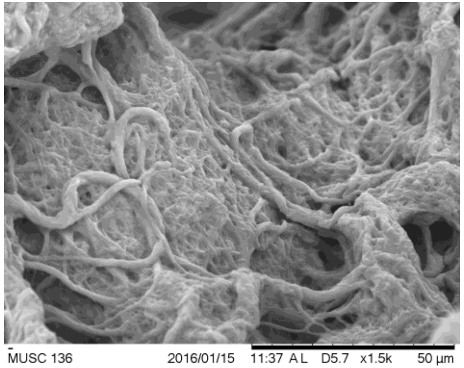




Streptomyces mangrovisoli MUSC 149^T

Streptomyces antioxidans MUSC 164^T





Streptomyces malaysiense MUSC 136^T

Results – 16S rRNA sequencing of MUSC 136^T

Comparison of 16S rRNA genes on EzBiocloud server

⬇

Table 2: 16S rRNA gene similarities between MUSC 136^T and closely related strains.

Type strains	**16S rRNA similarities with MUSC 136^T**
Streptomyces misionensis NBRC 13063^T	99.6%
Streptomyces phaeoluteichromatogenes NBRC 5799^T	99.6%
Streptomyces rutgersensis NBRC 12819^T	98.9%

↑ similarity level

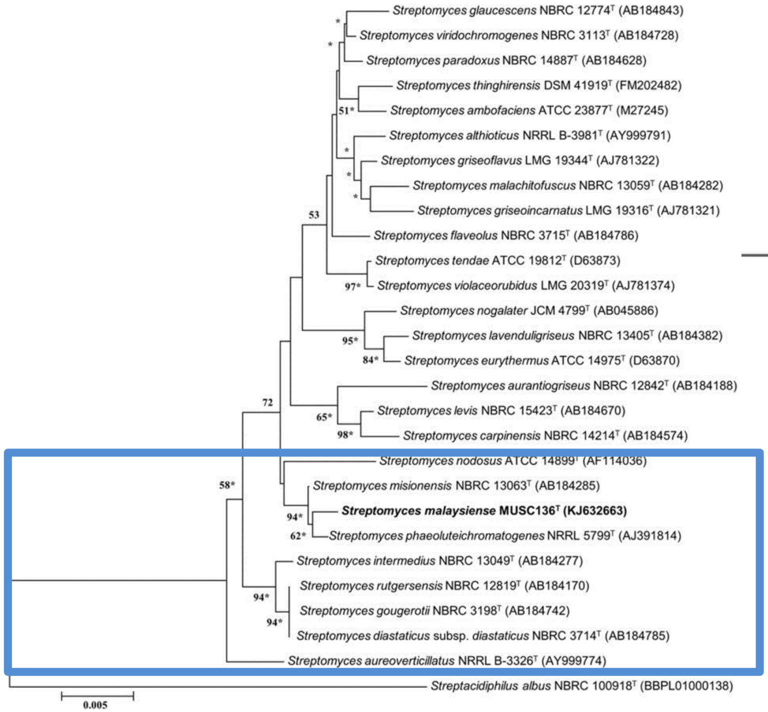
⬇

↑ number of species in *Streptomyces* genus
(823 species described)

⬇

Phylogenetic tree construction

Results – Phylogenetic analysis of MUSC 136^{T}



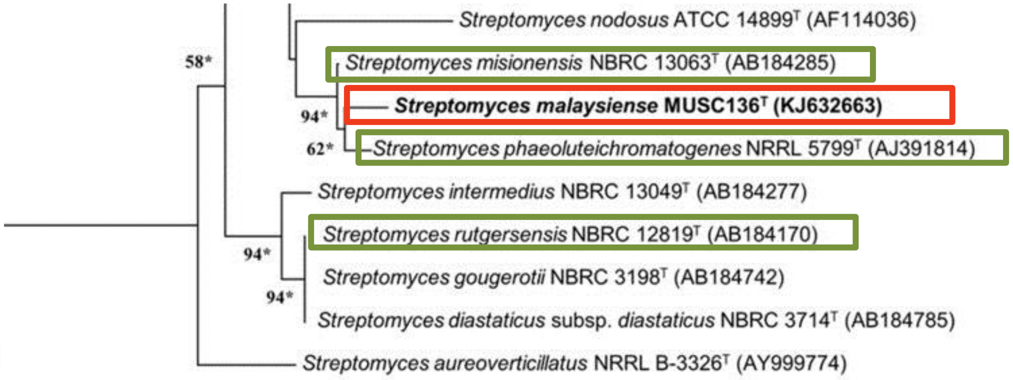


Figure 1: Neighbour-joining phylogenetic tree of 16S rRNA sequences.

- 1000 bootstrap re-samplings, only values above 50% are shown.
- Asterisks – corresponding nodes were also observed in neighbour-joining tree
- *Streptacidiphilus albus* NBRC 100918^{T} was used as an outgroup.

Results – DNA-DNA hybridization of MUSC 136^T and type strains

Table 3: DNA-DNA relatedness between MUSC 136^T and closely related strains.

Type strains	**DNA-DNA relatedness with MUSC 136^T**
S. misionensis NBRC 13063^T	46.5 ± 0.2 %
S. phaeoluteichromatogenes NBRC 5799^T	44.5 ± 0.4%
S. rutgersensis NBRC 12819^T	22.7 ± 0.5%

DNA-DNA hybridization value of
70% is used for **delineation of bacterial species.**
(Wayne et al., 1987)

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Results – Chemotaxonomic characterization of MUSC 136^T

Table 4: Respiratory quinones and cell wall composition of MUSC 136^T.

Properties	**Values**
Diaminopimelic acid	LL-diaminopimelic acid (Xu et al., 2009; Lee et al., 2014)
Predominant menaquinones	MK-9(H_8) and MK-9(H_6) (Kim et al., 2003)

G+C content: 72.3 mol%

Within 67.0 – 78.0 mol% for species of the **genus *Streptomyces***

Results – Chemotaxonomic characterization of MUSC 136^T

Table 5: Predominant cellular fatty acid composition (>10%) of strain MUSC 136^T and its closely related *Streptomyces* species.

Fatty acid	MUSC 136^T	*S. misionensis* NBRC 13063^T	*S. phaeoluteichromatogenes* NBRC 5799^T	*S. rutgersensis* NBRC 12819^T
iso-$C_{15:0}$	12.2	7.2	12.3	5.2
anteiso-$C_{15:0}$	35.3	40.1	35.5	32.2
iso-$C_{16:0}$	12.4	14.4	17.7	10.3
anteiso-$C_{17:0}$	11.8	19.3	13.4	13.9

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Results – Chemotaxonomic characterization of MUSC 136^T

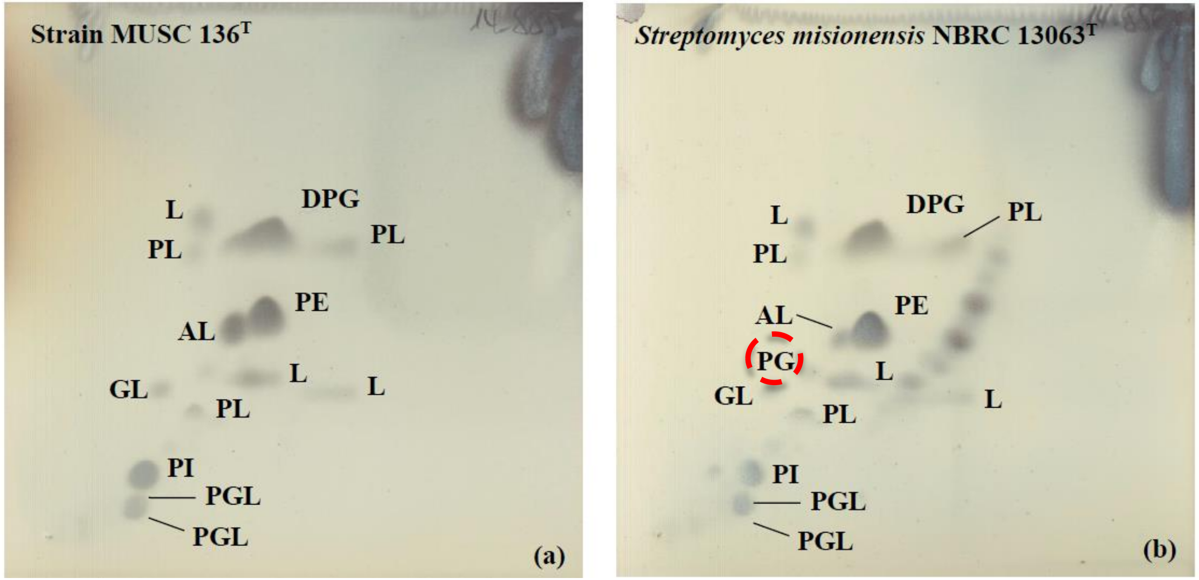

Figure 2: Two dimensional total lipid profile of strain MUSC 136^T and *Streptomyces misionensis* NBRC 13063^T. AL, Aminolipid; DPG, Diphosphatidylglycerol; GL, Glycolipid; PL, Phospholipid; PI, Phosphatidylinositol; PE, Phosphatidylethanolamine; PG, Phosphatidylglycerol; PGL, Phosphoglycolipid; L, Lipid.

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Results – Phenotypic characterization of MUSC 136^T

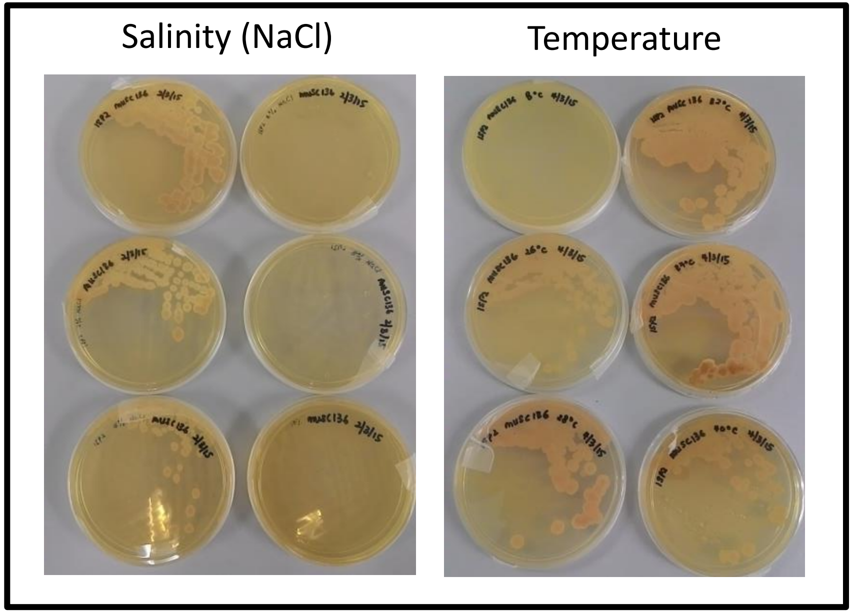

Figure 2: Growth of MUSC 136^T at different salinity and temperature.

Table 6: Cultural characteristics of strain MUSC 136^T on different media at 28 °C after 7-14 days of incubation.

Medium	**Growth**	**Colony color**	
		Aerial mycelium	**Substrate mycelium**
Yeast malt agar (ISP 2)	Good	Yellowish white	Grayish yellow
Oat Meal agar (ISP 3)	Moderate	Yellowish white	Dark grayish yellow
Glycerol Asparagine Agar Base (ISP 5)	Good	Pale yellow	Yellowish white
Peptone Yeast Extract Iron agar (ISP 6)	Good	Yellowish white	Pale yellow
Tyrosine agar base (ISP 7)	Good	Pale yellow	Grayish yellow

Results - Antioxidant activities of *Streptomyces* extract

Table 7: Antioxidant activity of 2mg/mL of extract tested using different assays.

Strain	Antioxidant activity (%)		
	DPPH	ABTS	Metal-chelating
MUSC 26^T	N.D.	69.15	78.92
MUSC 149^T	36.53	17.8	4.94
MUSC 164^T	18.31	30.38	43.66
MUSC 136^T	26.72	27.87	37.01

Results – Antioxidant activity of MUSC 136^T extract

Table 8: Radical scavenging activity of MUSC 136^T evaluated using DPPH, ABTS, metal chelating and superoxide dismutase (SOD)-like assays (*, *p* >0.05).

Conc. (mg/mL)	Mean ± standard error(%)			
	DPPH	ABTS	SOD	Metal-chelating
0.25	4.87 ± 0.71	7.51 ± 2.19*	45.98 ± 2.81*	10.22 ± 1.58*
0.50	10.26 ± 2.44	11.59 ± 1.50*	56.93 ± 3.76*	17.00 ± 3.73*
1.00	11.15 ± 3.26	15.95 ± 2.34*	59.72 ± 6.19*	22.97 ± 1.51*
2.00	27.24 ± 1.91*	27.87 ± 2.19*	68.27 ± 3.67*	37.01 ± 2.59*

Increase in antioxidant activity with increasing concentration!

Results: Cytotoxic activities of *Streptomyces* strains

Table 9: Cytotoxic activities of mangrove-derived *Streptomyces* sp. extracts (400 µg/mL).

		MUSC 136^T	MUSC 164^T	MUSC 149^T	MUSC 26^T
Colon	HCT-116	48.8 ± 4.2	58.2 ± 5.1*	69.8 ± 5.7*	63.6 ± 3.6*
	HT-29	61.9 ± 3.4	83.1 ± 8.3*	85.8 ± 6.0	69.0 ± 6.4*
	Caco-2	55.4 ± 1.9*	71.7 ± 2.9*	95.6 ± 4.3	86.9 ± 4.4*
	SW480	68.6 ± 2.3*	65.2 ± 2.9*	79.9 ± 7.7*	67.5 ± 1.5*
Breast	MCF-7	65.2 ± 1.9*	66.0 ± 3.2*	80.7 ± 10.7	72.7 ± 3.6*
Prostate	DU145	80.2 ± 3.8	55.5 ± 3.6*	57.2 ± 5.7*	63.7 ± 6.8*
Lung	A549	67.1 ± 6.3	78.3 ± 6.9*	88.6 ± 9.0	86.7 ± 4.2*
Cervical	CaSki	55.7 ± 1.2	60.9 ± 5.2*	92.9 ± 2.1	79.1 ± 8.6*

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Results – Cytotoxic activity of MUSC 136^T

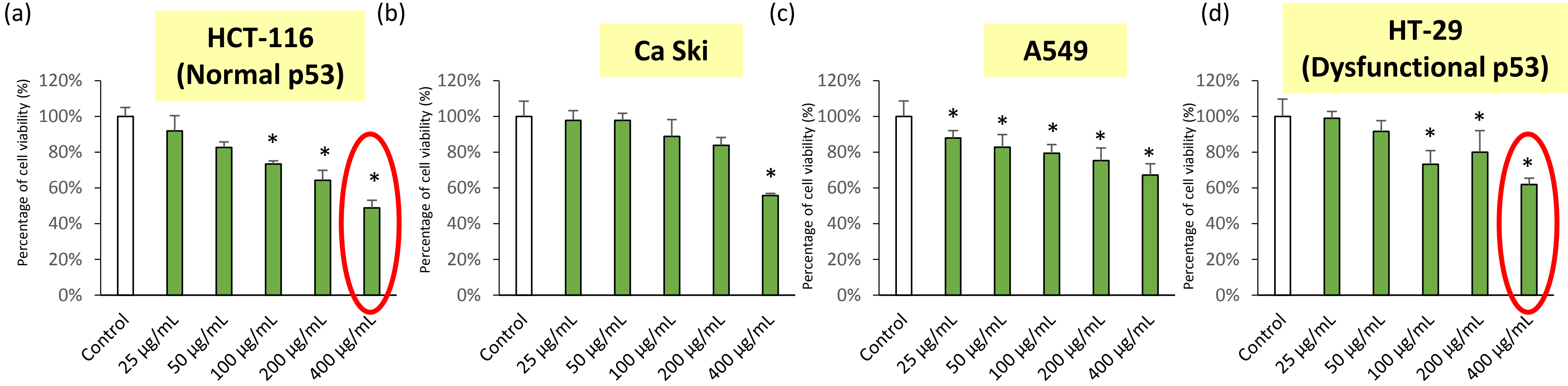

Figure 3: Cytotoxic activity of MUSC 136^T extract against human cancer cell lines. The measurement of cell viability was done using MTT assay.

Different susceptibility between colon cancer cell lines – HCT-116 and HT-29

Results – Apoptosis induction by MUSC 136^{T} extract

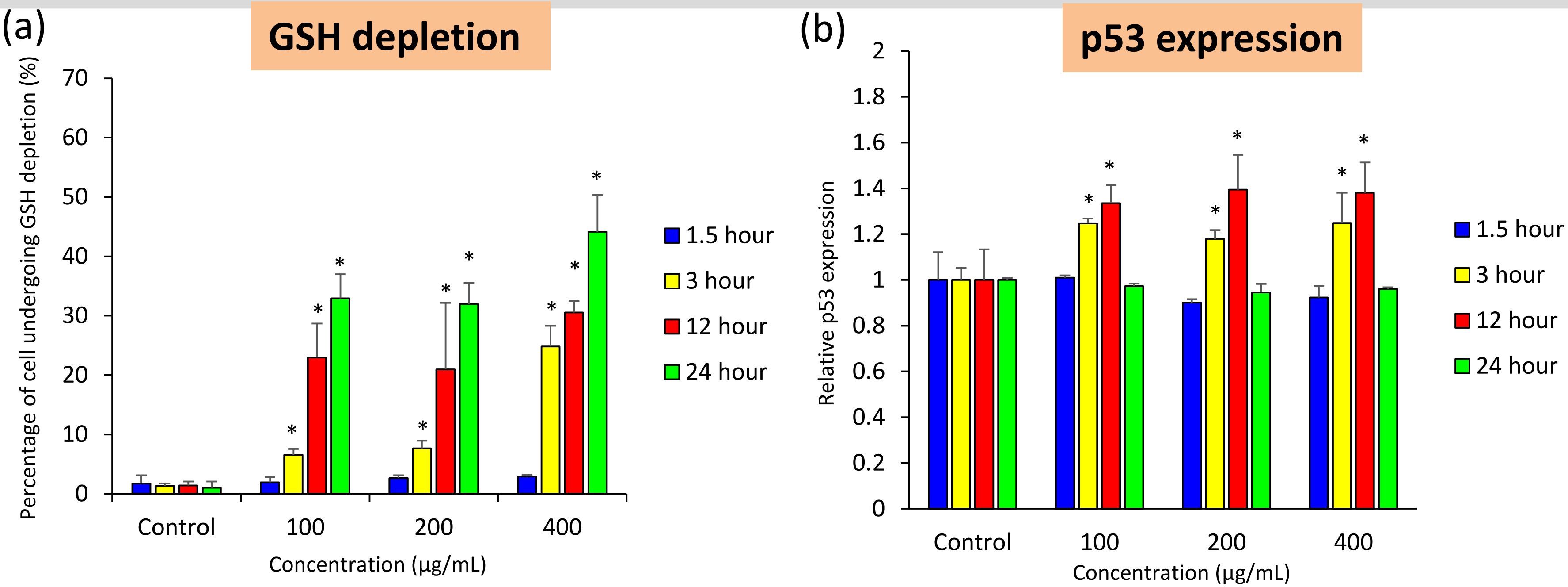

Figure 4: Effects of MUSC 136^{T} extract on (a) intracellular glutathione (GSH) content and (b) p53 protein in HCT-116 cells.

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Results – Apoptosis induction by MUSC 136^T extract

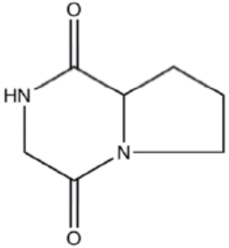
- When treated with MUSC 136^T extract, there is an increase in percentage of cancer cells experiencing GSH depletion. GSH is important in maintaining cell survival and decrease of this molecule could, in turn trigger apoptotic signalling cascades, including the activation of p53 signalling pathway.

- As seen in Figure (b), there was increase in p53 expression after treated with MUSC 136^T extract. All these results showed that the cytotoxic activity of MUSC 136^T extract could potentially acting via effects on p53 protein. However, further mechanistic studies on p53 associated cell death pathway would be required to fully understand the action target of MUSC 136^T extract.

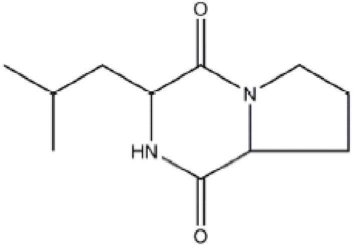
Results – Chemical profiling of MUSC 136^T extract

Table 10: GC-MS analysis of MUSC 136^T extract.

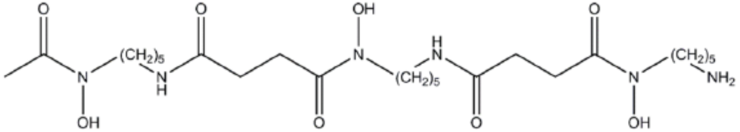
No	R.T. (min)	Compound	Formula	M.W.
1	14.370	Isomeric dihydro-methyl-furanone	$C_5H_6O_2$	98
2	39.484	1-Pentadecene	$C_{15}H_{30}$	210
3	44.502	Phenol, 2,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206
4	51.569	(3R,8aS)-3-methyl-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-a]pyrazine-1,4-dione	$C_8H_{12}N_2O_2$	168
5	53.074	1,4-diaza-2,5-dioxobicyclo[4.3.0]nonane	$C_7H_{10}N_2O_2$	154
6	54.956	Tetradecanoic acid, 12-methyl-, methyl ester	$C_{16}H_{32}O_2$	256
7	55.220	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	210
8	58.063	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270
9	71.544	Deferoxamine (metal chelating drug)	$C_{25}H_{48}N_6O_8$	560



(5)
Antioxidant



(7)
Anticancer



(9)

Bioactive compounds

Publication

SCIENTIFIC REPORTS

OPEN

Streptomyces malaysiense sp. nov.: A novel Malaysian mangrove soil actinobacterium with antioxidative activity and cytotoxic potential against human cancer cell lines

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Accepted: 23 March 2016
Published: 13 April 2016

Hooi-Leng Ser[1,2], Uma Devi Palanisamy[2], Wai-Fong Yin[3], Kok-Gan Chan[3], Bey-Hing Goh[1,2,4] & Learn-Han Lee[1,2,4]

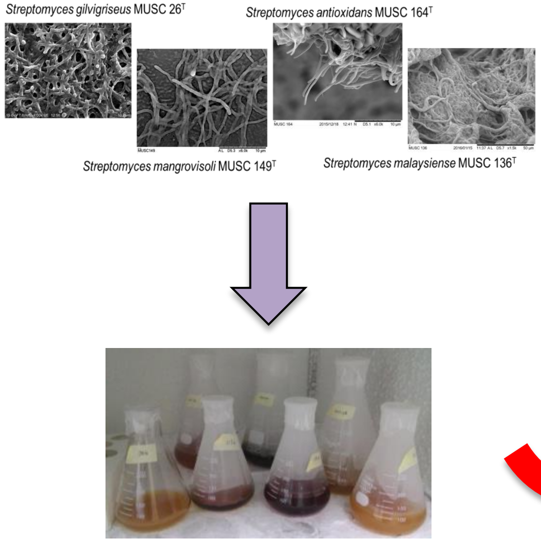
Actinobacteria from the unique intertidal ecosystem of the mangroves are known to produce novel, bioactive secondary metabolites. A novel strain known as MUSC 136^T (=DSM 100712^T = MCCC 1K01246^T) which was isolated from Malaysian mangrove forest soil has proven to be no exception. Assessed by a polyphasic approach, its taxonomy showed a range of phylogenetic and chemotaxonomic properties consistent with the genus of *Streptomyces*. Phylogenetically, highest similarity was to *Streptomyces misionensis* NBRC 13063^T (99.6%) along with two other strains (>98.9% sequence similarities). The DNA–DNA relatedness between MUSC 136^T and these type strains ranged from 22.7 ± 0.5% to 46.5 ± 0.2%. Overall, polyphasic approach studies indicated this strain represents a novel species, for which the name *Streptomyces malaysiense* sp. nov. is proposed. The potential bioactivities of this strain were explored by means of antioxidant and cytotoxic assays. Intriguingly, MUSC 136^T exhibited strong antioxidative activities as evaluated by a panel of antioxidant assays. It was also found to possess high cytotoxic effect against HCT-116 cells, which probably mediated through altering p53 protein and intracellular glutathione levels. Chemical analysis of the extract using GC-MS further affirms that the strain produces chemopreventive related metabolites.

Conclusion

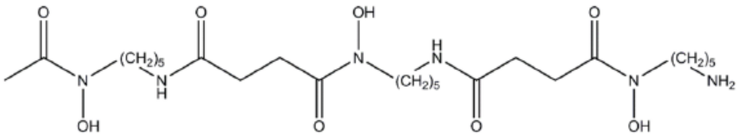
- Strain MUSC 136^T is novel, hence named as *Streptomyces malaysiense* sp. nov. (referring to country of isolation for this strain)

- Extracts of strain $MUSC136^T$ exhibited antioxidative and cytotoxic activity in various cancer cells

Chemical constituents of *Streptomyces* extracts



A total of **42** compounds were isolated from these **mangrove**-derived **novel streptomycetes**

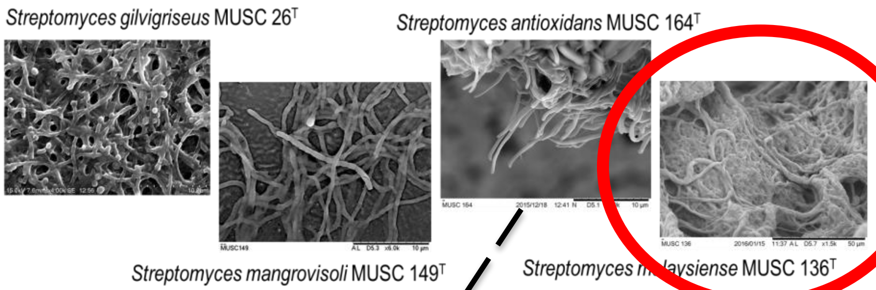


Deferoxamine

Expensive drug, costly to synthesis (*de novo*)

Summary of the study

Mangrove forest



Significant antioxidant activities across all four assays (DPPH, ABTS, SOD, metal-chelating)

Reduced cell viability of HCT-116 human colon cells to 48.8 %

Involvement of p53 signalling pathways → apoptosis

Neuroprotection against H_2O_2 insults

Antioxidant potential
of mangrove derived *Streptomyces* sp.

*Activities ranged from 4.94 - 78.92%^*

Cytotoxic potential

*Activities ranged from 4.4 – 51.2%**

*^tested with 2 mg/mL; *tested with 400 µg/mL*

Publications

frontiers in Microbiology

ORIGINAL RESEARCH
published: 20 August 2015
doi: 10.3389/fmicb.2015.00854

Presence of antioxidative agent, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- in newly isolated *Streptomyces mangrovisoli* sp. nov.

Hooi-Leng Ser[1], Uma D. Palanisamy[1], Wai-Fong Yin[2], Sri N. Abd Malek[3], Kok-Gan Chan[2], Bey-Hing Goh[1] and Learn-Han Lee[1]**

frontiers in Microbiology

ORIGINAL RESEARCH
published: 16 June 2016
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Streptomyces antioxidans sp. nov., a Novel Mangrove Soil Actinobacterium with Antioxidative and Neuroprotective Potentials

Hooi-Leng Ser[1,2], Loh Teng-Hern Tan[1,2], Uma D. Palanisamy[2], Sri N. Abd Malek[3], Wai-Fong Yin[4], Kok-Gan Chan[4], Bey-Hing Goh[1,5] and Learn-Han Lee[1,5]**

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Impact factor: 4.165

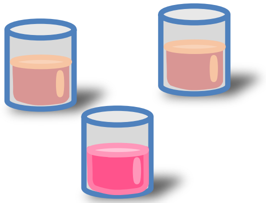
Future work

Novel *Streptomyces*
MUSC 26^{T}, 136^{T}, 149^{T}, 164^{T}

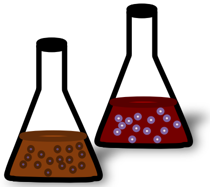
→

Production of bioactive compounds
(e.g. Deferoxamine – drug listed on WHO List of Essential Medicines)

Next generation sequencing
Identification of biosynthetic gene clusters



Isolation of bioactive compound(s)
Bioassay-guided fractionation



Media optimization
Maximizing production of bioactive compound(s)

Main Research Areas

- Biosystematics of Bacteria
- Discovery of Bioactive Secondary Metabolites

Foodborne Pathogens
- *Vibrio Parahaemolyticus*

Actinobacteria
- Streptomyces
- Non-Streptomyces

Bioactive secondary metabolites
- Antimicrobial
- Antioxidant
- Anticancer

frontiers in Microbiology

Streptomyces Bacteria as Potential Probiotics in Aquaculture

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Ajaree Rayanakorn

Hefa Kemung

University of Malaya

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UMBI, UKM

Dr Nurul Syakima Ab Mutalib

DSMZ, Germany

Dr Susanne Verbarg

US-FDA, USA

Prof Dr Om V Singh

Prof Dr Marc Allard

Wuhan University, China

Prof. Hong Kui

Dr. Xie Qing-Yi

Lin Hai-Peng

Tang Yi-Li

Hu Hu

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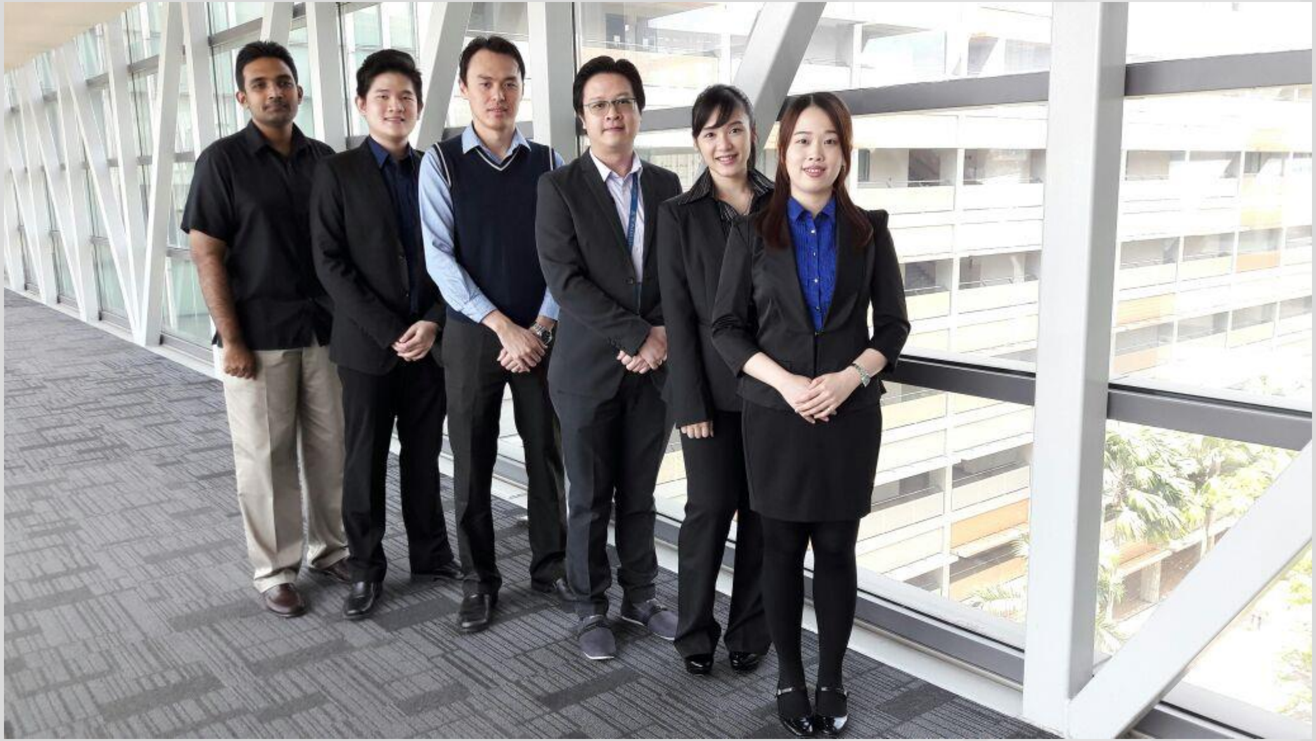

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