I: Discovery and Isolation of Bioactive Natural Products from Rare Non-Streptomyces Actinomycete Bacteria
II: Isolation of a New Antibiotic from a Commercial Feed Additive

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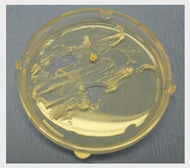
I. Introduction

Natural product isolation and identification is a necessary task to implement the development of novel compounds for uses such as drug discovery. Searching for novel natural products begins with finding unique strains of microorganisms. Actinomycetes are widely distributed in the natural environment, and synthesize numerous natural products. These natural products or derivatives thereof are widely used in medicine to fight bacterial, viral and fungal infections, cancer, and immune system disorders[1]. The order *Actinomycetales* constitutes of mostly Gram-positive, aerobic, and chemoorganotrophic bacteria. They grow from branching filaments resembling mycelia which sometimes break off into rod shaped structures. This fungus like characteristic once wrongly classified these bacteroides as fungi, but proves to be a very beneficial trait. Their metabolic diversity and particular growth characteristics, forming mycelia and relatively rapid colonization of selective substrates, signify them as well suited agents for bioremediation of metal and organic compounds[3]. Actinomycetes are also the current source of anti-tumor metabolites and various natural drugs including rapamycin, lipstatin, and thienamycin[2]. Natural products produced by actinomycetes most importantly include antibacterial activity against resistant strains of pathogens, which have been on the rise since the introduction of modern antibiotic chemotherapy in the 1930s and 1940s. The chance of finding genuinely new biologically active molecules from the common *Streptomyces* spp. actinomycetes is greatly reduced because many produce similar compounds[1]. Therefore rare, non-*Streptomyces* actinomycetes from soil samples collected from a Black Water Ecosystem in Indonesia are being screened for the production of novel bioactive natural products.

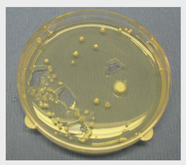
Experimental Actinomycete Strains

Strain 8316 : (-) Bioactivity
Amycolatopsis albidoflavus



Strain 8368 : (-) Bioactivity
Nocardia jiangxiensis

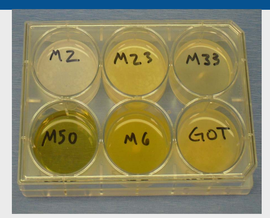
Strain 8400 : (-) Bioactivity
Amycolatopsis echigonensis



Strain 8352 : (+) Bioactivity
Amycolatopsis halotolerans

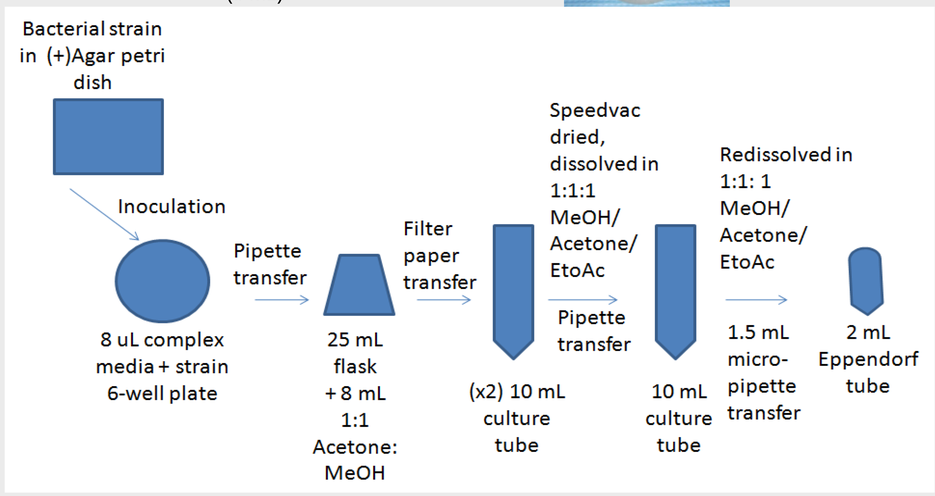
Strain 8340 : (+) Bioactivity
Nocardia seriolaes

Culture/Harvest

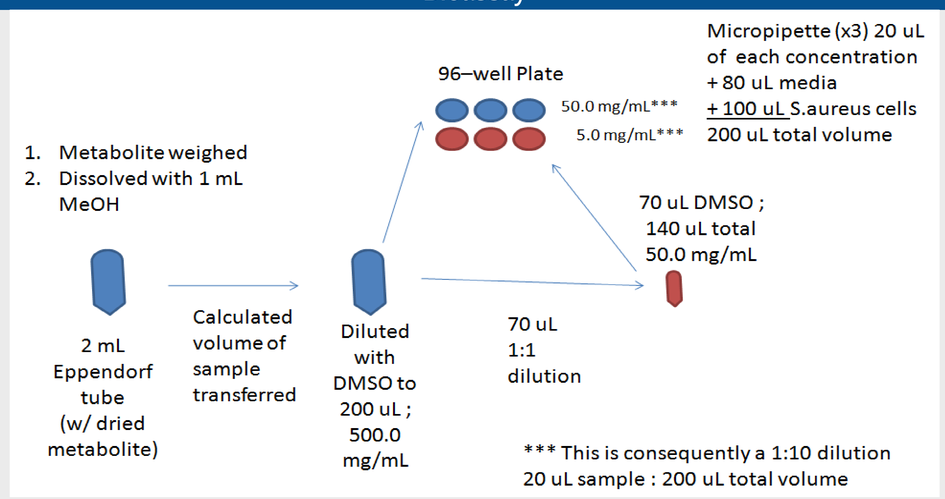


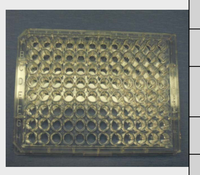
Complex Media 6-well Plate
(8 mL)

Specific Complex Media Culture for Bioactivity Confirmation (100 mL)



Bioassay





Bioassay Model Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	256 ug/mL	128 ug/mL	64 ug/ml	32 ug/mL	16 ug/mL	8 ug/mL	4 ug/mL	2 ug/mL	1 ug/mL	← (+) Control Kanamayacin :DMSO Serial dilution		
B	1C – 83X1; 50 mg/mL	1C	1C	2C – 83X1 ; 50 mg/mL	2C	2C	3C – 83X1 ; 50 mg/mL	3C	3C	4C – 83X1 ; 50 mg/mL	4C	4C
C	1C ; 5 mg/mL	1C	1C	2C ; 5 mg/mL	2C	2C	3C ; 5 mg/mL	3C	3C	4C ; 5 mg/mL	4C	4C
D	5C – 83X1; 50 mg/mL	5C	5C	6C – 83X1 ; 50 ug/mL	6C	6C	1C – 83X2 ; 50 mg/mL	1C	1C	2C – 83X2 ; 50 mg/mL	2C	2C
E	5C ; 5 mg/mL	5C	5C	6C ; 5 mg/mL	6C	6C	1C ; 5 mg/mL	1C	1C	2C ; 5 mg/mL	2C	2C
F	3C – 83X2 ; 50 mg/mL	3C	3C	4C – 83X2 ; 50 mg/mL	4C	4C	5C – 83X2; 50 mg/mL	5C	5C	6C – 83X2 ; 50 mg/mL	6C	6C
G	3C ; 5 mg/mL	3C	3C	4C ; 5 mg/mL	4C	4C	5C ; 5 mg/mL	5C	5C	6C ; 5 mg/mL	6C	6C
H							(-) Control 20 uL DMSO; 80 uL media; 100 uL S.aureus	(-) Control	(-) Control	Blank 20 uL DMSO ; 180 uL broth	Blank	Blank

Results

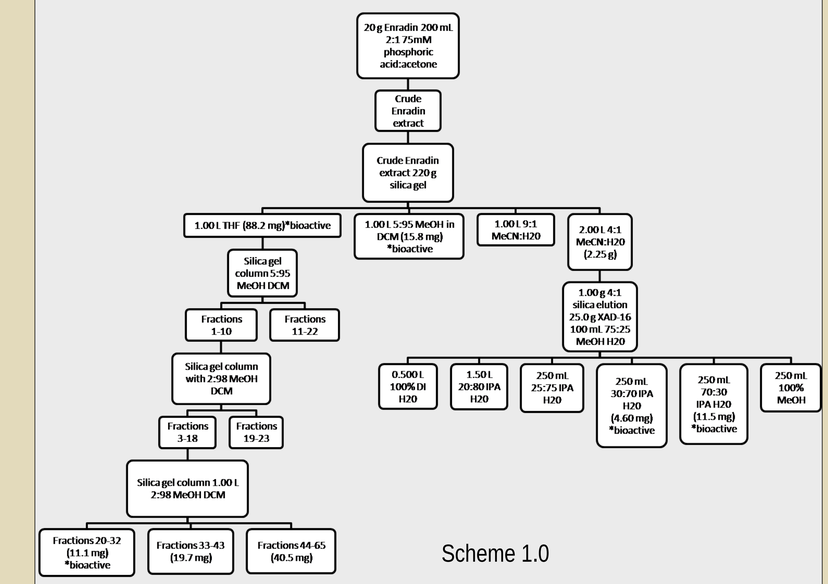
	1	2	3	4	5	6	7	8	9	10	11	12
A	256 ug/mL -15%	128 ug/mL -15%	64 ug/ml -15%	32 ug/mL -10%	16 ug/mL -16%	8 ug/mL -15%	4 ug/mL 24%	2 ug/mL 41%	1 ug/mL 92%	← (+) Control		
B	1C - 8340 30 mg/mL 31%	1C 45%	1C 53%	2C - 8340 1.1 mg/mL 102%	2C 90%	2C 110%	3C – 8340 .7 mg/mL 101%	3C 109%	3C 112%	4C – 8340 .7 mg/mL 109%	4C 132%	4C 137%
C	1C 3 mg/mL 90%	1C 96%	1C 94%									
D	5C – 8340 2 mg/mL 126%	5C 98%	5C 98%	6C – 8340 5 mg/mL 89%	6C 80%	6C 74%	1C – 8352 5 mg/mL 92%	1C 111%	1C 92%	2C – 8352 1.1 mg/mL 103%	2C 103%	2C 139%
E				6C .5 mg/mL 90%	6C 33%	6C 95%	1C .5 mg/mL 98%	1C 97%	1C 90%			
F	3C – 8352 1.8 mg/mL 122%	3C 125%	3C 127%	4C – 8352 5 mg/mL 109%	4C 102%	4C 82%	5C – 8352 1.5 mg/mL 110%	5C 104%	5C 103%	6C – 8352 50 mg/mL 16%	6C 15%	6C 15%
G										6C 5 mg/mL 81%	6C 99%	6C 105%
H							(-) Control	(-) Control	(-) Control	Blank	Blank	Blank

96-well plate results for strains 8340 and 8352 (8.20.10)

II. Introduction

Purification of the potent antibiotic enduracidin from the commercial poultry feed additive, Enradin® (Schering-Plough) is outlined by Scheme 1.0. This method is similar to the purification of confirmed bioactive metabolites from Project I. On route to extracting enduracidin, a new bioactive compound was found from the THF elution. Determination of the structure of the new antibiotic is underway.

Purification



Scheme 1.0

References

1. Bredholdt, *et al. Environmental Microbiology* 9.11 (2007): 2756-764. Print.
2. Kim, *et al. Pharmacology* 77 (2009): 1694-701. Print.
3. Polti, *et al. Chemosphere* 67 (2006): 660-67. Print.

Acknowledgements

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