DIVERSITY OF CULTURABLE ACTINOMYCETES FROM A LIMESTONE CAVE IN MALAYSIA

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Introduction

Actinomycetes form a well-defined clade of filamentous, high G+C Gram-positive bacteria which are widely distributed in rhizosphere, soil, caves, marine and freshwater systems. Geomicrobiology of caves is becoming a popular area of research due to its extreme environmental conditions. However, it is still in its infancy in Malaysia although she has some of the biggest and longest limestone caves in the world, mostly concentrated in the northern states of Peninsular Malaysia and Borneo.

In the realm of drug discovery, important new metabolites with biological activities have been and are still being discovered from actinomycetes and many of these are described as being produced by polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS). These biosynthetic gene clusters encode for important biochemical activities in pharmacology especially in the production of metabolites [1]. Rapid screening for the presence of potential antibiotic production biosynthesic gene clusters in actinomycetes can be done using an expedient tool of PCR screening [2].

Metals are essential nutrients for living organisms where they participate in a variety of cellular processes. However, metals such as lead, zinc and nickel are harmful even though they exist at low levels of concentration [3]. Heavy metals have largely contributed to environmental contamination and are a major concern to the public health. Technologies have developed to cope with the presence and accumulation of heavy metals in metal contaminated sites; however such remediation approaches may suffer from serious shortcomings due to high cost, high maintenance requirements, complicated procedures and the extended duration of the operation. A more practical and cost effective method using bioremediation involving the use of microorganisms to detoxify environmental contaminants should therefore be devised as an effective biotechnological approach to treat heavy metal contaminated environments [4].

Objectives

The objectives of the study are:

- to isolate actinomycete strains from environmental samples collected from Kandu Cave
- to screen the isolates for Type I & II PKS and NRPS genes as well as antibacterial activities
- to detect tolerance of the isolates towards lead, nickel and zinc.

Results

Various selective isolation media (HYEA, SM3, ISP4, and MM + 1% $CaCO_3$) used to isolate actinomycetes from samples collected from five selected sampling sites yielded populations of actinomycetes ranging from $1.0x10^5$-$3.8x10^6$ cfu/g (wall), $1.4x10^5$-$2.9x10^5$ cfu/g (column), $2.0x\ 10^5$-$2.9x10^5$ cfu/g (soil), $2.0x10^5$-$1.8x10^7$ cfu/g (stalagmite), to $1.7x\ 10^6$-$2.8x10^6$ cfu/g (flowstone). A total of 192 actinomycete strains were isolated and most of the strains have morphological properties similar to *Streptomyces* spp., i.e. dry, coarse, leathery or crusty colony surface, and smooth spiral chain spores. The strains were dereplicated according to the colours of aerial and substrate mycelium, as well as diffusible pigmentation. Nearly ¾ of the strains (72%) contained LL-DAP in their cell walls whereas the remaining contained *meso*-DAP. 16S rRNA gene sequence analysis on selected strains showed that most of them were members of genera *Streptomyces* and *Nocardia* (Fig. 1).

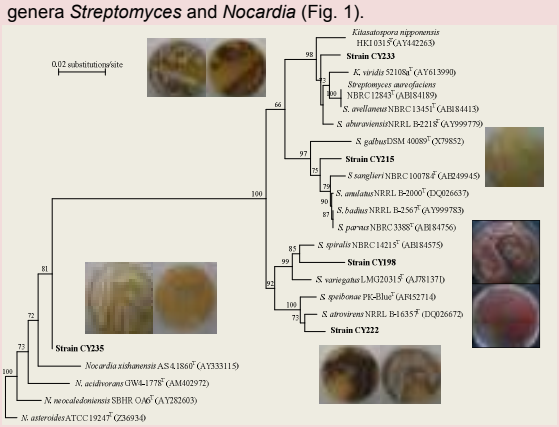


Fig. 1. Phylogenetic tree showing relationships between selected actinomycete strains and representatives of closely related species. The numbers at the nodes indicate the level of bootstrap support (%) based on 1000 resampled datasets.

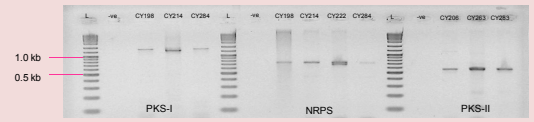


Fig. 2. Detection of NRPS, PKS-I and PKS-II biosynthetic genes.
L: 100bp marker; -ve: negative control.

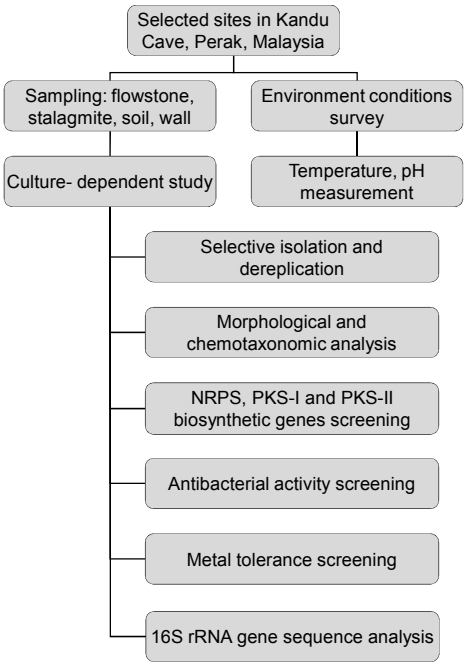
Seventy-five strains were screened for antibacterial activities. 63% inhibited at least one of the test bacteria, mostly against *K. pneumoniae* and *B. cereus*. For the biosynthetic gene sequences, the size length of the amplified fragments depend on the variable interdomain region extension (Fig. 2). Many of the strains (68%) showed presence of at least one type of biosynthetic gene, and 18 strains (24%) contained the NRPS and PKS-I genes. We observed that the number of bioactive strains were slightly lower than the number of strains possessing the different types of biosynthetic genes (Table 1). The antibacterial activities might also be activated by other domains of biologically active polyketides and peptide compounds instead of the screened genes.

Table 1. Screening of bioactive compound and biosynthetic genes.

Total strains	NRPS	PKS I	PKS II	Bioactive strains	Antibacterial activity			
					S. aureus	*B. cereus*	*K. pneumoniae*	*P. aeruginosa*
75	46	14	9	47	27	46	44	31
12	+	+	–	11	6	10	10	10
6	+	–	+	6	6	5	5	5
28	+	–	–	21	7	18	17	19
3	–	–	+	1	0	1	1	1
2	–	+	–	1	1	1	1	1
24	–	–	–	7	6	7	6	7

Metal tolerance results showed that 80 strains could grow in the presence of lead and zinc at high concentrations (<0.4 mg/ml) but only 19 were tolerant towards nickel at < 0.3 mg/ml. These strains may have metal tolerance mechanisms, such as metal binding proteins and reducing enzymes, to withstand high metal concentrations.

Methods



Summary

Actinomycete strains isolated from Kandu Cave are identified to be members of the genera *Streptomyces* and *Nocardia*. Most of the strains possess biosynthetic genes and have the ability to inhibit bacteria. In addition, they are tolerant to lead and zinc at high concentrations. Therefore, some of these strains may have potential applications in bioremediation processes. These strains will also be subjected to further studies on tolerance to other metals such as copper and mercury, and resistance to antibiotics.

Acknowledgements

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