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Antimicrobial Activity of Actinomycetes Isolated from Paya Maga, Sarawak

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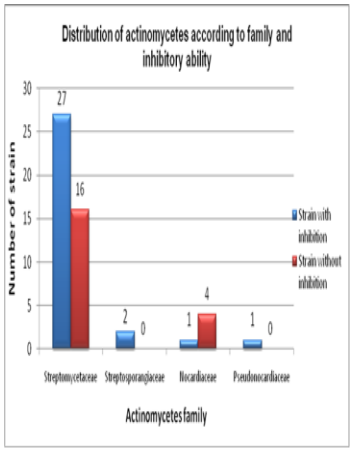
Graphical abstract



Abstract

A study was carried out to evaluate the antimicrobial activity of 51 actinomycetes strains isolated from environmental samples collected during an expedition at Paya Maga, located in Ulu Trusan, Lawas, Sarawak. These actinomycetes strains were isolated from 20 soil and 15 plant samples. Their ability to inhibit the growth of Gram positive bacteria (*Staphylococcus aureus, Micrococcus luteus*), Gram negative bacteria (*Escherichia coli*) and yeast (*Saccharomyces cerevisiae*) were tested by using co-culture method. Twenty five percent of the isolates (13 out of 51) were active against at least one bacteria or one yeast standard strain while 18 (35%) were active against at least two standard strains, indicating broad spectrum activities. The results also showed that 23% (7 strains) of the positive isolates with single or broad spectrum activities were able to show better result in comparison to 100 mg/L of Chloramphenicol and Nystatin used (by forming inhibition zone more than 20 mm in diameter). The 31 inhibitory strains were sequenced and partial 16S rDNA sequences were derived for taxonomic identification. The majority of these strains (27 out of 31) belong to the family of Streptomycetaceae, followed by 2 strains from Streptosporangiaceae and 1 strain each from Nocardiaceae and Pseudonocardiaceae. Sixteen of the isolates can only be classified up to the genus level so they are potentially novel species which are targets for further study to isolate antimicrobial agents.

Keywords: Actinomycetes; co-culture method; antimicrobial activity

Abstrak

Kajian telah dijalankan untuk menilai aktiviti antimikrob oleh 51 actinomycetes yang dipencil daripada sampel alam sekitar yang dikumpul sempena expedisi ke Paya Maga, Ulu Trusan, Lawas, Sarawak. Strain actinomycetes ini dipencil daripada 20 sampel tanah dan 15 sampel tumbuhan. Keupayaan strain tersebut untuk menghalang pertumbuhan bakteria Gram positif (Staphylococcus aureus, Micrococcus luteus), bakteria Gram negatif (Escherichia coli) serta yis (Saccharomyces cerevisiae) telah dikaji melalui kaedah ko-kultur. Dua puluh lima peratus strain tersebut (13 daripada 51) adalah aktif terhadap sekurang-kurangnya satu standard strain bakteria atau yis diuji manakala lapan belas (35%) strain tersebut adalah aktif terhadap sekurang-kurangnya dua strain standard yang menunjukkan aktiviti spektrum yang luas. Keputusan daripada kajian ini juga menunjukkan 23% (7 strain) daripada strain positif adalah lebih berkesan untuk menghalang pertumbuhan standard strain berbanding dengan 100 mg/L of Chloramphenicol dan Nystatin yang digunakan (dengan pembentukkan zon penghalang yang lebih besar daripada 20 mm diameter). Semua 31 stains yang positif telah dihantar untuk penjujukan DNA dan jujukan 16S rDNA yang dihasilkan diguna untuk pengenalan taksonomi. Majoriti strain tersebut (27 daripada 31) tergolong kepada famili Streptomycetaceae, diikuti dengan 2 strain dari Streptosporangiaceae dan1 strain dari Nocardiaceae dan Pseudonocardiaceae masing-masing. Enam belas strain tersebut hanya dapat dikenal pasti hingga ke tahap genus sahaja, mereka berpotensi dikenali sebagai spesies baru yang akan dijadikan sasaran dalam kajian lajutan untuk pemencilan agen antimikrob.

Kata kunci: Actinomycetes; kaedah ko-kultur; aktiviti antimikrob

1.0 INTRODUCTION

New drugs, especially antibiotics, are urgently needed to counter rapidly increasing multidrug resistance (MDR) amongst bacterial pathogens.[1] It is a serious global health alarm as existing antibiotics may no longer be effective in treatments. One of the most productive resources for the discovery of new antibiotics is from natural products. In prokaryotes, actinomycetes, remarkably Streptomycetes, remain the richest natural source of antibiotic.[2] Currently, the uncommon and less studied rare actinomyctes from unique ecosystem are targeted to improve the discovery of new antibiotics.

Efforts had been carried out to search for new modalities including sampling at underexplored ecological niches.[3] Some researchers believe that the more novel the actinomycetes the greater are the chances of discovering new antibiotics and this can be done by sampling unexplored location/untapped geographical sources.[4,5]

Paya Maga is a highland area located at Ulu Trusan, Lawas at an altitude of 1500–1800 m. This unexplored location has various types of habitat such as forest, swamp, exposed cliff, waterfall and lake. It was selected as one of the expeditions to the Heart of Borneo Sarawak organized by Forest Department Sarawak and Sarawak Forestry Corporation in year 2010.

2.0 EXPERIMENTAL

A total of 51 actinomycetes isolated from 20 soil and 15 plants samples collected from habitats of waterfall, cave and forest (1,590 m, 1,728 m and 1,732 m) were studied. The isolation methods of the strains were as reported earlier.[6]

2.1 Antimicrobial Activity Screening

Antimicrobial activity screening was conducted using co-culture method. The standard strains were grown for 24 hours in Luria Bertani broth for bacteria and Sabouraud Dextrose broth for yeast. The standard strains at a working concentration of 10^6 cells/ml were inoculated in 1% Luria Bertani agar (LBA) for bacteria and 1% Sabouraud Dextrose agar (SDA) for yeast respectively. An agar plug was prepared by using sterilized straw of 5 mm in diameter. Agar plugs of seven-day-old cultures of isolated actinomycetes were placed on the LBA and SDA plate respectively (five plugs/plate). After incubation of 24 hours at 28°C, the inhibition zone was measured. The standard strains tested were *Staphylococcus aureus* NBRC 12732, *Micrococcus luteus* NBRC 12708, *Escherichia coli* NBRC 3301 and *Saccharomyces cerevisiae* ATCC 9763.

The positive control for bacteria strains are 100 mg/L of Chloramphenicol and 100 mg/L of Nystatin for yeast strain which are able to form inhibition zones at the diameter of 20 mm.

2.2 DNA Amplification and Analysis

Genomic DNA extracted from the actinomycetes isolates was subjected to amplification using polymerase chain reaction (PCR) performed in a 20 µl mixture containing 16.8 µl of sterilized water, 2 µl of 10X PCR buffer (Fermentas), 0.2 µl of 10 mM dNTP (Fermentas), 0.4 µl for both 20 µM/ml of SRR181F (5'GTTTGATCCTGCTCAGGAC3') and SRR182R (5'CAGATATCAGGAGGAACACCG3') primer respectively,[7] 0.01 unit of Pfu polymerase (Fermentas) and 40 ng of genomic DNA. PCR was performed under the following condition: initial denaturation step at 96°C for 1 min, followed by 30 cycles of denaturation at 96°C for 45 s, annealing at 53°C for 1 min, extension at 72°C for 2 min and a final extension at 72°C for 7 min. Sequencing of the purified PCR products was carried out by First Base Laboratories Sdn. Bhd. (Kuala Lumpur, Malaysia). The 16S rRNA sequences obtained were compared to sequences in the GenBank database with the Basic Local Alignment Search Tool (BLAST). Sequences of strains with maximum identity ≤ 98% are cutoff points in species determination. This is based on Muramatsu (2008), who used slightly higher 98.6% for species determination.

3.0 RESULTS AND DISCUSSION

A total of 31 out of 51 actinomycetes isolates were able to form inhibitory zones on the standard strains tested. Thirteen of the isolates (25%) were able to inhibit single bacteria or single yeast standard strain. Inhibitions toward two or more standard strains were shown by 18 isolates (35%). The ability of the actinomycetes to inhibit two or more standard strains indicates a broad spectrum activity. None of the isolates could inhibit the Gram negative bacteria exclusive. However, there were 2 isolates (4%) that displayed activity against the growth of Gram positive and Gram negative bacteria at the same time. The antimicrobial activity result is summarized in Figure 1.

Strains that actively inhibit Gram negative bacteria are of more interest to some researchers as more than 60% of known antibiotics are active only against Gram positive bacteria. Baltz and his team (2007) screened against *Escherichia coli* allowing them to concentrate on what they believe to be the serious and most clinically important challenge, particularly the MDR Gram negative bacteria.

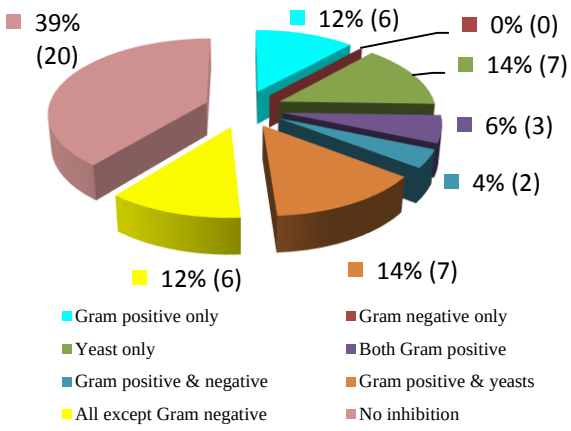


Figure 1 Antimicrobial activity of 51 actinomycetes isolated from Paya Maga

From the antimicrobial screening result, 7 out of the 31 positive isolates (23%) were able to show better inhibition in comparison to 100 mg/L of Chloramphenicol and Nystatin used (by forming inhibition zone more than 20 mm in diameter). The result is shown in Table 1.

The 51 actinomycetes isolated can be classified into four families namely Streptomycetaceae, Streptosporangiaceae, Nocardiaceae and Pseudonocardiaceae. Majority of the positive isolates (27 out of 31) belong to the family of Streptomycetaceae, followed by 2 positive isolates from Streptosporangiaceae and 1 positive isolate each from Nocardiaceae and Pseudonocardiaceae (Figure 2).

Table 1 Antimicrobial activity of 7 positive isolates by the co-culture method against the four standard strains tested[a]

Isolates	Inhibition zone (mm)			
	SA[b]	ML[c]	EC[d]	SC[e]
PM 39	-	-	-	25
PM 62	27	-	-	-
PM 74	6	12	-	32
PM 87	-	-	-	23
PM 90	-	-	-	25
PM 181	-	30	-	-
PM 193	-	15	-	33

[a] The inhibition zone includes the diameter (5 mm) of the actinomycetes agar plug
[b] SA = *Staphylococcus aureus NBRC 12732,*
[c] ML = *Micrococcus luteus NBRC 12708,*
[d] EC = *Escherichia coli NBRC 3301,*
[e] *SC = Saccharomyces cerevisiae ATCC 9763*

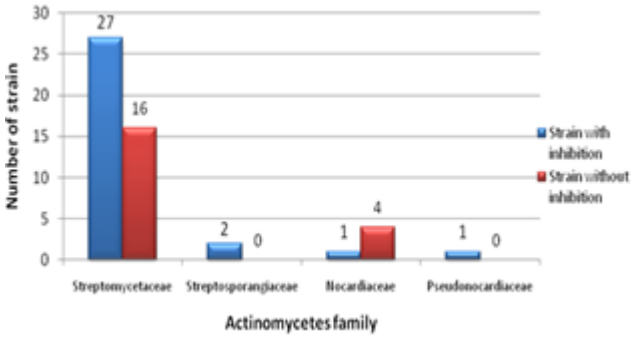


Figure 2 Distribution of actinomycetes according to family and inhibitory ability

Based on BLAST maximum identity ≤ 98%, 26 out of 51 of the isolates (51%) can only be classified to the genus level. Sixteen out of the twenty six isolates (62%) are positive isolates. Among the 16 positive isolates, 12 isolates from Streptomycetaceae and 4 isolates from the rare actinomycetes family (2 isolates from Streptosporangiaceae; 1 isolate from Nocardiaceae and 1 isolate from Pseudonocardiaceae). Streptomycetaceae are the most abundant actinomycetes in the environment[8] which also reflected in this study. They are normally dominant in collections and easily isolated compared to the rare actinomycetes that are difficult to isolate and cultivate.

4.0 CONCLUSION

Thirty-one out of the actinomycetes isolated from Paya Maga inhibited the growth of the standard bacterial and yeast strains tested. Thirteen of the isolates were active against at least one bacteria or one yeast standard strain. Meanwhile, 18 isolates were able to inhibit at least two standard strains tested. Seven of the positive isolates with single or broad spectrum activities were able to show better results than the positive control. Majority of the positive isolates belonged to the family of Streptomycetaceae. A total of 16 positive isolates can only be classified up to the genus level so they are potential candidates for further studies especially the rare actinomycetes isolates. Co-culture method is an effective and simple method to provide information on antimicrobial activity before proceeding to the fermentation processes. The antimicrobial information is also useful in strain selections.

Acknowledgements

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