Isolation and Characterization of Antibiotic-producing Actinomycetes from hot spring sediment of Thailand

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Abstract

In the course of our investigation for the antibiotic-producing actinomycetes, thirty-two actinomycete strains were isolated from Thai hot spring sediment. The generic identities of these isolates were determined by using a procedure that combined morphological, chemotaxonomic and 16S rDNA sequence-based phylogenetic analyses. Actinomycetes belonging to a total of 4 genera were identified. They were members of *Streptomyces, Micromonospora, Microbispora* and *Planosporangium*. The fermentation broths of these isolates were extracted with ethyl acetate and were tested for antimicrobial activity. The results showed that more than 50% of the actinomycete strains inhibited at least one of the tested micro-organisms. Based on these results, we conclude that actinomycete diversity in the hot spring sediment is great and should represent an excellent source for discovery of the bioactive compounds.

Key words: Actinomycetes, Hot spring, Antibiotic.

Introduction

The actinomycetes are Gram-positive bacteria having high G+C (>55%) content in their DNA. These organisms were originally considered to be an intermediate group between bacteria and fungi but now are recognized as prokaryotic organisms. The actinomycetes are well-known sources of secondary metabolites that can be developed as a resource for biotechnology (Fiedler et al., 2005 and Blunt et al., 2007). Since hot spring environment differs greatly from terrestrial habitats, the biological characteristics of actinomycetes and their distribution are expected to be different from those of soil actinomycetes. Research on the biodiversity of hot spring actinomycetes is not only important for basic studies but also necessary in practice for its exploitation. Furthermore, the hot spring environment is proving to be a major source of new natural products, especially antimicrobial compounds, most notably those expressed by actinomycetes (Bull et al., 2005). New metabolites produced by actinomycetes have increasingly been reported, a promising source for pharmaceutical and biotechnology products.

During investigation for bioactive metabolites from actinomycetes isolated from hot spring sediment in Krabi province, Thailand, we successfully isolated thirty-two actinomycete strains. These strains showed typical morphological, chemotaxonomic and genotypic characteristics of the genera *Streptomyces, Micromonospora, Microbispora* and *Planosporangium*. Here we report on isolation, taxonomic characterization of these actinomycete strains and also report on their antimicrobial activity.
Materials and methods

A. Sample collection and isolation of the actinomycetes

The hot spring sediments were collected from Krabi province, Thailand. The samples were air-dried at room temperature for four days. The sample (1 g) was treated with 0.05% (w/v) sodium lauryl sulfate in distilled water (9 ml) at 30 °C for 30 minute and then serially diluted in sterile distilled water as recommended by Thawai et al., 2005. The strain was isolated on humic acid-salts vitamin agar (HV) supplemented with 25 mg l\(^{-1}\) nalidixic acid, 50 mg l\(^{-1}\) cycloheximide and 50 mg l\(^{-1}\) nystatin and the pure cultures were preserved by freezing at -80 °C and freeze-drying.

B. Identification of strain GDN 8-88

Strain GDN 8-88 was subjected to polyphasic taxonomy, including morphology of hyphae and spores, 16S rRNA gene sequence analysis and so on (Thawai et al., 2005).

C. Extraction of antimicrobial metabolites

A loopful of representative actinomycete strain in each group on ISP2 agar slant was inoculated in to 100 ml of the seed medium (YM) for 3 days. One percent of seed culture was inoculated in to production medium (YM, 1 L) at room temperature for 10 days. The YM fermentation broth was filtrated and partitioned with ethyl acetate for three times. The ethyl acetate layer was concentrated under reduced pressure at 40 °C to yield crude ethyl acetate extract.

D. Anti-microbial activity

The crude ethyl acetate extracts of each representative actinomycete strain were tested against different test organisms by agar disc diffusion method as described by Lorian et al., 1980, to evaluate the anti-microbial activities

Results and discussion

Thirty-two actinomycete strains were isolated from Thai hot spring sediments. These strains were grouped using morphological characteristic, physiological, biochemical properties. The generic identities of these selected actinomycete isolate in each group were determined by using a procedure that combined morphological, chemotaxonomic and 16S rRNA gene sequence-based phylogenetic analyses.

The actinomycete in group I consisted of nine strains, including HSS5-1, HSS5-2, HSS5-3, HSS5-4, HSS4-2, HSS4-3, HSS4-4, HSS4-5, HSS10-11. They typically produced the longitudinally paired spores on their aerial hyphae having an approximate diameter of 0.5-0.6 µm. The spore surface was smooth and non-motile (Fig 1a). The colors of the substrate mycelium were yellowish white to brown and turned to pinkish white to pink after sporulation. The morphological characteristics of these isolates were consistent with their classification in the genus Microbispora. The representative strain, HSS5-1, was most closely associated with Microbispora rosea subsp. aerata subsp. aerata NBRC 14624\(^T\) (Miyadoh et al., 1990) in the neighbor-joining analysis (Fig 1b) and shared the highest 16S rRNA gene sequence similarity percentage of 99.7 with Microbispora rosea subsp. aerata NBRC 14624\(^T\). Their secondary metabolites inhibited growth of methicillin resistant Staphylococcus aureus (MRSA), Micrococcus luteus ATCC 9341 and Staphylococcus aureus ATCC 25923.
The actinomycete in group II consisted of eighteen isolates, HSS9-1 - HSS9-18. All isolates in this group produced the long spiral chain of spores on their aerial hyphae. The spore surface was smooth and non-motile (Fig 2a). The colors of the substrate mycelium were pale yellow and turned to white after sporulation. The morphological characteristics of these isolates were consistent with their classification in the genus *Streptomyces*. The representative strain, HSS9-1, was most closely associated with *Streptomyces xinghaiensis* (Zhao *et al.* 2009) in the neighbor-joining analysis by moderate bootstrap value (Fig 2b) and shared the highest 16S rRNA gene sequence similarity percentage of 98.7. These isolates could produce the active secondary metabolite against *Micrococcus luteus* ATCC 9341 and *Bacillus subtilis* ATCC 6633.

The actinomycete in group III consisted of four isolates, HSS1-1, HSS1-5, HSS2-2, HSS2-4. All isolates in this group produced a single spore directly on their substrate hyphae. The spore surface was warty and non-motile (Fig 3a). The colors of the substrate mycelium were vivid orange and turned to brown after sporulation. The morphological characteristics of these isolates were consistent with their classification in the genus *Micromonospora*. The representative strain, HSS1-1, was most closely associated with *Micromonospora eburnea* (Thawai *et al.*, 2005) in the neighbor-joining analysis (Fig 3b) and shared the highest 16S rRNA gene sequence similarity percentage of 99.4. The secondary metabolite of these isolates exhibited the anti-bacterial activity against *Micrococcus luteus* ATCC 9341.

The actinomycete in group IV consisted of one isolate, HSS8-18. This isolate produced single globose bodies and short finger-like sporangium directly from substrate mycelium (Fig 4a). Colonies are strong orange yellow to reddish brown on ISP 2. Each smooth sporangium contains motile spores. Globose bodies and pale yellowish white aerial mycelium are observed on ISP2, modified soil extract agar and humic acid–vitamin agar after 7 weeks of cultivation. A reddish brown soluble pigment is also produced in ISP 2. The morphological characteristics of these isolates were consistent with their classification in the genus *Planosporangium*. The representative strain, HSS8-18, was most closely associated with *Planosporangium flavigriseum* (Wiese *et al.*, 2008) in the neighbor-joining analysis by high bootstrap value (Fig 4b) and shared the highest 16S rRNA gene sequence similarity percentage of 98.3.

![Figure 1](image1.png)

**Figure 1.** (a) Scanning electron micrograph of strain HSS5-1; (b) Neighbour-joining tree of strain HSS5-1

![Figure 2](image2.png)

**Figure 2.** (a) Scanning electron micrograph of strain HSS9-1; (b) Neighbour-joining tree of strain HSS9-1
Conclusion

In this study, we successfully isolated the actinomycetes from the sediments collected from hot spring pond located in Krabi and Trang province, Thailand. These actinomycete strains were identified using the morphological property and 16S rRNA gene sequence analysis. They belonged to the member of genera *Streptomyces*, *Micromonospora*, *Microbispora* and *Planosporangium*. The crude ethyl acetate extract from the fermentation broth of the representative strain in each group with exception in the genus *Planosporangium* exhibited the anti-bacterial activity against Gram-positive bacteria i.e. methicillin resistant *Staphylococcus aureus* (MRSA), *Micrococcus luteus* ATCC 9341 and *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633. This result implied that the hot spring sediments are a great source for discovery of new actinomycetes and the bioactive compounds.

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References


