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Poster:

Isolation and characterization of antimicrobial constituents from a terrestrial *Streptomyces* sp.: A preliminary result

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Abstract. Five phenolic derivatives, namely 2-methoxybenzene-1,3-diol (1), 2,3-dihydroxy-benzamide (2), benadrostin (4), 2,3-dihydroxy-benzoicacid (5) and tyrosol (6) were isolated from the terrestrial *Streptomyces* sp. Ank75. Three of them are new natural products. A further new antifungal is an azoxy derivative 3. All structures were elucidated based on spectroscopic and MS data and will be discussed here.
Keywords: Fermentation, algae, *Streptomyces* sp, derivatives

Introduction

The genus of *Streptomyces* has been reported as the largest source of novel antibiotics from bacteria since the first report of streptomycin in 1942. Currently, many microbial antibiotics have been reported from genus *Streptomyces*, and the number of microbial antibiotics has increased exponentially for the last two decades (Watve *et al*., 2001). In 2001, more than 70% of antibiotics had been isolated from genus *Streptomyces*, and 4% of them were used clinically as human drugs (Clardy *et al*., 2006).

In this research, we reported the secondary metabolites isolated from terrestrial *Streptomyces* sp.Ank75. One of the secondary metabolites from the strain Ank75 was an azoxy derivative, compound **3**, which was also active as antifungal. In addition, five phenolic compounds, namely 2-methoxybenzene-1,3-diol (**1**), 2,3-dihydroxy-benzamide (2), benadrostin (**4**), 2,3-dihydroxy-benzoicacid (**5**) and tyrosol (**6**) were isolated from the terrestrial *Streptomyces* sp. Ank75 (Figure 1). Due to the complexity of the structure, only compound **3** will be discussed in more detail here.

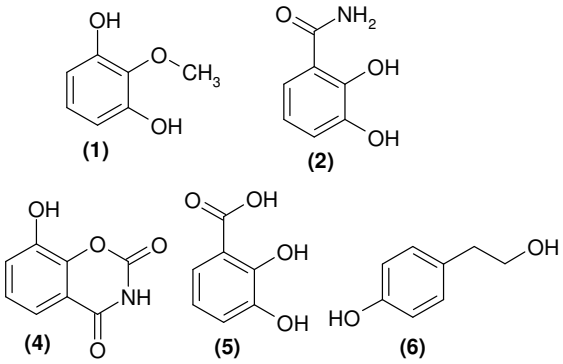

Figure 1: Isolated compounds from *Streptomyces* sp. Ank75

Materials and Methods

Isolation and bioassay

The *Streptomyces* sp.Ank75 strain was fermented in 25 L scale on M_2 medium for 5 days at 28 ºC. The culture broth was filtered over Celite and the water phase was adsorbed on resin Amberlite XAD-16 column and eluted with methanol after washing with demineralised water prior to the elution with methanol. The biomass was exhaustively extracted with ethyl acetate and acetone. TLC of crude extract from each organic residues was identical, thus the three extracts (6.0 g) were combined together for further work-up procedure (Figure 2).

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The separation of crude extract (6.0 g) was performed by silica gel column chromatography (3 × 75 cm, 200 g) using aCH_2Cl_2/CH_3OH gradient. Based on the spot pattern on the TLC, four fractions were selected for further purification. The 2-methoxybenzene-1,3-diol (**1**) was obtained from fraction II *via* Sephadex LH-20 column using CH_3OH as a solvent. From fraction III, benadrostin (**4**) was isolated *via* Sephadex LH-20 column using CH_3OH as a mobile phase. Compounds **2**, **3**, **5** and **6** were isolated from fraction IV after being subjected to Sephadex LH-20 (MeOH) and RP-18 (MeOH:H_2O) columns, respectively. The isolation procedure from crude extract of *Streptomyces* sp.Ank75 was shown in Figure 2.

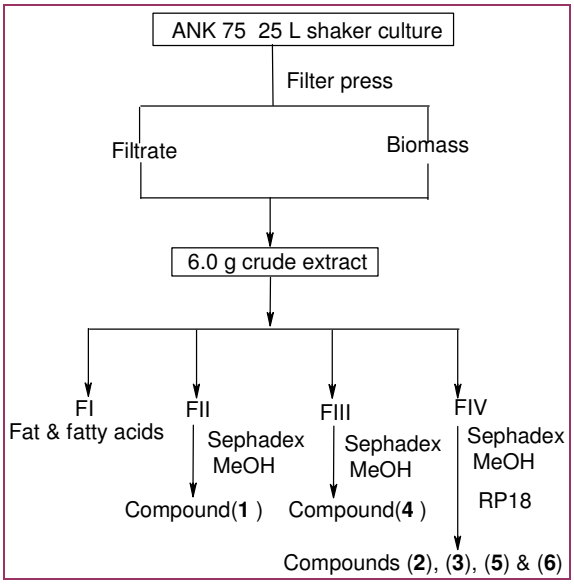

Figure 2: Work up scheme of *Streptomyces* sp. Ank75

Biological activity

The terrestrial *Streptomyces* sp. Ank75 formed on M_2 agar medium a white aerial mycelium within 3 days at 28 ºC. The crude extract of the strain showed activities against *Candida albicans*, *Mucor miehei* (Tü 284), *Chlorella vulgaris*, *Chlorella sorokiniana*, but was inactive against bacteria and *Artemia salina* (brine shrimp test) (Table 1).

Table 1: Anti-microbial activity of the crude extract *Streptomyces* sp. Ank75 (1 mg/ml, diameter of inhibition zones in mm).

Tested microorganisms	Inhibition zone ∅ [mm]
Candida albicans	15
Mucor miehei (Tü 284)	25
Chlorella vulgaris	33
Chlorella sorokiniana	31
Artemia salina	0%

Result and Discussion

ESIMS of **3** afforded 255.2 ($[M^+ + Na]^+$, 100), and HRESI MS delivered the formula $C_{10}H_{20}N_2O_4$. One of the two double bond equivalents is due to a CO group, the other one belongs to a ring, as double bond signals are missing. Three alternative structures A, B or C were taken into account, where A and C are azoxy derivatives, and B is a highly strained oxadiaziridine (Figure 3). According to the literature, structure B is thermally labile and less stabile than structures A and C (Hecht and Greene, 1967: Swigert and Taylor, 1971). Structure C is a tautomer of an instable primary nitrosamine. The ^{13}C NMR spectrum showed an sp^3 signal at δ_C 56.1 for a carbon, which is possibly attached to nitrogen. Kariya *et al.* (2006) have reported a related compound bearing an azoxy moiety, which corresponds to structures A or C.

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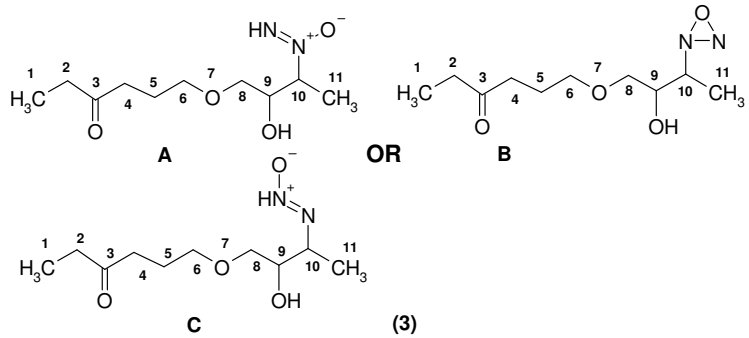

Figure 3: Plausible structures of compound **3**.

Conclusions

To sum up, five phenolic and one azoxy derivatives have been isolated from the terrestrial *Streptomyces* sp.Ank75. In addition, compound **3** (azoxy derivative) has also shown antifungal activity in this research.

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