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Desert actinobacteria as a source of bioactive compounds production with a special emphases on Pyridine-2,5-diacetamide a new pyridine alkaloid produced by *Streptomyces* sp. DA3-7

Krishnasamy Nithya[a], Chinnasamy Muthukumar[b], Bhaskar Biswas[c], Naiyf S. Alharbi[d], Shine Kadaikunnan[d], Jamal M. Khaled[d], Dharumadurai Dhanasekaran[a,e,*]

[a] Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India
[b] Department of Botany, National College (Autonomous), Tiruchirappalli 620001, Tamil Nadu, India
[c] Department of Chemistry, Surendranath College, Kolkata 700009, India
[d] Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[e] Department of Molecular, Cellular and Biomedical Sciences, University of New Hampshire, Durham, NH, 03824-2617, USA

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ABSTRACT

In the present study, 134 morphologically distinct actinobacteria isolates were obtained from soil samples from 10 different localities in the Saudi Arabian desert. The preliminary screening revealed that 16 of these isolates possessed antimicrobial activity. One isolate, which was identified as *Streptomyces* sp. DA3-7, possessed broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria, as well as against fungi, and modified nutrient glucose medium was suitable for *Streptomyces* sp. DA3-7 to produce extracellular metabolites. The ethyl acetate extract of *Streptomyces* sp. DA3-7 exhibited antimicrobial activity against *Enterococcus faecalis* and *Salmonella typhimurium*, with minimum inhibitory concentrations of 78 and 156 µg/mL, respectively, as well as strong cytotoxicity (24 h IC_{50} 85 µg/mL) against MCF-7 human breast adenocarcinoma cells. The active compound was separated, purified, and identified as Pyridine-2,5-diacetamide ($C_9H_{11}N_3O_2$ + H+, 194.21), which possessed a lowest minimum inhibitory concentration (31.25 µg/mL) against both *Escherichia coli* and *Cryptococcus neoformans*. The antimicrobial activities of this novel compound are reported here for the first time.

1. Introduction

Actinobacteria provide an unlimited source of bioactive compounds that can be used for a variety of applications, and the genus *Streptomyces*, in particular, has yielded several important bioactive compounds, including antibiotics, bio-herbicides, and enzymes, all of which possess high commercial value. In fact, nearly two-thirds of the antibiotics known today have been obtained from *Streptomyces*. However, even though the number of antimicrobial compounds obtained from this genus increased exponentially each year between 1960 and 1970, the identification of such compounds progressed at a slower pace during the 1980s and 1990s (Watve et al., 2001).

This declining trend in the discovery of new antimicrobial compounds and the enigmatic development of antibiotic resistance in bacteria has increased the need for scientists to investigate unexplored habitats, in order to identify novel actinobacterial isolates and bioactive compounds. Recently, researchers have focused on extremophilic actinobacteria with the hope that these organisms would add an innovative dimension to antimicrobial natural products research (Zitouni et al., 2004; Vijayakumar et al., 2012; Dhanasekaran et al., 2014), and as a result, bioactive compounds have been identified in actinobacterial isolates from the Algerian Saharan desert (Badji et al., 2006), Atacama desert (Rateb et al., 2011), Egyptian desert (Koberl et al., 2011), Qinghai-Tibet Plateau (Ding et al., 2013), and Thar desert (Thumar et al., 2010).

In Saudi Arabia, more than 90% of the land area is occupied by desert. However, the diversity of actinobacteria in this habitat and the bioactive compounds that they ostensibly produce have been poorly explored (Atta et al., 2010; Ara et al., 2012; Nithya et al., 2015). Therefore, the aim of the present study was to screen the soil of Saudi Arabian deserts for actinobacteria, with a special emphasis on a bioactive compound produced by the desert actinobacterium *Streptomyces* sp. DA3-7.

* Corresponding author at: Department of Microbiology, School of Life Sciences, Bharathidasan University, Tiruchirappalli 620024, India.
E-mail addresses: dhansdd@gmail.com, ddhanasekaran@bdu.ac.in (D. Dhanasekaran).

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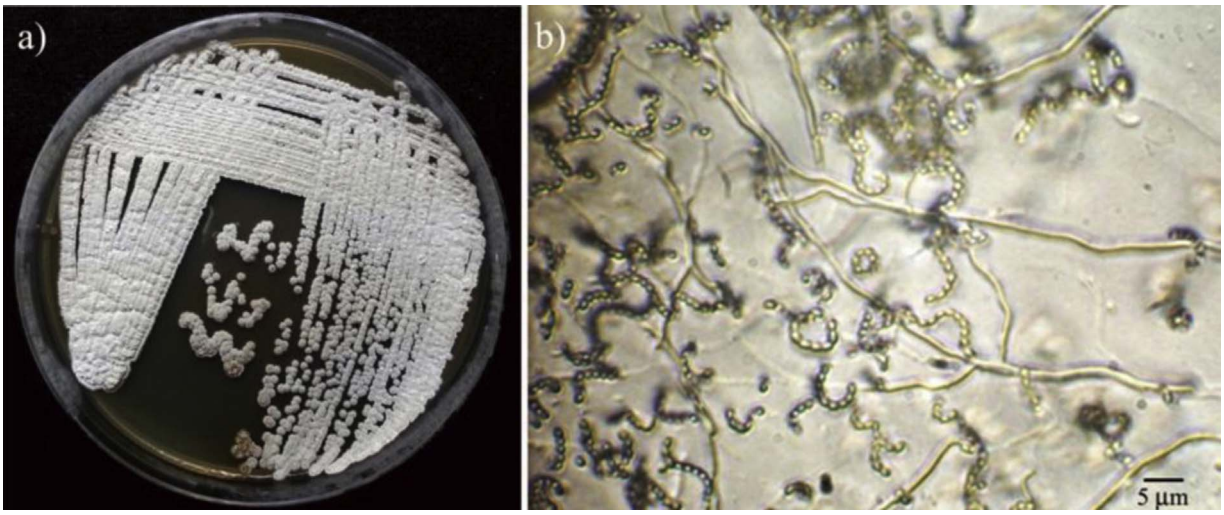

Fig. 1. Colony morphology and microscopic view of *Streptomyces* sp. DA3-7. (a) Isolate DA3-7 grown on ISP-2 medium; (b) Phase contrast microscopic view of isolate DA3-7.

Table 1
Morphology of isolate DA3-7 on different media.

Media	Aerial mycelium	Substrate mycelium	Diffusible pigment	Growth
ISP 1	Greyish white	Pale yellow	−	+ + +
ISP 2	Ash grey	Brown	−	+ + +
ISP 3	Ash grey	Golden yellow	−	+ +
ISP 4	Ash grey	Greyish white	−	+ + +
ISP 5	Ash grey	Greyish white	−	+ + +
ISP 6	Pale yellow	Pale yellow	−	+
ISP 7	Ash grey	Ash grey	−	+ + +
SCA	Yellowish grey	Pale yellow	−	+ + +
NA	Pale yellow	Pale yellow	−	+
GYEA	Greyish white	Brown	−	+ + +

+ + +, good; + +, moderate; +, poor; −, absent. ISP, International *Streptomyces* Project; GYEA, glucose yeast extract agar; NA, nutrient agar; SCA, starch casein agar.

2. Materials and methods

2.1. Isolation of actinobacteria

The chemicals and media used for the experimental analysis were of analytical reagent grade and are purchased from Himedia (Mumbai, India) and Sigma-Aldrich (Bengaluru, India). Soil samples were collected from 10 different locations in the desert of the Riyadh Province (24° 49′01.3″N; 46° 43′01.2″E), Saudi Arabia. Starch casein agar (SCA) medium that was supplemented with amphotericin B (20 µg/mL) and nalidixic acid (10 µg/mL) was used to isolate the actinobacteria (Ellaiah et al., 1996). The soil samples were serially diluted by aseptically adding 10 g of soil in 90 mL of sterile distilled water (10^{-1}), mixed by shaking and further tenfold dilutions were made up to 10^{-6}. The 0.5 mL aliquots of each soil, from the dilutions 10^{-4}, 10^{-5} and 10^{-6} were spread over the agar plates in triplicates and incubated at 28 °C for 15 days. Three replicates were used for each dilution and the average colony count of actinobacteria formed on a plate was calculated. Data were expressed as a colony forming units (CFU/g) of soil dry weight. Colonies were recognized by their cultural and morphological features, the actinobacterial isolates were purified. Stock cultures of these isolates were maintained in cryotubes with 1.5 mL 20% (w/v) sterile glycerol solution at −20 °C (Wellington and Williams, 1978).

2.2. Primary screening

Pathogenic bacteria and fungi were obtained from the American type culture collection (ATCC; Virginia, USA) (Table 2). Bacterial and fungal inocula were prepared using Mueller-Hinton (MH) broth and Sabouraud dextrose (SD) broth, respectively, the antimicrobial activities of the actinobacterial isolates were assessed using the cross streak method (Egorov, 1985).

2.3. Characterization of isolate DA3-7

The morphological characteristics of isolate DA3-7 was examined as recommended by the International *Streptomyces* Project (ISP). The growth, colour of the aerial and substrate mycelia, and the production

Table 2
Minimum inhibitory concentrations of the DA3-7 extract against various pathogenic microorganisms.

S. No.	Test organism	Strain number	MIC	
			DA3-7 extract (mg/mL)	Control (µg/mL)
	Bacteria			Streptomycin
1.	*Klebsiella pneumoniae*	ATCC 12882	0.312	25
2.	*Enterococcus faecalis*	ATCC 49532	0.078	12.5
3.	*Escherichia coli*	ATCC 10536	0.312	50
4.	*Proteus vulgaris*	ATCC 33420	0.312	25
5.	*Salmonella typhimurium*	ATCC 13311	0.156	50
6.	*Pseudomonas aeruginosa*	ATCC 27883	0.312	50
7.	*Staphylococcus aureus*	ATCC 6538P	0.312	25
	Fungi			Ketoconazole
8.	*Candida albicans*	ATCC 2091	0.625	25
9.	*Cryptococcus neoformans*	ATCC 90113	0.625	50
10.	*Saccharomyces cerevisiae*	ATCC 9763	0.625	25