Biologicals 42 (2014) 305–311

Contents lists available at ScienceDirect

Biologicals

journal homepage: www.elsevier.com/locate/biologicals

Antimicrobial potential of phylogenetically unique actinomycete, *Streptomyces* sp. JRG-04 from marine origin

Ganesan Govindarajan, Velayudhan Satheeja Santhi, Solomon Robinson David Jebakumar*

Department of Molecular Microbiology, School of Biotechnology, Madurai Kamaraj University, Madurai 625021, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 4 June 2014
Received in revised form
15 August 2014
Accepted 16 August 2014
Available online 6 September 2014

Keywords:
Mangrove
Streptomyces
16S rRNA
Polyketide
Benzoisochromanequinone

ABSTRACT

Due to the emergence of severe infectious diseases and thriving antibiotic resistance, there is a need to explore microbial-derived bioactive secondary metabolites from unexplored regions. Present study deals with a mangrove estuary derived strain of *Streptomyces* sp. with potent antimicrobial activity against various pathogens, including methicillin resistant *Staphylococcus aureus*. Bioactive compound was effective even at low MIC level, damages the membrane of methicillin resistant *S. aureus* and causes cell death, however it has no cytotoxic effect on H9C2 cells. 16S rRNA shared 99.5% sequence similarity to *Streptomyces longispororuber*. Optimum biomass and antimicrobial compound production were observed in production medium supplemented with 1.0% maltose and 0.5% yeast extract. The active compound purified from the chloroform extract of the cell-free supernatant was studied by FT-IR, 1H NMR, 13C NMR and LC ESI-MS and identified as aromatic polyketide. β-ketosynthase (KS) domain of the *Streptomyces* strain revealed 93.2% sequence similarity to the benzoisochromanequinone, an actinorhodin biosynthetic gene cluster of *Streptomyces coelicolor* A3(2). However, the region synthesizing the secondary metabolite produced by the *S. longispororuber* was not related to the KS domain of the strain, due to the phenomenon of horizontal gene transfer over the period of evolutionary process, thus generating metabolic compound diversity.

1. Introduction

The emerging infectious diseases and antibiotic resistance pathogens create a major health concern all over the world. In order to overcome these problems, search for a potent, new antibiotic compound with novel mode of action is urgently needed. The secondary metabolites from marine bacteria, particularly the members those belonging to the phylum Actinobacteria are endowed with a wide variety of chemical structures possessing strong biological activities [1]. *Streptomyces* is the GC rich, Gram-positive, filamentous member of the phylum, Actinobacteria and they produce many pharmaceutically important secondary metabolites such as therapeutic enzymes, antibiotics, immunosuppressants, anti tumor agents and vitamins [2].

Unexplored marine environments usually have the more chances of identifying the Streptomyces with chemically unique structures for combating the emerging infectious microbial pathogens [3,4]. Estuary regions are part of a coastal body of marine ecosystem that facilitates a wide variety of organic compounds which are effectively metabolized by the microorganisms for their growth, development and metabolite production, thereby cleaning up their habitats [5]. Mangrove forests are believed to be a resource material for promising extraordinary metabolites due to the continuous changes in the environmental conditions such as salinity and tidal forces [6]. The unexploited microbial biodiversity of the mangrove sediment is a potential resource for exploring the novel bioactive compounds. India has many such unexplored regions, including marine and mangrove environments with rich source of novel metabolites.

The nature of microbial inhabitants with potential secondary metabolite production is not well understood and recent studies reported the invention of compounds with a unique chemical structure and function from newly isolated marine *Streptomyces* [7–9]. Although many reports suggested the importance of the pharmaceutical activity of *Streptomyces* obtained from different habitats, only a few reports are available to deal with the pharmaceutically important *Streptomyces* from mangrove ecosystem

* Corresponding author. Tel.: +91 452 2459480; fax: +91 452 2459105.
E-mail addresses: jsolomon_mrna@yahoo.com, get_rob2001@yahoo.com (S.R.D. Jebakumar).

http://dx.doi.org/10.1016/j.biologicals.2014.08.003
1045-1056/

[10–12]. In the present study, we report the bioactive potential of the polyketide compound produced by the newly isolated *Streptomyces* sp. JRG-04 from mangrove sediment in combating the pathogenic microorganisms including methicillin resistant *Staphylococcus aureus*. In addition, the evolutionary context of the active compound was compared with the β-ketosynthase domain of the type II polyketide gene cluster.

2. Materials methods

2.1. Sample collection and isolation of microorganisms

The soil sediments were collected from the Karangadu mangrove forest in Ramanathapuram district in Tamil Nadu, India (Latitude: 9° 36′N; Longitude: 78° 83′E). The sediment samples were collected near the root zone of the mangroves at 0.7–10 cm depth and transferred to the laboratory in aseptic containers for further use. The soil samples were serially diluted and plated on inorganic starch agar medium containing soluble starch-20 g, NaCl – 0.5 g, KNO_3 – 1.0 g, K_2HPO_4 – 0.5 g, $MgSO_4.7H_2O$ – 0.5 g, $FeSO_4$ – 20 μM, and agar – 15 g [5]. To inhibit the fungal and Gram-negative bacterial growth, 75 μg mL^{-1} of cyclohexamide and 15 μg mL^{-1} of nalidixic acid were supplemented in the medium and the plates were incubated at 30 °C for 7–10 days. The bacterial isolates were pure cultured by repeated streak plate technique and stored at 15% glycerol stock for further characterization. Twenty different bacterial isolates were differentiated based on their colony morphology and all these isolates were screened for the antimicrobial activity by agar overlay method [13].

2.2. Physiological and cultural characteristics

An efficient bioactive compound producing isolate was selected for further characterization in terms of morphological, physiological, and biochemical parameters. The biochemical properties such as starch, casein, lipid, gelatin liquefaction, catalase, nitrate reduction, citrate utilization, triple sugar iron, MR-VP tests were carried out according to the previous report [5]. The physiological studies such as salinity tolerance (NaCl), pH and temperature were also tested for the isolate. The carbon source utilization test, diffusible pigment production, spore formation, aerial and substrate mycelium examination were done in different Internal Streptomyces Project media [14]. The morphological observations of mycelia and spores were conducted via light microscopy (SUSWOX, India) and scanning electron microscopy (Hitachi – S3400N).

2.3. Amplification and sequence analysis of 16S rRNA gene

The isolate was grown in nitrate defined yeast extract medium for 36 h and genomic DNA was isolated by standard method [15]. The 16S rRNA gene was amplified by PCR according to the report [16] using universal primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′- TAC GGC TAC CTT GTT ACG ACT T-3′). The amplified product was sequenced by Big Dye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, USA) and the phylogenetic analysis was performed as per the previous report [16].

2.4. Fermentation extraction and purification of bioactive compound

The production medium consisted of (L^{-1}): Maltose 10 g, Na_2HPO_4 2.9 g, KH_2PO_4 2.3 g, NH_4Cl 1.0 g, $MgSO_4.7H_2O$ 0.5 g, $FeSO_4$ 20 μM, $CaCO_3$ 0.5, yeast extract 5.0 g and trace element salt solution of 5.0 mL ($ZnSO_4.7H_2O$ 100 mg, H_3BO_3 300 mg, $COCl_2.6H_2O$ 200 mg, $MnCl_2$ $4H_2O$ 30 mg, $Na_2MO_4 \cdot 2H_2O$ 30 mg, $NiCl_2.6H_2O$ 20 mg, $CuCl_2.2H_2O$ 10 mg and distilled water 1.0 L). 1% spore suspension of *Streptomyces* strain was inoculated into 250 mL Erlenmeyer flask containing 50 mL of production medium and incubated at 28 °C for 7 days. After incubation, the cell free culture supernatant was collected by centrifugation at 12,000 RPM and acidified to pH 3 using 1 N HCl [17]. The bioactive compound was extracted twice with equal volume of chloroform and concentrated by rotational vacuum concentrator RVC 2-18 CD (CHRIST, Germany). The antimicrobial compound was purified by silica column chromatography using stepwise gradient solvent system (methanol: chloroform gradient 10: 90) and fractions were validated by thin layer chromatography (chloroform/methanol 9:1). All the fractions collected were assayed for the antimicrobial activity against the test microorganisms by disc diffusion method [18] and the active fraction with antimicrobial property was further purified by high-Performance liquid chromatography [19].

Table 1
Growth characteristics of the isolate JRG-04.

Medium	Growth	Substrate mycelium	Aerial mycelium	Spore	Soluble pigments
ISP-2	Good	Grayish brown	Dark grayish brown	Good	Absent
ISP-3	Good	Strong red	Strong red	Good	Light pink
ISP-4	Good	Brownish orange	Dark purplish blue with white halo	Good	Absent
ISP-5	Moderate	Dark yellowish pink	Light pink	Absent	Absent
ISP-6	Good	Brown	Black	Absent	Brown
ISP-7	Good	Brownish black	Deep brown	Poor	Dark Grayish Brown

Abbreviation ISP denotes, International Streptomyces Project medium.

2.5. Antimicrobial effect of active compound

The antibacterial activity of the purified active compound was examined against different test organisms by disc diffusion method [18] on Mueller–Hinton agar medium (HiMedia, India). The test organisms (Table 2) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and Microbial Type Culture Collection (MTCC, IMTECH, Chandigarh, India). Methicillin resistant *S. aureus* is a clinical isolate obtained from the Madurai Kamaraj University Hospital, India. The antibiotic resistance of the methicillin resistant *S. aureus* strain was confirmed by disc diffusion and broth dilution methods according to the Clinical and Laboratory Standards Institute (CLSI) against methicillin and oxacillin and detection of the *mecA* gene by PCR [20] (data not shown). The MIC

Table 2
Antimicrobial activity and minimal inhibitory concentration (MIC) determination against various test organisms.

Test organism	Zone of inhibition (mm)	MIC value (μg/ml^{-1})		
		Compound	Streptomycin	Erythromycin
S.aureus ATCC 6518	17 ± 0.1	1.25–2.5	1.5–2.5	1.25–2.5
S.aureus MTCC 3160	17 ± 0.1	1.25–2.5	1.5–2.5	1.25–2.5
MRSA	18 ± 0.2	1.25–2.5	30–60	30–60
P. aeruginosa ATCC 10145	20 ± 0.1	0.3–0.6	0.6–1.25	0.6–1.25
E. coli ATCC 3739	20 ± 0.2	0.6–1.25	1.25–2.5	1.25–2.5
S. typhi MTCC 733	18 ± 0.1	0.6–1.25	2.5–5.0	1.25–2.5
B. subtilis MTCC 441	20 ± 0.3	0.3–0.6	0.6–1.25	0.1
P. vulgaris MTCC 426	20 ± 0.2	0.3–0.6	1.25–2.5	0.63–1.25
K. pneumoniae MTCC109	17 ± 0.2	1.25–2.5	2.5–5.0	1.25–2.5

The values are represented as mean of triplicate experiments.