Microbiological Research 185 (2016) 55–60

Contents lists available at ScienceDirect

Microbiological Research

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

Penicillium chrysogenum DSOA associated with marine sponge (*Tedania anhelans*) exhibit antimycobacterial activity

Amarendra Visamsetti [a], Santhosh Sarojini Ramachandran [a,b,*], Dhevendaran Kandasamy [a]

[a] Centre for Research in Infectious Diseases, School of Chemical and Biotechnology, SASTRA University, Thanjavur, Tamilnadu 613401, India
[b] Genetic Engineering Lab, ASK302, Anusandhan Kendra, SASTRA University, Thanjavur, Tamilnadu 613401, India

ARTICLE INFO

Article history:
Received 18 April 2015
Received in revised form 3 October 2015
Accepted 6 November 2015
Available online 23 November 2015

Keywords:
Tedania anhelans
Penicillium
Antibacterial activity
Mycobacterium
Diketopiperazines

ABSTRACT

A strain of *Penicillium chrysogenum* was isolated from *Tedania anhelans* (marine sponge) collected from Indian Ocean (8°22′30″N latitude and 76°59′16″ longitude) and deposited in culture collection centers. The strain subjected to different culture conditions for production of extrolites were extracted using ethyl acetate and chloroform. When both extracts were subjected for antibacterial activity, latter had high activity. Minimum inhibitory concentration of chloroform extract ranged from 31.25–1000 μg/mL in tested microbes such as, *Mycobacterium tuberculosis* H37Ra, *Mycobacterium avium*, *Mycobacterium fortuitum*, *Mycobacterium smegmatis*, *Mycobacterium vaccae*, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Vibrio cholerae*. No cytotoxicity was observed in Vero cell line up to 399.10 μg/mL. Antibacterial activity previously reported by Parameswaran et al. in 1997 from ethyl acetate extract of *T. anhelans* might be due to the diketopiperazines, Cyclo-(L-Pro-L-Phe) and Cyclo-(L-Leu-L-Pro) produced by the associated fungi-*P. chrysogenum* DSOA. It is producing a metabolites having antimycobacterial activity, a first report.

1. Introduction

Microbial primary and secondary metabolites or its derivatives are currently in use as antibiotics for treatment of diseases. Presently, isolation and characterization of more compounds became essential due to emergence of drug resistant forms of microbes. According to WHO, the eradication of tuberculosis and leprosy in developing countries is possible by preventing the emergence of multidrug drug resistant forms of bacilli using new antimycobacterial compounds and vaccines. On land, plants (Santhosh and Suriyanarayanan, 2014) and in ocean several free livings and symbionts (Amarendra et al., 2013) are source for new antimycobacterial compounds; in that few are in clinical trials. *Penicillium* sp., generally a contaminant, were isolated from both terrestrial and marine habitats. They were reported to have antibacterial (Devi et al., 2009), antifungal (Edrada et al., 2002), cytotoxic (Xin et al., 2005), antileukemic (Jadulco et al., 2004); (Bringmann et al., 2007) and antiHIV compounds (Bringmann et al., 2007). The microbial habitats are the ecological battlefields to acquire novel enzymes to produce potent antimicrobials. Hence, microbes belonging to same species from different habitats can produce different metabolites. *Aspergillus* produce large number of extrloites followed by *Penicillium* (Frisvad, 2014)

Penicillium, a saprophyte exposed to all environmental challenges produce metabolites with several bioactivities. Marine derived *Penicillium* exhibit great diversity in extrolites produced. Marine gorgonian coral-associated *Penicillium* sp. SCSGAF 0023 produce polyketides with antibacterial and antifouling activities (Bao et al., 2013). A macroalgae, *Monostroma hariotii*, from coastal line of Antarctica harbor *Penicillium* sp. UFMGCB 6120 that produce compounds against *Cladosporium sphaerospermum*-fungus causing allergic responses in patients having respiratory tract diseases and *Trypanosoma cruzi* causing Chagas disease (Godinho et al., 2013). *Penicillium chrysogenum* FF001 isolated from *Melophlus* sp. (marine sponge) produce citrinin which is effective against drug resistant forms of *Styphalococcus aureus* and *Enterococcus faecium*. This compound can also inhibit the growth of *Cryptococcus neoformans* and cytotoxic as tested from brine shrimp larvae (LD_{50} = 96 μg/mL) (Subramani et al., 2013). Marine *P. citrinum* strain SFC20140101-M662 can be used for controlling plant diseases caused by *Colletotrichum acutatum* and *Fusarium oxysporum* (Park et al., 2014). New isochromans were obtaind from *Penicillium* sp.

* Corresponding author at: School of Chemical and Biotechnology, SASTRA University, Thanjavur, Tamilnadu 613401, India. Fax: +91 4362 264120.
E-mail addresses: santhosh@biotech.sastra.edu, rs.santhosh@gmail.com (S.S. Ramachandran).

http://dx.doi.org/10.1016/j.micres.2015.11.001
0944-5013/

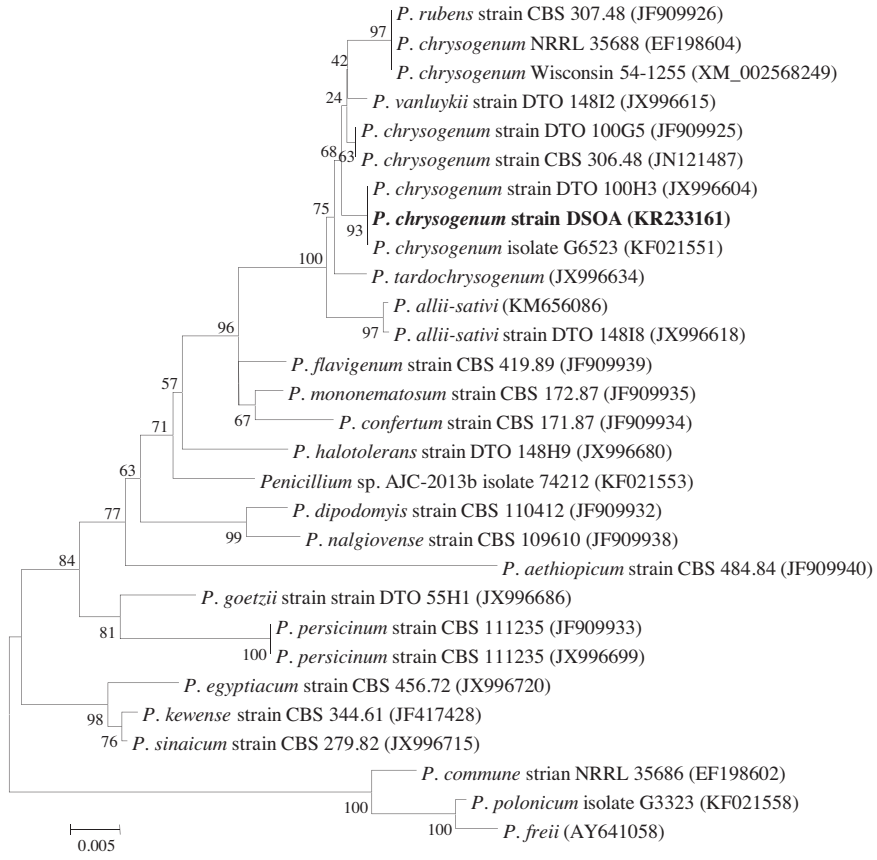

Fig. 1. Phylogenetic trees based on *rpb2* gene sequence data of *P. chrysogenum* DSOA and other taxa of the genus *Penicillium*. GenBank accession numbers are shown in parentheses; sequences obtained in the study are highlighted in bold. Numbers near the branches are boot strap values (1000 pseudoreplicates). Bar value indicates substitutions per nucleotide position.

WN-11-1-3-1-2 isolated from hypersaline lake sediments (Orfali et al., 2015). Active compounds present in organic extract of *Penicillium* sp. CYE-87associated with marine tunicate, *Didemnum* sp., collected from Suez Canal, Egypt were anticandid and antiproliferative (Shaala and Youssef, 2015). Shark gill associated *P. crustosum* AP2T1 produce quinolinones which inhibited HCT116 cancer cell line and weakly inhibited *S. aureus* (Zhang et al., 2015). A novel benzoic acid from marine *P. chrysogenum* SYP-F-2720 was found better than aspirin (Li et al., 2015). Cillifuranone and furanones were first isolated from *P. chrysogenum* LF066 associated with marine sponge, *Tethya aurantium* (Wiese et al., 2011). An antileukemic Sorbicillactone A was isolated from *P. chrysogenum* DSM 16137 associated with Mediterranean sponge, *Ircinia fasciculate* (Bringmann et al., 2005).

We have isolated *P. chrysogenum* DSOA (DSOA) producing antimycobacterial compound from a marine sponge, *Tedania anhelans*.

2. Materials and methods

2.1. Chemicals and media

Chemicals from Merck; culture media, sugars and growth supplements from Hi-Media; and glassware from Borosil, India were purchased.

2.2. Isolation of associated microbes

Marine sponge, *T. anhelans*, was collected from Vizhinjam port (8°22′30″N latitude and 76°59′16″ longitude), India. Sponge was crushed and extract was diluted with sterilized sea water. One milliliter of 10^{-2} dilution was mixed with glycerol asparagine top agar medium (pH 7.4 ± 0.2) and plated in 90 mm petri dishes. Pure colonies selected were maintained on nutrient agar. PCR primers for RNA polymerase beta (*rpb2*) was designed from *P. chrysogenum* strain P2niaD18 chromosome II (GenBank CM002799.1, 7736675 to 7737889).

2.3. Determination of antibacterial activity

Two days old broth culture was subcultured into 1 L of nutrient broth in haffkine flask (3 L) and incubated in static condition for 20 days at 30 °C. Biomass was separated by filtering and culture supernatant was in mixing with equal volume of chloroform for 2 days. Chloroform layer was separated and concentrated using Rota Evaporator (Buchi R250 with V700 vacuum pump, Switzerland) and the extract was stored at 4 °C to determine antibacterial activity by disc diffusion method.

Gram +ve (*Mycobacterium avium,* MTCC 1723; *Mycobacterium fortuitum,* MTCC 1902; *Mycobacterium smegmatis,* MTCC 6; *Mycobacterium vaccae,* MTCC 272; *Staphylococcus aureus,* MTCC 3160) and Gram −ve (*Aeromonas hydrophila, Vibrio cholerae*, *Psuedomonas aeruginosa,* MTCC 424) bacteria were cultured using standard media recommended. Cell suspension having 15×10^5 CFU/mL was used for the assay (Kuete et al., 2008). Test cell suspension (100 μL) was plated on Müller Hinton Agar and on that placed whatman filter discs (4.5 mm) impregnated with 500 μg of extract and chloroform as solvent control. The plates were incubated at 37 °C for 1 day (*A. hydrophila*, *P. aureginosa*, *S. aureus* and *V. cholerae*), 2 days (*M. fortuitum* and *M. smegmatis*), 5 days (*M. vaccae*)