Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 11, 2014

Original Article

ANALYSIS OF BIOACTIVE CONSTITUENTS FROM A NEW *STREPTOMYCES VARIABILIS STRAIN SU5* BY GAS CHROMATOGRAPHY - MASS SPECTROMETRY

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Received: 15 Sep 2014 Revised and Accepted: 14 Oct 2014

ABSTRACT

Objectives: To analyze the bioactive constituents found in the crude extract of a new *Streptomyces variabilis strain SU5* by gas chromatography - mass spectrometry.

Methods: A total of fifty two actinomycetes were isolated from sediment samples of South east coast of Tamil Nadu by the serial dilution method. A new strain was identified based on cultural and molecular analysis as *Streptomyces variabilis* strain SU 5 and submitted in GENBANK with the accession number KF551876. The ethyl acetate extract of the new strain was produced by submerged fermentation and has been subjected to GC-MS analysis.

Results: The major chemical constituents are Diisobutyl phthalate (31.84), Monoethylhexyl phthalate (20.33), tributylamine (13.24), Dibutyl phthalate (5.51), 2-Bromotetradecane (3.79) with the retention time 15.635, 21.606, 7.164, 16.608, 12.657 respectively.

Conclusion: The presence of some of these constituents in the actinomycetes extract provides the scientific evidences for eliminating tumor cells in bone marrow, purging agent in autologous bone marrow transplantation, cytotoxic activity, stimulates adipogenesis and glyceroneogenesis, affects the differentiation of Human Liposarcoma.

Keywords: *Streptomyces variabilis*, GC-MS, Diisobutyl phthalate, Tributylamine, Monoethylhexyl phthalate.

INTRODUCTION

Natural products have played an important role in the development of drugs and drug leads to various diseases including cancer [1]. The secondary metabolites from natural sources are good candidates for drug development because being elaborated within the living systems. They are perceived to exhibit more similarities to drugs and show more biological friendliness than totally synthetic drugs [2]. Actinomycetes are prolific producers of antibiotics and majority of the antibiotics in clinical use today are produced by them. Apart from antibiotics, actinomycetes also produce other bioactive secondary metabolites**,** anticancer (mitomycin and daunomycin) [3] and immunosuppressive agents (rapamycin and FK506) [4]. Approximately, 60% of the approved chemotherapeutic drugs are derived from natural compounds [5]**.** Among those, 50% of the natural antibiotics are produced from actinomycetes. Marine actinomycetes are proving to be a rich source of novel bioactive compounds [6,7]. Success in recovering members of new actinomycete taxa from marine ecosystems is crucially dependent on the use of improved selective isolation strategies, sample pre-treatments, and the dereplication of isolate collections. Thus the objective of the study was to identify the active compounds from a new marine sediment inhabitant isolate Streptomyces variabilis strain SU 5 by GC-MS analysis.

MATERIALS AND METHODS

Isolation of Actinomycetes

Marine sediment samples were collected from Pulicat, Ennore, Muttukadu, and Veerampattinam costal areas and were immediately transferred to the laboratory condition. Approximately one gram of soil sample was aseptically transferred into 99 ml of pre sterilized 50% seawater and serially diluted. 100 µl of diluted samples were transferred to molten starch casein agar medium (10g.1^{-1} soluble starch, 1 g.1^{-1} of casein and 18 g.1^{-1} of agar made up with 50% of sea water) and incubated at 27±2°C for 7- 8 days. After incubation, colonies appeared on the agar medium were re-streaked in the same agar medium.

Identification of actinomycetes

The isolates were identified based on morphological characters followed by the methods of Bergey's manual of determinative bacteriology and confirmed by 16S rRNA sequencing.

The morphological, cultural, physiological and biochemical characterization of the isolate was carried out as described in International *Steptomyces* Project (ISP) [8]. The morphological characters of the selected isolate was examined by using light microscope as well as scanning electron microscope. The cultural characters of the isolate was studied by cultivating it on different media namely ISP1, ISP2, ISP4, ISP5 and ISP7 and incubated for 7-10 days at 28°C. colony morphology including color of the aerial mycelium, substrate mycelium, reverse side color, melanin pigment production and production of diffusible pigments were recorded.

The physiological characters such as growth at different pH (5, 7, 9, 10 and 11), temperature (10°C, 20°C, 20°C, 40°C, and 50°C) was also recorded. The biochemical characterization of the isolate was also studied by the procedures of [9].

Molecular sequencing

Genomic DNA was isolated from cells as described by [10]. The 16S rRNA gene of strain SU 5 was amplified by polymerase chain reaction, using two universal bacterial primers, 1492R (5 - GGTTACCTTGTTAC GACTT-3′) and Eubac27F (5 - AGAGTTTGATCCTGGCTC AG-3′); [11].

The amplified products were purified using TIAN gel mini purification kit, ligated to MD18-T simple vector (TaKaRa), and transformed into competent cells of Escherichia coli DH5α.16S rRNA gene fragment was sequenced using forward primer M13F (−47) and reverse primer M13R (−48). The derived 16S rRNA gene sequence was compared to the GENBANK database (NCBI), to search for similar sequences using the basic local alignment search tool algorithm.

Extraction of cell free crude extracts

A loopful of selected actinomycetes strain was inoculated into 150 ml of ISP2 medium (4.0g.1^{-1} of glucose, 10.0 g.1^{-1} of malt extract and 4.0 g.1^{-1} of yeast extract, pH 7.2±2°C made up with 50% sea water) for 10 days under continuous shaking (100 rpm). After that, cell free broth was adjusted to pH 5.0 with 1N hydrochloric acid and equal volume (1:1) of ethyl acetate was added and mixed by vigorous shaking and kept without disturbance. The organic phase was collected and evaporated in an incubator at 60-70°C and the residue was stored at-20°C for further use.

GC - MS analysis

Preparation of extract

2 µl of the ethyl acetate extract of selected isolate was employed for GC/MS analysis. The compound was characterized by using GC-MS [SHIMADZU QP2010] instrument at GC column oven Temperature 70°C, Injector Temperature 200°C at spilt mode ratio 40 with a flow rate of 1.51 ml/min. The MS with Ion source temp 200 °C, Interface temp: 240°C, Scan range: 40 – 1000 m/z, Event time: 0.5 Sec, Solvent cut time: 5 min*s, MS start time: 5 (min), MS end time: 35 (min), Ionization: EI (-70ev).

Identification of components

Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULT AND DISCUSSION

Based on different cultural characters on SCA media 52 actinomycetes isolates were isolated from the coastal soil samples of Pulicat, Ennore, Muttukadu, Veerampattinam and Parangipettai, Tamil Nadu, South east coast of India, some of the isolated actinomycetes were presented in **fig. 1**. Among the fifty two isolates based on cytotoxic activity against brine shrimp larvae [12, 13] the isolates were selected for identification. The selected active isolate was identified based on classical and molecular analysis. Fig. **2** shows the scanning electron micrography of identified isolate SU 5. **Table 1** shows the cultural and biochemical characteristics of isolate SU 5. The isolate SU5 was identified as a divergent strain of *Streptomyces variabilis.* The isolate shows 99% similarity with the species *Streptomyces variabilis* and it was submitted in GENEBANK as *Streptomyces variabilis* strain *SU 5* with the accession number KF551876.

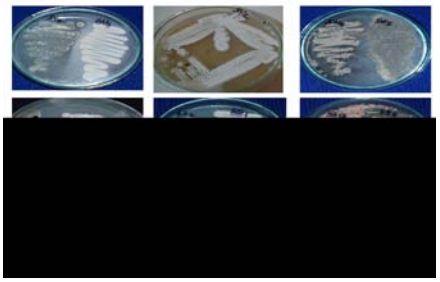

Fig. I: Isolated Actinomycetes

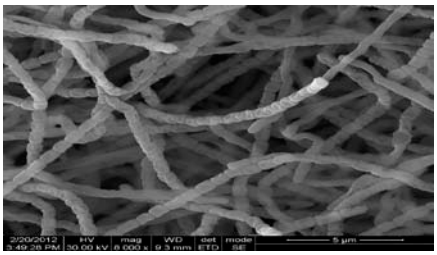

Fig. I: Scanning electron micrography of isolate SU 5

Table 1: A comparative study of the identification properties of the isolate SU 5 in relation to the reference strains

Characteristics	Isolate SU 5	*Streptomyces variabilis*
Cultural characteristics		
Aerial mass colour	Grey	Grey
Melanoid pigment	-	-
Reverse side pigment	Brownish black	-
Soluble pigment	-	-
Spore morphology	Straight, smooth	Straight, smooth
Utilization of		
Sucrose	+	+/-
Rhamnose utilization	+	+
Mannitol	+	+
Inositol	+	+
Nitrate reduction	+	+
H_2S production	+	+
Growth at 45°C	+	±
Growth at NaCl 7% (w/v)	-	±

Table 2: Activity Of Identified Chemical Constituents In The Ethyl Acetate Extract Of The Isolate *Streptomyces Variabilis Strain SU 5* By GC-MS Analysis

RT	Name of the compound	Activity
15.635	Diisobutyl phthalate (31.84),	Eliminate tumor cells on bone marrow, purging agent in autologous bone marrow transplantation. Cytotoxic activity.
21.606	Monoethylhexyl phthalate (20.33),	Stimulates Adipogenesis and Glyceroneogenesis, Affects the Differentiation of Human Liposarcoma
7.164	Tributylamine (13.24),	Solvent
16.608	Dibutyl phthalate (5.51)	Eliminate tumor cells on bone marrow, purging agent in autologous bone marrow transplantation, Cytotoxic activity.
12.657	2-Bromotetradecane (3.79)	*NR (Not yet reported)

The major constituent was found to be phthalate at retention time of 15.635, 21.606and 16.608. Tianshan [14] reported Dibutyl Phthalate, diisobutyl phthalate were active against partially four selected immortal cell lines. In a similar study the natural occurrence of 1, 2-Benzenedicarboxylic acid bis(2-ethylhexyl) phthalate has been isolated from a marine alga, *Sargassum weightii*, and apart from its plasticizing ability it was also found to have antibacterial effect on a number of bacteria [15]. Bis (ethyl hexyl) phthalate reported from *Streptomyces bangladeshiensis* show antimicrobial activity against gram positive bacteria and some

pathogenic fungi [16]. Adipogenesis and glyceroneogenesis activity of Monoethylhexyl Phthalate in human adipocytes were reported by [17]. Enrico Campioli [18] stated that mono-(2-ethylhexyl) phthalate affects the differentiation of human liposarcoma cells (sw 872).

CONCLUSION

The presence active constituents' in the actinomycetes extract was detected by an interpretation of mass spectrum of GC-MS with the database of the National Institute Standard and Technology (NIST). The comparison provides the scientific evidences for the presence of chemical classes with various biological activity like eliminating tumor cells in bone marrow, purging agent in autologous bone marrow transplantation, cytotoxic activity, stimulates adipogenesis and glyceroneogenesis, affects the differentiation of Human Liposarcoma.

ACKNOWLEDGMENT

The authors wish to convey their sincere sense of gratitude to the management of Sathyabama University for their constant support and encouragement.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Cragg GM, Newman DJ. Plants as source of anticancer agents. J Ethnopharmacol 2005;100(1-2):72-9.
2. Shoeb M. Anticancer agents from medicinal plants. Bangladesh J Pharmacol 2006;1:35-41.
3. Galm U, Hager MH, Lanen SGV, Ju J, Thorson JS, Shen B. Antitumor antibiotics: Bleomycin, Enediynes, and Mitomycin. Chem Rev 2005;105:739-58.
4. Simmons TL, Andrianasolo E, McPhail K, Flatt P, Gerwick WH. Marine natural products as anticancer drugs. Mol Cancer Ther 2005;4:333-42.
5. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981–2002. J Nat Prod 2003;66:1022–37.
6. Fiedler HP, Bruntner C, Bull AT, Ward AC, Goodfellow M, Potterat O, Pudar C, *et al*. Marine actinomycetes as a source of novel secondary metabolites. Antonie Van Leeuwenhoek 2005;87:37–42.
7. Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR. Marine natural products. Nat Prod Rep 2007;24:31–86.
8. Shirling EB, Gottlieb D. Methods for characterization of Streptomyces species. Int J Syst Bacteriol 1966;16:313-40.
9. Vengadesh prabhu K, Sundaramoorthi C, Gupta saurabh, Karthick K, Tamilselvi N. Int Res J Pharm 2011;2(4):114-18.
10. Hopwood DA, Bibb MJ, Chater KF, Kieser T, Bruton CJ, Kieser HM, *et al*. Genetic manipulation of Streptomyces: a laboratory manual. The John Innes Foundation, (Norwich, UK); 1985.
11. Jiang HL, Tay JH, Tay STL. Bacterial diversity and function of aerobic granules as a microbial response to high phenol loading. Appl Environ Microbial 2004;70:6767-75.
12. Sudhakesavan S, Vijayalakshmi S, Usha Nandhini S, Bhavani Latha M, Masilamani Selvam M. Application of brine shrimp bioassay for screening cytotoxic actinomycetes. Int J Pharm Pharm Sci Res 2011;1(3):104-7.
13. Sudha S, Masilamani Selvam M. *In vitro* cytotoxic activity of Bioactive metabolite and crude extract from a new Actinomycete Steptomyces avidinii strain SU4. Int J Pharm Pharm Sci 2013;5(3):612-6.
14. Tianshan Wang, Yuan Yuan, Jing Wang, Changri Han, Guangying Chen. Anticancer activities of constituents from the stem of *polyalthia rumphii.* Pak. J Pharm Sci 2012;25(2):353-6.
15. Sastry VMVS, Rao GRK. Dioctyl phthalate and antibacterial compound from the marine brown alga Sargassum wightii. J Appl Physiol 1995:7:185-6.
16. Al-Bari MAA, Sayeed MA, Rahman MS, Mossadik MA. Characterization and antimicrobial activities of a phthalic acid derivative produced by *Streptomyces bangladeshiensis*-A novel species in Bangladesh. Res J Med Med Sci 2006;77-81.
17. Sandrine Ellero-Simatos, Sandrine P Claus, Chantal Benelli, Claude Forest, Franck Letourneur, Nicolas Cagnard, *et al*. Beaune, and Isabelle de Waziers. Combined transcriptomic–^{1}h NMR metabonomic study reveals that monoethylhexyl phthalate stimulates adipogenesis and glyceroneogenesis in human adipocytes. J Proteome Res 2011;10(12):5493–502.
18. Enrico Campioli, Amani Batarseh, Jiehan Li, Vassilios Papadopoulo. The endocrine disruptor mono-(2-ethylhexyl) phthalate affects the differentiation of human liposarcoma cells (SW 872). J Pone 2011;6:12.