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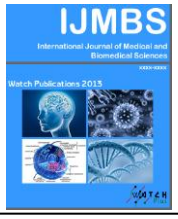

ASSAY OF GENOTOXIC ACTIVITY OF SOIL ACTINOMYCETES EXTRACT

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Abstract

Actinomycetes are Gram positive group of branching unicellular microorganisms, known for their ability to produce variety of antibiotics. The aim of the present study was to screen the genotoxic effect of actionmycetes isolated from soil samples collected from Amirthi National Park-river bank, Tamil Nadu, India. Out of ten actinomycetes isolates obtained the isolate named BT-7 was found to be active and the ethyl acetate (EA) extract obtained from the isolate was screened for genotoxic effect on human leucocyte culture. The effect of EA extract on human chromosomes was observed under microscope and noted for any aberrations. The active isolate showed significant genotoxicity on human chromosomes at higher concentration (100 µg) and the genotoxicity was compared with mitomycin, used as a positive control. Based on the results of this study it can be concluded that the secondary metabolites produced by actinomycetes species possess significant genotoxic effect on human chromosomes.

Introduction

Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil. They provide many important bioactive compounds of high commercial value and are routinely screened for new bioactive compounds (Berdy 2005). These searches have been remarkably successful and about two thirds of naturally occurring antibiotics, including many of medical importance; have been isolated from actinomycetes (Fenical and Jensen 2006). Almost 80% of the world's antibiotics are known to come from actinomycetes, mainly from the genera *Streptomyces* and *Micromonospora* (Donia and Hamann 2003). According to the World Health Organization, over-prescription and the improper use of antibiotics has led to the generation of antibiotic resistance in many bacterial pathogens. At present the drug resistant pathogens emerge more quickly than the rate of discovery of new drugs and antibiotics and because of this, many scientists and pharmaceutical industry have actively involved in isolation and screening of actinomycetes from different untouched habitats, for their secondary metabolites(antibiotics). Serious infections caused by bacteria have become resistant to commonly used antibiotics and become a major global healthcare problem in the 21st century. *Staphylococcus aureus*, for instance, a virulent pathogen that is responsible for a wide range of infections, has developed resistance to most classes of antibiotics. Clinicians and public health officials have faced hospital acquired drug resistant *S.aureus*, which also bears resistance to many antibiotics. Hence there is need to rediscover new drugs active against these drug resistance pathogens.

Most of the actinomycetes in soil that are potential drug sources remain uncultivable, and therefore inaccessible for novel antibiotic discovery (Grabley and Thiericke 1999). A lot of the antibiotics in use today are derivatives of natural products of actinomycetes and fungi (Demain and Sanchez 2009.). Actinomycetes can be isolated from soil and marine sediments, although soils have been screened by the pharmaceutical industry for about 50 years, only a small fraction of the surface of the globe has been sampled, and only few are discovered (Amador et al 2003). Worldwide unexplored habitats need to be studied for novel secondary metabolites and antibiotics. The drug resistance problem demands that to discover new antibacterial agents effective against pathogenic bacteria resistant to current antibiotics (Bush and Macielag 2000). Hence there is a need to screen more and more actinomycetes from different habitats (Anderson and Wellington 2001) for antimicrobial activity and screening would get some actinomycetes isoltes that produce antibiotics and are active against drug resistant pathogens (Anderson and Wellington 2001).They produce two types of branching mycelium such as substrate mycelium and aerial mycelium. Among actinomycetes, the streptomycetes

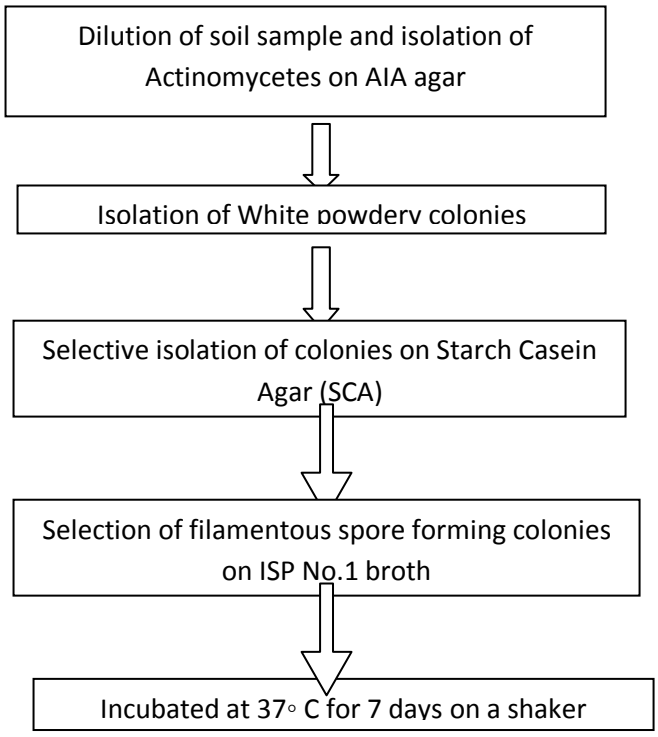

Figure 1. Scheme for isolation of soil actinomycetes

are the dominant (Hwang et al 2001). Actinomycetes are also commonly referred to as Actinobacteria, they are called so because they tend to produce secondary metabolites, many of which have been successfully isolated and turned into useful drugs and other organic chemicals (Ikeda et al 2003; Hopwood 2007). The present study was undertaken to isolate actinomycetes from soil samples collected from wasteland and gardens of Vellore. All isolates were screened for their anti-bacterial and genotoxic properties. In the present study the genotoxic effect of the selected actinomycetes isolate on human chromosomes was studied through leucocyte cell culture study.

Materials and Methods

Sample collection and isolation of actinomycetes

Soil samples were collected from habitats of Amirthi National Park river bank, Vellore, Tamil Nadu, India. All samples were air-dried and then used for isolation of actinomycetes. The samples were dried to minimize the bacterial contaminants. Soil sample (1g) was serially diluted (10^6 dilution) and 0.1 ml of the diluted suspension was spread over the surface of starch casein agar (SCA) medium prepared in 50% sea water to enhance the isolation of actinomycetes. After 7 days of incubation at room temperature white powdery colonies of actinomycetes formed were isolated and sub-cultured and the strain was maintained on ISP 1 media as reported earlier (Deepika et al 2011). A scheme for isolation of actinomycetes is given in Figure 1. The obtained culture in the medium was filtered using Whattman's filter paper no. 1. To the obtained supernatant ethyl acetate was added and was kept on boiling water-bath at 70◦C for evaporation. After complete evaporation, the residue was collected as secondary metabolite.

Characterization and identification of isolates

Macroscopic observations including morphological, cultural, physiological and biochemical characterization of the isolate was carried out using ISP as described earlier (Thenmozhi et al. 2013). Based on their cultural (growth on SCA medium) and morphological (electron microscopy) characteristics the isolate was studied and identified. Actinomycetes named as BT-1 to BT-10 (10 isolates) isolated from soil samples were used to study their genotoxic effect.

Assay of Genotoxicity

A readymade RPMI media of 5ml was taken in centrifuge tubes. To this solution, 50 drops (0.5ml) of blood was added. Then the solution was kept for incubation at 37^oC (room temperature) for 72 h. Carbon dioxide was being

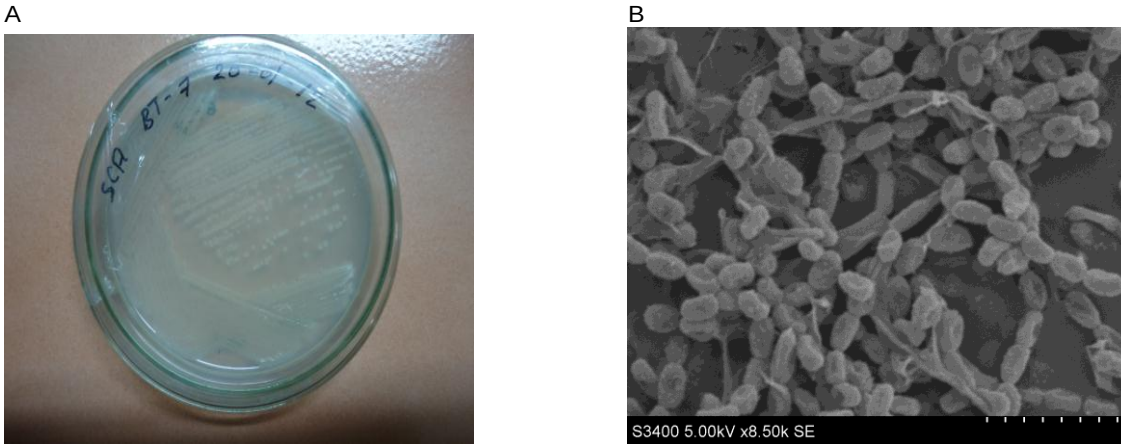

Figure 2. Culture and spore chain morphology of *Streptomyces* sp. VITBT7. A) Culture of the isolate on starch casein agar medium. B) spore chain morphology under Scanning Electron Microscope (3400N Hitachi) showing matured smooth surfaced spore chains

Table 1: Genotoxic effect of ethyl acetate extract of actinomycetes isolates on human leukocyte culture

Actinomycetes isolates	Genotoxicity
BT-1 (100 µg)	-
BT-2 (100 µg)	-
BT-3 (100 µg)	-
BT-4 (100 µg)	-
BT-5 (100 µg)	-
BT-6 (100 µg)	-
BT-7 (100 µg)	+
BT-8 (100 µg)	-
BT-9 (100 µg)	-
BT-10 (100 µg)	-
Mytomicin (10 µg) (Positive control)	+
Human blood sample (Negative control)	-

+ positive; -negative:

released from the tubes every 24 hours (in laminar-flow chamber). At 70th h secondary metabolites (EA extract) obtained from actinomycetes were added at different concentrations and incubated. Harvesting is done on the 72nd h in which 2-3 drops of colchicine (0.01%) was added to the solution, after which it was incubated at room temperature for 20 minutes. Then centrifugation of the tubes at 1500 rpm for 5 minutes was followed from which the supernatant was discarded. To the pellet, approximately 6ml of hypotonic solution of KCl (0.560g KCl in 100ml double distilled water) was added. The pellet was then disturbed and incubated at room temperature for 6-7 minutes. After that, centrifuge the tubes again at 1500 rpm for 5 minutes. After centrifugation, discard the supernatant and pellet was collected. To the acquired pellet, approximately 6ml of fixative [methanol (3): acetic acid (1)] was added and mixed well with the help of Pasteur pipette. Centrifuge the tubes twice at 1500 rpm for 5 minutes. Then incubate at room temperature for 30 minutes. And finally, the slides were being prepared, stained (Giemsa stain) and scored for any chromosomal damages or abnormalities (Saurabh et al 2010). Mitomycin was used as positive control and human blood was used as negative control.

Results and discussion

Actinomycetes were cultured in actinomycetes isolation agar (AIA) as isolated colonies formed white powdery in nature. These colonies were inoculated on the starch casein agar (SCA) medium for obtaining a pure culture. All isolates grew on starch casein agar media showing typical actinomycetes morphology with filamentous spore formation and based on culture morphology (Figure 2A) and electron microscopy (Figure 2B) the strain was identified as *Streptomyces* species.

The EA extracts (different concentrations) prepared from all actinomycetes isolates were reated with leukocyte culture and observed for genotoxic effect. The EA extract obtained from the isolate BT-7 showed chromosomal aberrations at higher (100 µg) concentrations (Table 1). The EA extract obtained from

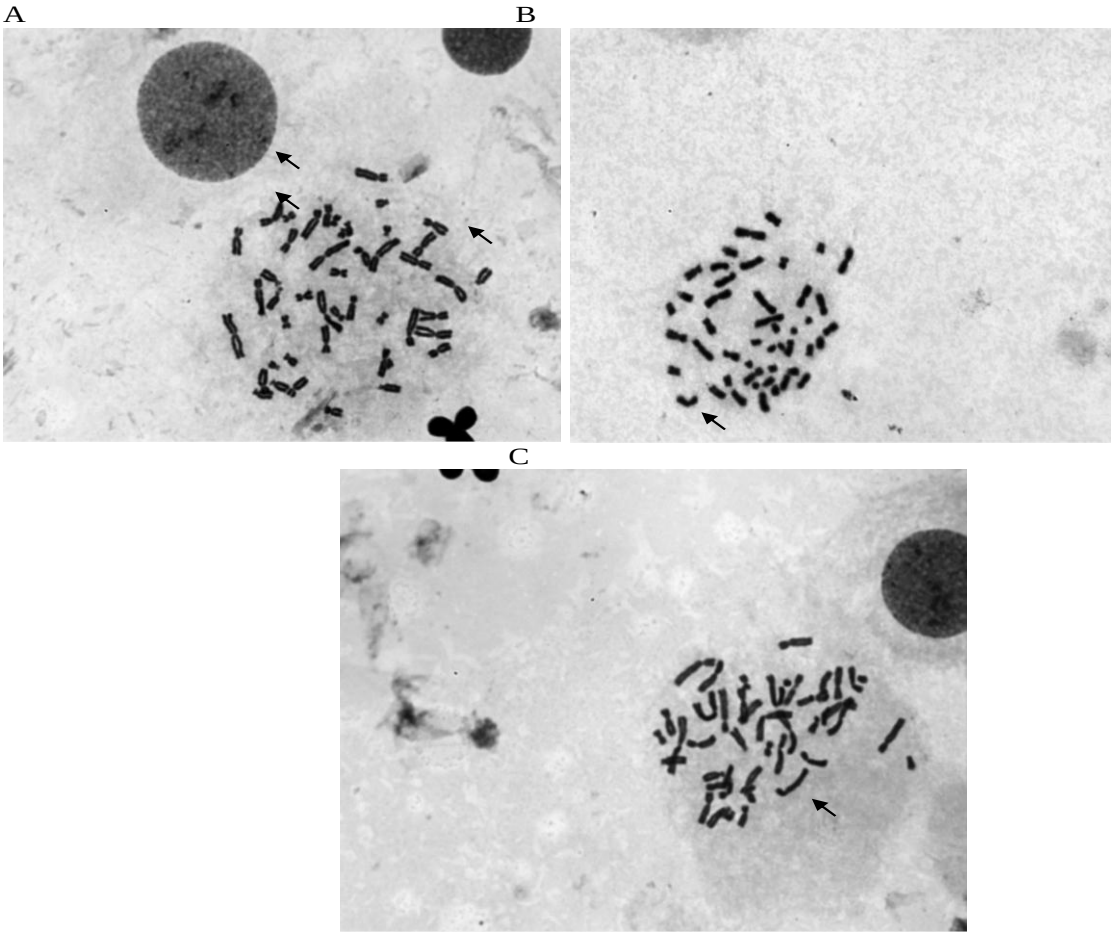

Figure 3. Structure of human chromosomes observed under light microscopy (100 X magnification) A) Normal Human chromosomes. B) Human chromosomes treated mitomycin (10 µg) and C) Human chromosomes treated with ethyl acetate extract (100 µg) of BT- 7 actinomycetes isolate. A bend in p-arm in most of the human chromosomes was shown and indicated by arrow.

other isolates (except BT-7) failed to show any chromosomal abnormalities. The human leukocyte culture tubes treated with EA extract of actinomycetes isolate is shown in Figure 3. Human chromosomes observed under light microscope (100X magnification) are shown in Figure 3A. The chromosomes observed under microscope appearing normal and no abnormalities were observed.

Human chromosomes treated with mitomycin (10 µg) are shown in Figure 3B. Mitomycin is used as positive control and being a known genotoxic agent, it produced chromosomal abnormalities indicated by arrow in Figure 3 B. The EA extract (100µg) of BT-7 produced chromosomal abnormalities indicated by arrow in Figure 3C. Genotoxic effects of the fungicide thiophanate-methyl on *Podarcis sicula* was already reported (Capriglione et al. 2011). Cytogenetic analysis of chromosomal aberrations has been suggested to be a useful tool to determine the safe maximum allowable concentration (MAC) of any and drug (Sram 1981). Abamectin and Ivermectin are two(2) closely related members of the avermectin family of 16-membered macrocyclic lactones derived from the actinomycete *Streptomyces avermectinius* have been reported to exhibit extraordinary anthelmintic activity. They are used worldwide in veterinary and human medicine as well as in agriculture. These two compounds have been reported to exert genotoxicity and cytotoxicity on several cellular systems (Molinari et al. 2010). The compound extracted from marine *Streptomyces* species have been shown to less cytotoxic and genotoxic to human chromosomes (Balaji et al. 2011). Several chemical agents have already been shown to be chromosome breaking agents called chromosomoclastogens (Shaw . 1970). Bleomycin isolated from mutant strain of *Streptomyces verticillus* have been reported to be capable of inducing chromosomal aberrations which include formation of chromatid fragments and translocations (Paika and Krishan 2001). Streptonigirin, antitumor antibiotic isolated from *Streptomyces flocculus* reported to cause chromosome damage and sister chromatid exchange (Bolzan and Bianchi 2001).

Conclusions

Based on the results of this study, it can be concluded, that the secondary metabolite produced by actinomycetes isolate BT-7 has genotoxic effect on human chromosomes.

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References

Berdy J (2005). Bioactive microbial metabolites- A personal view. J. Antibiotics 58, 1- 26.

Fenical W, Jensen PR (2006). Developing a new resource for drug discovery: Marine actinomycete bacteria, Nature Chemical Biol., 2 (12): 666–673

Donia M, Hamann MT (2003). Marine natural products and their potential applications as anti-infective agents. Lancet. Infect. Dis., 3:338-348.

Grabley S, Thiericke R (1999). The impact of natural products on drug discovery. In: Grabley, S. and R.Thiericke (eds.) Drugs discovery from nature, Springer-Verlag, Berlin. pp. 1-37.

Demain AL, Sanchez S (2009). Microbial drug discovery: 80 years of progress. J. Antibiotics 62: 5-16.

Amador ML Jimeno, J, Ares LP, Funes HC, Hidalgo M, (2003). Progress in the development and acquisition of anticancer agents from marine sources. Annals of Oncol., 14: 1607-1615.

Bush K, Macielag M (2000). New approaches in the treatment of bacterial infections. Current Opinion in Chemical Biol. 4: 433–439.

Anderson AS, Wellington EMH (2001). The taxonomy of *Streptomyces* and related Genera. International Journal of Systemic Evol. Microbiol., 51: 797-814.

Watve MG, Tickoo R, Jog MM, Bhole B (2001). How many antibiotics are produced by the genus *Streptomyces*? Archives of Microbiol. 176: 386-390.

Hwang BK, Lim SW, Kim BS, Lee JY, Moon SS (2001). Isolation and *in vivo* and *in vitro* antifungal activity of phenylacetic acid and sodium phenylacetate from *Streptomyces humidus*. Appl, Environ.Microbiol., 67: 3739-3745.

Ikeda H, Ishikawa J, Hanamato A, Shinose M, Kikuchi H, Shiba T, Sakaki Y, Hattori H, Omura S (2003). Complete genome sequence and comparative analysis of the industrial microorganism *Stretomyces avermitilis*. Nature Biotechnol. 21: 526-531.

Hopwood DA (2007). Therapeutic treasures from the deep. Nature Chemical Biology 3: 457-458.

Deepika TL, Kannabiran K, GopieshKhanna V, Rajakumar G, Jayaseelan C, Santhoshkumar T, Rahuman AA (2011). Isolation and characterization of acaricidal and larvicidal novel compound (2S, 5R, 6R)-2-hydroxy-3,5, 6-trimethyloctan-4-one from *Streptomyces* sp. against blood-sucking parasites. Parasitology Res.2012; 111:1151-1163.

Thenmozhi M, Kannabiran K, Rajakumar G, Rahuman AA, Velayutham K (2013). Eco-friendly approach using marine actinobacteria and its compounds to control ticks and moskitoes. Parasitol. Res. 112:719-729.

Saurabh KB, Radha S, Sivakumar E (2010). Genotoxic assessment in peripheral blood lymphocytes of 313 post-polio individuals using sister chromatid exchange analysis and micronucleus assay. Exp. Toxicol. doi: 10.1177/0960327110376983.

Capriglione T, De Iorio S, Gay F, Capaldo A, Vaccaro MC, Morescalchi MA, Laforgia V (2011). Genotoxic effects of the fungicide thiophanate-methyl on *Podarcis sicula* assessed by micronucleus test, comet assay and chromosome analysis. Ecotoxicology. 20(4):885-91.

Sram RJ (1981). cytogenetic analysis of peripheral lymphocytes as a method for monitoring environmental levels of mutagens, in: I. Gut, M. Cikrt, G.L. Plaa (Eds), Industrial and environmental xenobiotics: Metabolism and pharmacokinetics of Organic chemicals and methods, Springer-Verlag, Berlin.187-193.

Molinari G, Soloneski S, Larramendy ML (2010) New ventures in the genotoxic and cytotoxic effects of macrocyclic lactones, abamectin and ivermectin.Cytogenet Genome Res,.128:37-45.

Balaji S, Saurav K, Gopieshkhanna V, Kannabiran K (2011). Assay of genotoxic and cytotoxic potential of a compound extracted from marine *Streptomyces*. Pharmacologyonline, 2:263-271.

Shaw MW (1970). Human chromosome damage by chemical agents. Annual Rev. Med., 21:409-432.

Paika KD, Krishan A (1973). Bleomycin-induced chromosomal aberrations in cultured mammalian cells. Cancer Research 33: 961-965.

Bolzan AD, Bianchi SM (2001). Genotoxicity of Streptonigrin: a review. Mut. Res., 488: 25-37