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ANTIBACTERIAL ACTIVITY OF *PENICILLIUM SPINULOSUM* TTMF2 ISOLATED FROM MARINE SOILS OF CHIDIYA TAPU IN ANDAMAN AND NICOBAR ISLANDS, INDIA.

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ABSTRACT

The marine fungus *Penicillium spinulosum* TTMF2 was isolated from Chidiya Tapu in Andaman and Nicobar Islands, India. Diethyl ether, ethyl acetate and distilled water extracts of *P. spinulosum* TTMF2 were tested for their antibacterial efficacy against the five gram positive and five gram negative bacterial pathogens. The ethyl acetate extract *of P. spinulosum* (TTMF4) exhibited very promising antibacterial activity followed by diethyl ether and distilled water extracts. The antibacterial potentials of *P. spinulosum* TTMF2 were compared with standard antibiotic streptomycin.

KEYWORDS: Marine fungus, antibacterial efficacy, bacterial pathogens, streptomycin.

INTRODUCTION

Fungi are ubiquitous occurring, eukaryotic, heterotrophic organisms. Beside the well-known mushrooms, fungal life is found worldwide, in soil samples as well as deep sea vents and arctic ice, and often reveals symbiotic traits. Similar to plants, there is a long history of the utilization of fungi by mankind as remedies and in everyday life. Nearly 3000 years ago the Mayans used fungi to treat intestinal ailments (Strobel *et al*., 2004). The discovery of penicillin isolated from *Penicillium notatum* by Sir Alexander Fleming in 1928 which resulted in a breakthrough in the treatment of bacterial infections, that fungi became an important source of drugs for the treatment of a variety of diseases.

Marine fungi are prolific resources of natural products (Liberra and Lindequist, 1995; Pietra, 1997; Jensen and Fenical, 2002; Ebel, 2010). However, the potential of marine fungi has only been investigated to a limited extent. Recently, marine-derived fungi have been recognized as one of the most recent barely tapped sources for new biologically active secondary metabolites, (Liberra and Lindequist, 1995; Pietra, 1997; Biabani and Laatsch, 1998; Holler *et al.*, 2000; Jensen and Fenical, 2002;) including antitumor, antibacterial, antiviral, antifungal, anti-inflammatory and enzyme inhibitor compounds. This is probably because marine fungi have been explored to a much lesser extent than their terrestrial counterparts, which have been known for a long time as a very important source of biologically active and economically important natural products, such as those for use in treatment of human diseases as well as other biotechnological applications (Bennett, 1998; Strobel, 2002; Tan and Zou, 2001).

Only very little information is available concerning antimicrobial activities of isolates of marine-derived fungi. Hence, in the present investigation was designed to evaluate the antibacterial activity of marine isolate *Penicillium spinulosum* TTMF2.

MATERIALS AND METHODS

Isolation and identification of marine fungus

The marine fungus *Penicillium spinulosum* TTMF2 was isolated from Chidiya Tapu in Andaman and Nicobar Islands, India. Dilution plating technique described by Warcup (1950) was used to isolate the fungus from soils. Colony colour and morphology were observed besides hyphal structure, spore size, shapes and spore bearing structures. They were compared with the standard works of Manual of Soil fungi (Gillman, 1957).

Screening of fungi for antibacterial efficacy (Cuomo *et al.*, 1995)

The marine fungus *Penicillium spinulosum* TTMF2 was screened for antibacterial efficacy by well agar method (Perez *et al*., 1990.). The human pathogens gram - positive bacteria (*Bacillus subtilis, Enterobacter aerogenes, Streptococcus pyogenes* and *Staphylococcus aureus*) and gram - negative bacteria (*Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus vulgaris, Salmonella typhi,* and *Vibrio cholerae*) were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India.

Solvent extraction of fungal isolates

The conical flasks were taken and 150 ml of potato dextrose broth was prepared in each of the conical flask using seawater and distilled water mixture in a ratio of 1:1. The selected fungal cultures were inoculated in each of the conical flasks separately and incubated at 28°C for 5 days. After incubation, the fungal mats were taken from each of the flask and put into the each of the beakers. To this, each of the solvents (diethyl ether, ethyl acetate and distilled water) was added separately, crushed and centrifuged at 10, 000 rpm for 15 mins. The fungal mat extracts were tested against human pathogenic bacteria.

Assay

The nutrient agar medium (Beef extract - 3 gms, Peptone - 5 gms, Sodium chloride - 5 gms, Agar - 15 gms, Distilled water - 1000 ml and pH – 7) were poured into the sterile petri plates and allowed to solidify. The test bacterial cultures were evenly spreaded over the media by sterile cotton swabs. Then wells (6 mm) were made in the medium using sterile cork borer. 200 µl fungal extracts were transferred into the separate wells. The standard antibiotic (streptomycin) and solvents were used as positive and negative controls respectively. Then the plates were incubated at 37ºC for 24 hrs. After the incubation the plates were observed for formation of clear inhibition zone around the well indicated the presence of antibacterial activity. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well.

RESULTS AND DISCUSSION

Marine fungi are one of the most significant groups of organisms to be exploited for drug discovery purposes. Especially Fungi Imperfecti has provided mankind with numerous different bioactive secondary metabolites such as β-lactam antibiotics, griseofulvin, cyclosporine A or lovastatin. In agreement with the terrestrial fungi, marine fungi are producers of a high variety of biologically active secondary metabolites. In recent years, marine fungi have been explored more intensely to obtain novel and biologically active compounds. However, compared to marine sponges and bacteria, marine fungi are still less explored.

Marine fungi have been proved to be a rich source of bioactive natural products (Altamare *et al*., 2000; Abdel Lateff *et al.*, 2003; Bugni and Ireland, 2004; Chan *et al.*, 2006; Chen *et al.*, 2007; Gomes *et al.*, 2014). As these microorganisms grow in a unique and extreme habitat, they have the capability to produce unique and unusual secondary metabolites. It is believed

that the metabolites produced by these fungi would possibly act as a chemical defense as an adaptation mechanism of fungi competing for substrates (Fenical and Jensen, 1993; Gallo *et al.*, 2004). The process of new drug discovery is driven by the desire to identify structurally novel compounds that possesses important biological activity (Stephen and Horace, 2000; Holler *et al.*, 2000; Kohlmeyer and Volkmann-Kohlmeyer, 2003).

In the present investigation *Penicillium spinulosum* TTMF2 was isolated from marine soils of Chidiya Tapu in Andaman and Nicobar Islands, India and screened for its antibacterial efficacy. The results were summarized in table 1 and Plate 1 & 2.

The ethyl acetate extract *of Penicillium spinulosum* TTMF4 exhibited very promising antibacterial activity against *Klebsiella oxytoca* (22.7 mm). The distilled water extract showed higher antibacterial activity against *Vibrio cholerae* (18.7 mm) followed by *Klebsiella oxytoca* (17.7 mm), *K. pneumoniae* (16.7 mm) and *Escherichia coli* (13.0 mm). Several reports proved that there are number of antimicrobial drugs derived from different species of *Penicillium*. *Penicillium* species produce a much diversified array of active secondary metabolites, including antibacterial (Rancic *et al.* 2006; Lucas *et al.*, 2007), antifungal substances (Nicoletti *et al.*, 2007), immunosuppressants, cholesterol lowering agents (Kwon *et al.*, 2002) and also potent mycotoxins (Frisvad and Samson, 2004). Recently, Manimegalai *et al.* (2013) also reported *Penicillium citrinum* MF27 isolated from coastal region of Mahabalipuram exhibited significant antibacterial activity.

The antibiotic sensitivity test was studied against the tested bacterial pathogens. The results of antibiotic sensitivity test were presented in table 2. Streptomycin antibiotic exhibited higher antibacterial activity against *Streptococcus pyogenes*. The zone of inhibition was ranging between 8.5 - 14 mm. The result of antibacterial effect of three solvents revealed no activity against the tested pathogens.

To determine the effectiveness of the extraction methods, three different solvents (Distilled water, diethyl ether and ethyl acetate) were used for the extraction of antimicrobial metabolites from the selected fungi.

Organic solvents extraction of fungi showed higher antibacterial activity. It is accepted widely that the use of organic solvents always provides a higher efficiency in extracting antimicrobial compounds when compared with water extraction (Rosell and Srivastava,

1987). Similarly there is numerous reports emphasis, that use of ethyl acetate for the extraction of antimicrobial compounds from fungi is an effective method (Dalsgaard *et al.*, 2005; Lin *et al.*, 2005; Li *et al.*, 2006; Gallardo *et al.*, 2006). Evidently, Tarman *et al.* (2011) reported that the antibacterial activity of 11 fungal isolates from Indonesian marine habitats and *Aspergillus* sp. was the most active fungus against *Bacillus subtilis* and *Staphylococcus aureus*. Similarly the antibacterial efficacy of marine fungi was screened by many workers (Cuomo *et al.*, 1995; Newman *et al.*, 1998, Qi *et al.*, 2009; Mathan *et al.*, 2011; Geetha *et al.*, 2011; Radhakrishnan *et al.*, 2011; Manimegalai *et al.*, 2013).

The present investigation clearly showed that *Penicillium spinulosum* TTMF4 could produce potent antibacterial secondary metabolites and should be investigated for natural antibiotics.

Table 1. Antibacterial efficacy of *Penicillium spinulosum* TTMF2

S. No	Bacterial pathogens	Zone of inhibition (Diameter in mm		
		Distilled water	Diethyl ether	Ethyl acetate
1.	*Bacillus subtilis*	10.0±1.5	10.7±1.7	9.3±1.2
2.	*Enterobacter aerogenes*	-	-	11.7±2.9
3.	*Escherichia coli*	13.0±2.0	9.7±1.5	15.0±1.0
4.	*Klebsiella oxytoca*	17.7±2.5	21.3±1.5	22.7±2.5
5.	*K. pneumoniae*	16.7±2.9	10.7±2.9	9.3±0.6
6.	*Proteus vulgaris*	10.0±1.7	10.3±2.5	9.1±1.2
7.	*Streptococcus pyogenes*	-	-	9.3±1.5
8.	*Salmonella typhi*	-	10.7±2.9	10.6±2.9
9.	*Staphylococcus aureus*	-	10.7±2.9	11.0±2.6
10.	*Vibrio cholerae*	18.7±2.1	11.3±1.2	11.3±1.5

Results expressed as Mean ± SD (n=3), - - no inhibition zone

Table 2. Antibiotic sensitivity test on bacterial pathogens

S. No.	Bacterial Pathogens	Zone of inhibition (diameter in mm) Streptomycin(10 µg/ disc)
1.	*Bacillus subtilis*	9.5±1.5
2.	*Enterobacter aerogenes*	9.8±1.2
3.	*Enterococcus faecalis*	8.6±1.4
4.	*Escherichia coli*	9.1±1
5.	*Klebsiella oxytoca*	10.4±1.5
6.	*K. pneumoniae*	8.5±1.2
7.	*Salmonella typhi*	10.3±0.7
8.	*Staphylococcus aureus*	10.5±0.6
9.	*Streptococcus pyogenes*	14±1.1
10.	*Vibrio cholera*	10±1.5

Results expressed as Mean ± Standard Deviation (n - 3)

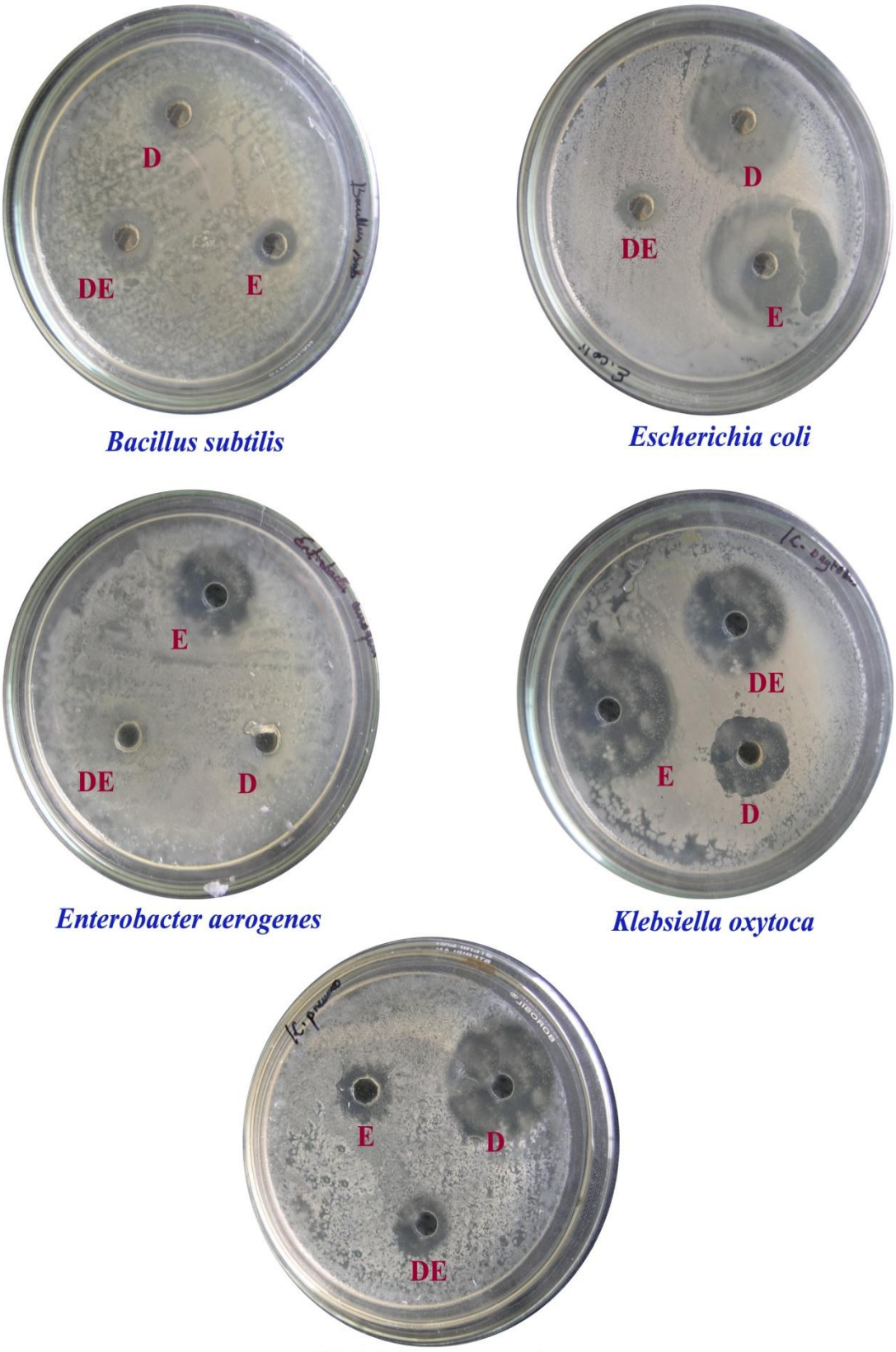

Plate 1. Antibacterial efficacy of *Penicillium spinulosum* TTMF2

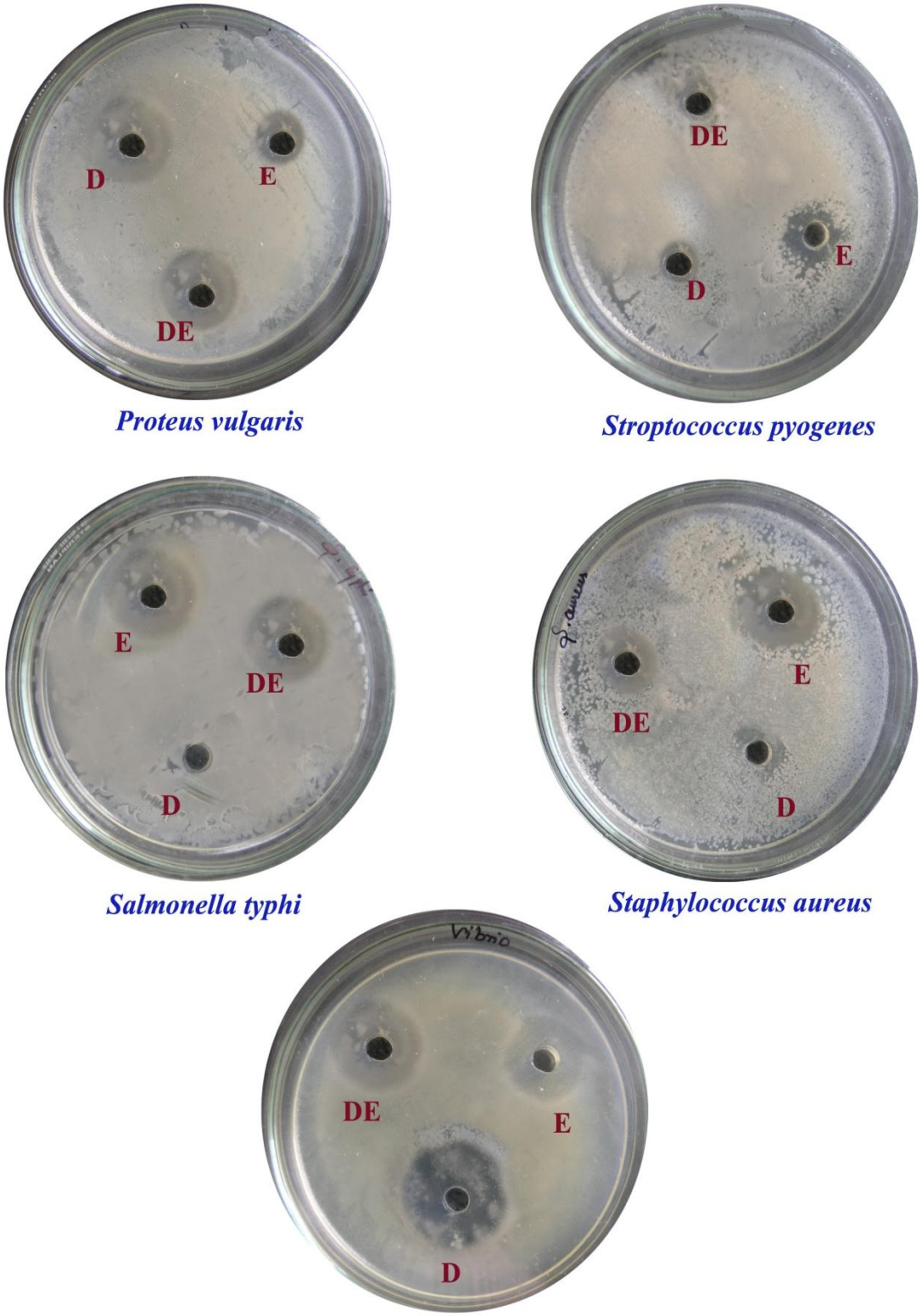

Plate 2. Antibacterial efficacy of *Penicillium spinulosum* TTMF2

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