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ANTIBACTERIAL ACTIVITY OF *PHAEOMARASMIUS ERINACEOUS*

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The present investigation deals with the antibiotic producing capacity of *Phaeomarasmius erinaceous* against *Bacillus subtilis* and *Staphylococcus aureus*. This paper also deals with the effect of temperature and incubation period on antibiotic production by the above fungus. The active compound has been isolated with solvent ether and its thermal stability has also been determined.

INTRODUCTION

Antibiotic activities of fleshy fungi were reported several times by a number of workers (Bose, 1953, Chakrabarti and Samajpati, 1979). During the routine screening of antimicrobial activities of locally available fleshy fungi, the authors noticed the antibacterial activities of culture filtrate of *Phaeomarasmius erinaceous*. The present paper also reports some preliminary data on the antibiotic substance produced by *Phaeomarasmius erinaceous*.

MATERIAL AND METHODS

During the rainy season, *P. erinaceous* was collectcd from the dry deciduous Sal forest (*Shorea robusta*) of Joypur, Dist. Bankura, West Bengal. *P. erinaceous* is a soil inhabitat mushroom. Tissue culture of the test fungus was prepared aseptically on potato dextrose agar medium from the fresh specimen. The culture was maintained on potato-dextrose agar slants at 30°C by regular subculturing.

For antibiotic screening, the fungus was grown in malt-peptone medium (malt-extract-20 g ; sucrose—20 g ; peptone—1 g and distilled water—1 litre, pH-5·5). After 15 days of growth, culture filtrates were obtained by filtration and were concentrated to one tenth the volume under reduced pressure. Antibiotic activity of the culture was then tested by 'cup-plate' (Bagyaraj and Sirsi, 1966) method on several plates containing 'nutrient peptone agar'[2] medium and each plate was seeded with the organisms *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*. The well developed inhibition zones indicated the antibiotic activity.

The optimum temperature for the maximum antibiotic activity of culture filtrate was studied by placing the different sets of flask (3 in each set) at different range of temperature (20°C, 24°C, 26°C, 30°C, 35°C and 40°C respectively) and then by testing the antibacterial activities of the culture filtrates by the 'cup-plate' method against *Bacillus subtilis* and *S. aureus*.

The optimum incubation period for maximum antibiotic activity was determined by growing the test fungus at varying intervals of incubation (3, 5, 7, 10. 15, 20, 25 and 30 days respectively) at 30°C and then the results were obtained by the testing the culture filtrates of different sets against *B. subtilis* and *S. aureus*.

For the isolation of active principles, solvent extraction procedure adopted was as follows.

The culture filtrate after 10 days incubation was extracted exhaustively several times with several solvents (solvent ether, petroleum ether, benzene and chloroform respectively). After extraction different solvents were evaporated to dryness under reduced pressure and the dried material were taken In water and screened for antibiotic activity against *B. subtilis*.

In order to study the thermal stability of the antibiotic compound, the compound extracted by solvent ether was treated at different range of temperature. i.e. 40°c, 60°c. 80°c, and 100°c for 1 hour and later on the property was tested against *B. subtilis*.

RESULTS AND DISCUSSIONS

The formation of well developed inhibition zones on nutrient agar plate seeded with *B. subtilis* (15 m.m.) and *S. aureus* (18 m.m.) showed the antibac-

terial activities of the culture filtrates of *P. erinaceous*. But the culture-filtrate failed to produce inhibition zones against *E. coli*.

The data on the effect of temperature on antibiotic production presented in Table I revealed that the culture filtrate exhibited antibiotic properties in all temperatures except 20°C and 40°C. The maximum zone of inhibition produced by the culture filtrate of 30°C are 16 m. m. against *B. subtiiis* and 18 m. m. against *S. aureus* respectively. So the optimum temperature revealed by the data is 30°C.

The data on the incubation period presented in Table II revealed that there is no production of antibiotic compound upto 3 days. After which the production starts, increased gradually upto 15 days (16 m.m. against *B. subtilis* and 18 m.m. against *S. aureus*) and then declines again. Hence the optimum Incubation period revealed by the data is 15 days.

Table 1. *Effect of temperature on antibiotic production by* Phaeomarasmius erinaceous *at 15 days incubation period*

Range of temperature	Microbes (zone of Inhibition in m. m.) *	
	Bacillus subtilis	*Staphylococcus* aureus
20°C	Nil	Nil
24°C	9.5	16.5
26°C	11.5	16.5
30°C	16	18
35°C	11	12
40°C	Nil	Nil

* Each value represents an average of five separate determinations.

Table 2. *Effect of incubation period on antibiotic produciion by* Phaeomarasmius erinaceous *at 30°C temperature*

Incubation period (days)	Microbes (zone of inhibition in m. m.) *	
	Bacillus subtilis	*Staphylococcus aureus*
3	Nil	Nil
5	9	10
7	12	12
10	13	15
15	16	18
20	15	16.5
25	14	16

* Each value represents an average of five separate determinations.

Among all the solvent extracts, solvent ether extract gave a good zone of inhibition (18 m. m.) against *B. subtilis* at the same concentration with the crude culture filtrate. Petroleum ether, benzene and chloroform extracts were inactive. So the compound is soluble in solvent ether only.

The data on the heating effect on the antibiotic compound reveals that the compound product similar zone of inhibition like normal after each heat treatment. Which indicated that the compound is heat stable.

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