Antimicrobial Activity of Mycoflora Inhabiting Wastes

Poonam Agrawal, Rekha Shukla and A. K. Pandey*

*Mycological Research Laboratory, Department of Bioscience, R.D University, Jabalpur-482001, M.P India; *Private University Regulatory Commission, M.P Bhoj University campus, Bhopal. E-mail:akpmycol@yahoo.com*

Abstract

Population, urbanization and industrialization generate waste making garbage pollution a serious problem to most cities. Waste provides an excellent site for colonization and multiplication of microorganisms including fungi. Fungi are known to produce a wide range of secondary metabolites of high therapeutic values. We investigated on the antimicrobial potential of fungi inhabiting hospital, sewage and domestic waste. Seventeen fungal isolates were tested against *Escherichia coli. Curvularia lunata* which showed a maximum inhibitory activity, were further evaluated against eight pathogenic bacteria by disc diffusion method. Maximum inhibitory activity by *C. lunata* against *Klebsiella pneumoniae, Vibrio cholera, E. coli* and *Enterococcus* sp. was detected in 20 days old fermented Richard's broth. Antibacterial metabolite of *C. lunata* was observed to be thermo-labile.

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Waste is any substance, solution, mixture or article for which no direct use is envisaged but which is transported for reprocessing, dumping, elimination by incineration or other methods of disposal (Yakowitz 1988). With increasing population, social changes urbanization and industrialization, production of waste is increasing rapidly making garbage pollution a serious problem (Yaliang 1996). In view of the development of resistance in pathogenic microorganisms, search of bioactive compounds with therapeutic potential is of great interest. Fungi are known to produce a wide range of secondary metabolites of high therapeutic values (Keller et al 2002). Substantial amounts of wastes that are generated from hospitals, domestic establishments, industries and other sources due to human activities are deposited in soil. Waste material accumulated in the natural environment provide an excellent site for colonization and multiplication of microorganisms including fungi (Mishra et al 1996; Obire et al 2002). Due to extreme environments presented by varied types of wastes, microorganisms interact with each other and produce certain metabolites for their survival. Therefore, an investigation was made to recover fungi in waste and evaluate them for antimicrobial potential.

Materials and Methods

Collection of soil samples. Soil samples from three different waste viz. Domestic waste, waste from hospital and sewage soil , situated at Jabalpur city were collected from Feb-Jun 2008. Samples were mixed thoroughly and categorized into three groups.

Isolation of fungi from soil samples. Five gram of each soil sample was dried at toom temperature and processed for isolation of fungi. Fungal cultures were maintained on Potato dextrose agar slants at 4±1C in a refrigerator. Identification of fungi was carried out with the help of available literature and experts (Subramanium 1956; Barnett 1972; Ellis 1971 1976; Gilman 1957). Frequencies of all the fungi were determined as per Agrawal and Hasija (1986).

Screening of antibacterial Activity. The reference bacterium, *Escherichia coli (MTCC# (*procured from IMT, Chandigarh). 0.5 ml test bacterial inoculum grown in 100 ml of nutrient broth (NA) at 37±2C for 24h was spread uniformly over surface containing PDA and nutrient agar and kept under aseptic conditions for 30 min (Weid et al 2003). Subsequently 6 mm disc of each fungal isolate obtained from 5d old actively growing culture on PDA was placed at the centre of plates containing bacterial culture in sterile conditions. Plates were then kept at upright position at 30±2C for 24 h. They were observed regularly and zone formation was recorded (Ayse et al 2004). Potential strain was selected for further studies.

Antibacterial activity of cell-free culture filtrate. Flask containing 50 ml of sterilized Richard's and Asthana and Hawker's broth separately were inoculated with five mm equal size disc obtained from 7 d old actively growing culture of the selected strain of fungi on PDA.

Table 1. Frequency (%) and antimicrobial activity of fungi in different types of wastes

Fung species	Fungi in different sites (%)			Antibacterial activity (against *E.Coli*)
	Domestic waste	Hospital waste	Sewage waste	
Curvularia geniculata	7.14	7.14	0	10-15 mm
Trichocladium opacum	0	0	42.85	<5 mm
Fusarium oxysporum	35.71	14.28	7.14	<5 mm
Aspergillus niger	42.85	21.42	21.42	<5 mm
Rhizopus nigricans	14.28	0	0	5-10 mm
Trichoderma viride	7.14	0	14.28	10-15 mm
Aspergillus nidulans	28.57	0	14.28	<5 mm
Fusarium roseum	7.14	42.85	0	<5 mm
Mucor hiemalis	14.28	7.14	0	0
Fusarium solani	7.14	14.28	0	<5 mm
Curvularia lunata	14.28	35.71	14.28	>15 mm
Chrysosporium indicum	14.28	0	0	<5 mm
Microsporum gypsum	21.42	7.14	7.14	5-10 mm
Aspergillus flavus	14.28	7.14	0	<5 mm
Dreschlera sp.	21.42	7.14	14.28	5-10 mm
Chaetomium globosum	21.42	0	7.14	5-10 mm
Aspergillus fumigatus	28.57	7.14	21.42	<5 mm

The flasks were incubated for 5, 10, 15, 20, 25 d at 25±2C. After desired incubation, the culture was filtered through Whatman No. 1 filter paper and centrifuged at 4000 rpm for 10 min. Supernatant was taken and its antibacterial activity was tested against all the eight bacterial strains by disc diffusion method (BIO 347) and spectrophotometric method (Ishikawa et al 2001). Thermal stability of cell free culture filtrate (CFCF) was also studied at 121C (Chakraborti and Samajpati 1981; Jonathan and Fasidi 2003). All the bacterial strains used in this study viz. *Bacillus subtilis* (MTCC 1789), *Staphylococcus aureus* (MTCC187), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 748), *Streptococcus* sp, *Pseudomonas aeruginosa*e (MTCC 779), *Vibrio cholerae* (MTCC 1068), *Enterococcus faecium* were procured from Institute of Microbial Technology, Chandigarh. All experiments were performed in triplicates and were statistically analyzed.

Results

Fungal isolates from waste. A total of 17 fungi represented by 10 genera were isolated from various wastes (Table.1). Domestic wastes yielded more fungi followed by hospital wastes and sewage sites. *Aspergillus niger* showed maximum frequency (87%) followed by *Fusarium solani, Curvularia geniculata*. *Mucor hiemalis, Rhizopus nigricans* and *Chaetomium globosum* were found only rarely. *A. niger, F. roseum* and *Trichocladium opacum* were the most frequently occurring fungi in domestic, hospital and sewage wastes, respectively. It was also interesting to note that *R. nigricans* and *Chrysosporium indicum* were isolated only from domestic waste while *T. opacum* was found only in sewage waste. Variation in occurrence of fungi may be due to difference in chemical composition of the wastes. It is evident from results (Table 1) that *C. lunata,* exhibited max. bacteriostatic activity against reference bacterium *E. coli. C. geniculata* and *T. viride* also showed significant activities (12 mm zone) against the bacterium when tetracycline used as reference antibiotic. Rest of the fungi also exhibited some activities while *M. hemalis* did not show any activity. Thus, *C. lunata* was selected for further evaluation.

Disc diffusion method. Significant variation in antimicrobial activities of *C. lunata* obtained from fermented broth after different incubation periods (Table 2). CFCF obtained from 5 days and 10 days old fermented broth did not show any bacteriostatic activity. CFCF applied in disc diffusion method showed significant reduction in growth of the test bacteria. Amongst all bacterial strains tested *K. pneumoniae, V. cholerae, E. coli* and *Enterobacter aerogenes* were found to be more sensitive to crude CFCF. 20 d old fermented Asthana and hawkers broth exerted max. activity against *E. coli.* No antibacterial activity was recorded against *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Significant difference in thermal stability of the CFCF was also recorded at 121C. (Table 3). Activity against *Bacillus subtilus* was completely lost. Similarly, CFCF obtained from Richard's and Aasthana and Hawkers did not show any antimicrobial activity against *Vibrio cholera* and *E. tedium* respectively.

Table 2. Antibacterial activity of cell-free culture filtrate of *Curvularia lunata* as indicated by zone of inhibition (mm) in disc diffusion method

Bacterial strains	5 d old		10 d old		15 d old		20 d old		25 d old	
	RB	AHB	RB	AHB	RB	AHB	RB	AHB	RB	AHB
Bacillus subtilis (MTCC 1789)	0	0	0	0	7± 0.23	5± 0.20	15± 0.28	12± 0.16	0	15 ±0.2
Staphylococcus aureus (MTCC 187)	0	0	0	0	0	0	0	0	0	-
Escherichia coli (MTCC 443)	0	0	0	0	0	5± 0. 31	18± 0.12	25± 0.11	15± 0.12	16± 0.12
Klebsiella pneumoniae (MTCC 748)	0	0	0	0	7± 0.23	9± 0.20	28 ±0.20	24± 0.20	31± 0.23	24± 0.12
Streptococcus sp	0	0	0	0	3± 0.23	8± 0.12	8± 0.23	8± 0.11	14± 0.17	11± 0.17
*Pseudomonas aeruginosa*e (MTCC 779)	0	0	0	0	0	0	0	0	0	-
Vibrio cholerae (MTCC 1068)	0	0	0	0	0	6± 0.34	21± 0.20	16± 0.23	15± 0.23	12± 0.12
Enterococcus faecium	0	0	0	0	3± 0.33	0	17± 0.26	10± 0.23	1± 0.28	7± 0.12

RB = Richard's broth; AHB = Asthana and Hawkers broth

Table 3. Effect of incubation temperature on antibacterial activity of 25 d old cell-free culture filtrate of *Curvularia lunata* by disc diffusion method

Bacterial strain	Zone of inhibition (in mm)	
	Richard's	Asthana
Bacillus subtilis (MTCC 1789)	0	0
Staphylococcus aureus (MTCC 187)	0	0
Escherichia coli (MTCC 443)	8±0.12	9±0.16
Klebsiella pneumonia (MTCC 748)	7±0.11	10±0.28
Streptococcus sp.	16±0.17	0
Pseudomonas aeruginosae (MTCC 779)	0	0
Vibrio cholerae (MTCC 1068)	0	9±0.12
Enterococcus faecium	9±0.12	0

Discussion

During the recent years serious efforts have been geared towards searching for new microbes with high therapeutic potential. Antimicrobials represent an important part of medicine today. The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. However, serious problems related to resistance against various drugs have arisen. Waste materials being rich in diverse nutrient provide novel situation to multiply and survive a large group of microorganisms including fungi. Through microbiological analysis of samples collected form hospitals, domestic and sewage water wastes yielded 17 fungi with varied frequencies. Variation in numbers and types of fungi might be due to difference in chemical composition of wastes. Similar observations have also been recorded by many earlier workers. Pandey et al (1989) reported 32 isolates of fungi from garbage soil among which *Chrysosporium, Fusarium, Aspergillus* and *Microsporum* were prominent. Gugnani et al (1978) recovered *Cladosporium* sp. and *D. trichoides* from Garbage soil. Obire et al (2002) reported several fungi from different wastes. Many keratinophilic fungi including *Chrysosporium* sp. and *Microsporium* sp. have also been reported from waste contaminated habitats (Ulfig and Korcz 1983; Ulfig et al 1996; Ulfig, 1985). Taiwo and Oso (2004) reported several fungi for municipal solid wastes. Adesemoye (2006) also reported on the identification of several fungi from wastes. Antimicrobial efficiency of all the test strains of fungi varied significantly against *E. coli*. *C. lunata* showed remarkable inhibitory action against this reference bacterium and thus was selected for further evaluation. The results showed a variable effect of extracts from this fungus on the target strains of bacteria. *C. lunata* was more active and produced a max. zone of inhibition than other fungal strains. It may presumably be due to the production of curvularin by this fungus as reported by Trigos et al (2006). Similar results have also been recorded by Bycroft (1988), Jonathan and Fasidi (2003), Patil et al (2007) Jung et al (2002). Trigos et al (2006) reported significant microbial activity in *C.lunata, Fusarium* sp. and *Rhizopus* sp. It was also recorded that CFCF of *C. lunata* had a broad spectrum of inhibitory activity against various bacteria. Thus, fungus producing secondary metabolites that may be used as antibiotics needs to be explored thoroughly. Disc diffusion method employed in the present study proved as an effective and efficient technique to identify and select fungi. However, Jonathan and Fasidi (2003) and Toda et al (1991) reported well diffusion method as the most sensitive method of evaluating antimicrobial activities as compared to disc diffusion method. CFCF which declined in antimicrobial activity after subjecting to a temp treatment clearly indicates its thermo-labile nature. Similar variations in activity of metabolites produce by various fungi have also been found by Weid et al (2003) and Chakrabarti and Samajpati (1981). Therefore, from the study it can be concluded that various fungi recovered from wastes sites have significant antimicrobial property and especially *C. lunata* needs further investigations to characterize and purify the antimicrobial moieties against multi-drug resistant bacteria.

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