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COMPARISON OF ANTIBACTERIAL PROPERTIES OF *PENICILLIUM* SPECIES

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ABSTRACT

Nine *Penicillium* species were isolated from different sources and were screened for antibacterial potential against ten common bacterial strains. *Bacillus* sp. (FCBP 234) and *Acetobacter xylinum* (FCBP 239) was the most sensitive to culture filtrates of *P. granulatum* (FCBP 1080), *P. verrucosum var. cyclopium* (FCBP 1052), *P. billii* (FCBP 1079) and *P. expansum* (FCBP 1102), with maximum inhibition zone diameters of 4.2 and 4.0 cm, respectively. On the other hand, filtrates of *P. simplicissimum* (FCBP 022), *P. citrinum* (FCBP 024), *P. oxalicum* (FCBP 025) and *Penicillium* sp. (FCBP 1069) were weak to least effective to control the growth of all tested bacteria. The preliminary data led us to conclude that, test *Penicillium* strains have remarkable antibacterial potential. These locally isolated *Penicillium* strains can be used for novel fungal natural products in biotechnological applications.

Key words: *Penicillium*, antibacterial, *Bacillus*, bacteria

INTRODUCTION

Fungi are recognized as active secondary metabolite producers. Many fungi have made available several bioactive compounds and chemical pathways currently used as pharmaceuticals, and soils are conventionally the major source of fungal genetic resources for bioprospection programs (Adrio and Demain, 2003). *Penicillium* is a anamorphic ascomycetous genus, widely distributed in most terrestrial environments. This fungal genus comprises more than 200 reported species and many are of common soil inhabitants, food borne contaminants as well as food ingredients used in the preparation of cheese and sausages (Frisvad and Samson, 2004; Pitt, 2000). *Penicillium* species are reported to produce a much diversified range of active secondary metabolites, including antibacterial (Lucas *et al.*, 2007; Rancic *et al.*, 2006), antifungal substances (Nicletti *et al*., 2007), immunosuppressants, cholesterol-lowering agents (Kwon *et al.*, 2002), and also potent mycotoxins (Frisvad and Samson, 2004).

Thousands of *Penicillium* isolates have been screened in bioprocessing since the discovery of penicillin. Numerous investigations have reported that various mycotoxins can be produced by *Penicillium* (Faid and Tantaoui-Elaraki, 1989) that also have strong antibacterial properties (Khaddor *et al.*, 2007). The purpose of this study was to investigate the anti- bacterial activity of a wide range of local *Penicillium* species. This study will also provide basis for novel fungal natural products targeting at metabolites with biotechnological applications for the pharmaceutical industry.

MATERIALS AND METHODS

Isolation of the *Penicillium* species

A total of nine *Penicillium* species were isolated from different sources (Table 1) The fungi were isolated by dilution plate method using the media and growth conditions specified by Pitt (2000), on Czapek yeast extract agar (CYA) at 25ºC and Malt Extract Agar (MEA) at 25ºC, incubated for 7 days in darkness. *Penicillium* species were identified on the basis of morphological features (Pitt, 2000), purified and preserved at 4ºC for further studies. *Penicillium* cultures were also deposited in First fungal Culture Bank of Pakistan (FCBP), University of the Punjab Lahore.

Test Bacterial strains

A total of ten bacterial strains, *Xanthomonas axonopodis* (FCBP 007), *Pseudomonas syringae* (FCBP 009), *Salmonella gallinarum* (FCBP 038), *Escherichia coli* (FCBP 046), *Xenorhabdus luminescens* (FCBP 119), *Carnobacterium mobile* (FCBP 245), *Acetobacter xylinum* (FCBP 239), *Oscillospira* sp. (FCBP 231), *Bacillus* sp. (FCBP 234) and *Xanthobacter autotrophicus* (FCBP 201) used for this study were obtained from First fungal Culture Bank of Pakistan (FCBP), University of the Punjab Lahore (Table 1). Cultures were revived on nutrient agar media at 37$\pm$ 2 ºC and used for the studies.

Preparation of *Penicillium* species extracts

A mycelia disc (8mm) from 7 days old culture of *Penicillium* sp. was inoculated in a 100 ml of 2% Malt extract (ME) broth. The fungal culture was incubated without agitation for 15 days at 25ºC. At the end of incubation the cultures mycelia was filtered off first through Whatman paper to remove the mycelium, and then through 0.45 mm millipore membrane for sterilization. Culture filtrate of each *Penicillium* sp. was tested against bacterial strains for antimicrobial activity using the well diffusion assay. The sterile culture filtrates were used for antibacterial activity tests.

Table. 1 List of *Penicillium* species Takahashi JA and Bacterial isolates

FCBP #	*Penicillium* species	Sources	FCBP #	bacterial species	Sources
022	*P. simplicissimum*	Onion	007	*Xanthomonas axonopodis*	*Citrus* fruit
024	*P. citrinum*	Bread	009	*Pseudomonas syringae*	Cherry fruit,
025	*P. oxalicum*	Rhizosperic soil of Grapes,	038	*Salmonella gallinarum*	Sugarcane stem,
1052	*P. verrucosum var. cyclopium*	Lemon fruit,	046	*Escherichia coli*	Sugarcane stem,
1069	*P.* species	Bread	119	*Xenorhabdus luminescens*	*Citrus sinensis* fruit,
1079	*P. billii*	Bread	245	*Carnobacterium mobile*	Vegetable rhizospheric soil,
1080	*P. granulatum*	Grape fruit,	239	*Acetobacter xylinum*	Vegetable rhizospheric soil,
1102	*P. expansum*	Garlic, Lahore	231	*Oscillospira* sp.	Citrus field soil,
1109	*P. implicatum*	Pomgranate fruit,	234	*Bacillus* sp.	Gram soil, Sargodha
			201	*Xanthobacter autotrophicus*	Wheat field soil,

Antimicrobial bioassays

A bacterial suspension from 24 h old culture, containing 10^6 cells /ml in sterile distilled water, was used for inocula. Petri dishes (90 mm) containing Luria Bertani (L.B) agar medium were surface inoculated with 0.2 ml of bacterial inocula. 15 min after inoculation, four wells of 8mm diameter were dug out in the agar medium, filled with 0.6 ml of sterile fungal filtrate. After 24 h incubation at 37°C, the antibacterial effect was determined by measurement of the inhibition zone diameters.

RESULTS AND DISCUSSION

Several metabolites have been purified from several *Penicillium* species (Larsen and Kn-chel, 1997). One of the important mechanisms that contributed to their biocontrol activities has been proposed to produce antibiotic compounds (Cortes, *et al.,* 1998; Larsen and Kno_chel, 1997; Onyegeme-Okerenta *et al.* 2009).

In this study, all *Penicillium* strains showed antibacterial potential (Table. 2). *Bacillus* sp. and *Acetobacter xylinum* was the most sensitive to culture filtrates of *P. granulatum* (FCBP 1080), *P. verrucosum var. cyclopium* (FCBP 1052), *P. billii* (FCBP 1079) and *P. expansum* (FCBP 1102), with maximum inhibition zone diameters of 4.2 and 4.0 cm, respectively (Table 2). *P. verrucosum* is a terverticillate species belonging to the subgenus *Penicillium*, a number of secondary metabolites, such as the already mentioned griseofulvin and penitrem A, are known to be produced by several species in subgenera *Penicillium* (Frisvad and Samson, 2004).

On the other hand, filtrates of *P. simplicissimum* (FCBP 022), *P. citrinum* (FCBP 024), *P. oxalicum* (FCBP 025) and *Penicillium* sp. (FCBP 1069) were weak to least effective to control the growth of all tested bacteria. Culture filtrates of *P. granulatum* (FCBP 1080) was also the most effective to control *Salmonella gallinarum* (FCBP 038), *Xanthobacter autotrophicus* (FCBP 201), *Oscillospira* sp (FCBP 231), *Bacillus* sp. (FCBP 234) and *Acetobacter xylinum* (FCBP 239). Other *Penicillium* species has also been reported to suppress the bacterial growth in a number of studies (Faid and Tantaoui-Elaraki, 1989; Carlton *et al.,* 1976). The production of antibiotic substances is regarded as one of the biochemical mechanisms regulating antagonism between soil fungi that may also influence fungistasis and the suppressive properties of certain soils toward plant pathogens (de Boer *et al.,*

2003; Frisvad and Samson, 2004; Vey *et al.*, 2001). *Penicillium* species produce a much diversified array of active secondary metabolites, including antibacterial (Lucas *et al.*, 2007; Rancic *et al.*, 2006). These preliminary data enable us to conclude that, test *Penicillium* strains have remarkable antibacterial potential. These locally isolated *Penicillium* strains can be used for novel fungal natural products in with biotechnological applications.

Table 2. Inhibition zones of bacterial species by the effect of *Penicillium* toxin.

		Inhibition Zone diameter (cm)								
FCBP #	**Bacterial species**	**FCBP 0022**	**FCBP 0024**	**FCBP 0025**	**FCBP 1052**	**FCBP 1069**	**FCBP 1079**	**FCBP 1080**	**FCBP 1102**	**FCBP 1109**
007	*Xanthomonas axonopodis*	1.8±0.09	2.0±0.09	1.7±0.17	3.2±0.17	1.8±0.09	2.0±0.98	2.4±0.09	3.4±0.17	1.8±0.09
009	*Pseudomonas syringae*	2.2±0.09	1.5±0.09	2.1±0.49	2.3±0.45	2.4±0.09	1.9±0.17	2.0±0.09	2.6±0.17	2.0±0.09
038	*Salmonella gallinarum*	1.4±0.09	1.1±0.09	1.1±0.17	2.3±0.09	1.5±0.45	4.3±0.45	3.5±0.09	2.0±0.09	2.5±0.17
046	*Escherichia coli*	1.1±0.17	1.4±0.09	2.3±0.35	2.5±0.05	2.3±0.35	2.0±0.09	2.7±0.35	1.8±0.09	1.6±0.09
119	*Xenorhabdus luminescens*	2.2±0.09	2.0±0.09	2.8±0.09	2.5±0.55	1.8±0.09	1.8±0.09	2.4±0.09	2.4±0.09	1.8±0.09
201	*Xanthobacter autotrophicus*	1.8±0.09	1.6±0.17	2.1±0.17	2.3±0.17	1.4±0.09	1.5±0.85	3.2±0.17	1.7±0.26	1.4±0.09
231	*Oscillospira* sp.	1.6±0.09	1.6±0.17	1.8±0.17	1.9±0.09	1.8±0.09	2.2±0.09	3.0±0.09	2.4±0.09	2.0±0.09
234	*Bacillus* sp.	2.0±0.09	2.7±0.35	2.8±0.09	4.2±0.43	2.5±0.17	3.8±0.09	4.2±0.45	3.7±0.45	3.2±0.17
239	*Acetobacter xylinum*	2.0±0.09	2.5±0.09	2.6±0.26	3.6±0.39	2.1±0.26	3.2±0.17	4.0±0.09	3.8±0.09	2.5±0.17
245	*Carnobacterium mobile*	1.3±0.17	1.6± 0.26	1.4±0.09	2.5±0.09	2.0±0.09	2.4±0.09	3.2±0.17	2.0±0.19	1.3±0.09

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