

Antibacterial activity of culture extracts of *Penicillium* species against soil-borne bacteria

Amna Ali*, Muhammad Saleem Haider, Ibatsam Khokhar, Uzma Bashir, Sobia Mushtaq and Irum Mukhtar

Institute of Agricultural Sciences (IAGS), University of the Punjab, Quaid-E-Azam campus, Lahore 54590 Pakistan.

*Corresponding Author: write2amna@gmail.com

Abstract

The present study was undertaken to test the efficacy of culture extracts of five *Penicillium* species viz. *P. citrinum*, *P. digitatum*, *P. expansum*, *P. verrucosum* and *P. viridicatum* against five different soil-borne bacteria namely *Salmonella gallinarum*, *Xenorhabdus luminescens*, *Xanthobacter autotrophicus*, *Acetobacter xylinum* and *Carnobacterium mobile*. The species of *Penicillium* showed marked antibacterial activity against all the bacterial species. *Xenorhabdus luminescens* was the most sensitive to culture filtrates of all the *Penicillium* species except *P. viridicatum* with minimum inhibition zone of 2.2 cm. On the other hand, *S. gallinarum*, *X. autotrophicus* and *A. xylinum* exhibited moderate inhibitory activity against all the tested *Penicillium* species.

Key words: Antibacterial, culture extracts, *Penicillium*, soil-borne bacteria.

Introduction

Natural products are an important source of novel active agents. These natural compounds play a significant role in the discovery and development of drugs for the treatment of diseases and microbial environment. (Newman *et al.*, 2003). Many of these products currently used are produced by microbial fermentation, or are derived from chemical modification of a microbial product (Donadio *et al.*, 2002). Fungi are recognized as prolific secondary metabolite producers. Fungi have provided several bioactive compounds and chemical models currently used as pharmaceuticals, and soils are traditionally the main source of fungal genetic resources for bioprospection programs (Adrio and Demain, 2003). *Penicillium* is a large anamorphic (asexual state) ascomycetous fungal genus with widespread occurrence in most terrestrial environments. This 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). *Penicillium* species produce a much diversified range of active secondary metabolites, including antibacterial (Lucas *et al.*, 2007; Rancic *et al.*, 2006), antifungal substances (Nicoletti *et al.*, 2007), immunosuppressants, cholesterol-lowering agents (Kwon *et al.*, 2002), and also potent mycotoxins (Frisvad and Samson, 2004). These secondary metabolites of *Penicillium* species have been identified as well as proved their biological activities (Silva *et al.*, 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) those also have strong antibacterial properties (Khaddor *et al.*, 2007). The purpose of this study was to investigate the anti-bacterial activity of some local *Penicillium* spp.

Materials and Methods

Isolation of the *Penicillium* species

A total of five *Penicillium* species were isolated from different sources (Table 1). These fungi were isolated by dilution plate method using the media and growth conditions specified by Pitt (1979), on Czapek yeast extract agar (CYA) at 25 °C and Malt Extract Agar (MEA) at 25 °C, incubated for 7 days in darkness. *Penicillium* species were identified on the basis of morphological features (Pitt, 1979), and purified and preserved at 4 °C for further studies. *Penicillium* cultures were also deposited in Fungal Culture Bank of Pakistan (FCBP), University of the Punjab Lahore, Pakistan.

Procurement of bacterial strains

A total of five soil-borne bacterial strains, *Salmonella gallinarum* (FCBP 038), *Xenorhabdus luminescens* (FCBP 119), *Xanthobacter autotrophicus* (FCBP 201), *Acetobacter xylinum* (FCBP 239) and *Carnobacterium mobile* (FCBP 245) used for this study were obtained from FCBP

(Table 1). Cultures were revived on nutrient agar media at 37 ± 2 °C.

Preparation of culture filtrates of *Penicillium* spp.

Mycelial discs (8 mm) from 7 days old cultures of *Penicillium* spp. were inoculated in 100 mL of 2% Malt Extract (ME) broth. The fungal cultures were incubated without agitation for 15 days at 25 °C. At the end of incubation the cultures mycelia was filtered off through Whatman paper No: 1 to remove the mycelium. Culture extracts of each *Penicillium* sp. was tested against bacterial strains for antimicrobial activity using the well diffusion assay.

Determination of inhibitory activity

A bacterial suspension (10^6 cells mL^{-1}) for inoculum was prepared in sterilized water from 24 h old culture. Bacterial inoculum (0.2 mL) was surface inoculated on Luria Bertani (LB) agar medium in petri dishes (90 mm). Four wells of 8 mm diameter were dug out in the agar medium, filled with 0.6 mL of culture filtrate, after 15 min of bacterial inoculation. For control, streptomycin ($30 \mu\text{g m}^{-1}$) was filled in the wells against tested soil-borne bacteria. After 24 h incubation at 37 °C, the antibacterial effect of fungal filtrate was determined by measurement of the inhibition zone diameters.

Results and Discussion

In present study, all *Penicillium* strains showed antibacterial potential (Table 2). *X. luminescens* (FCBP 119) was the most sensitive to culture filtrates of *P. verrucosum* (FCBP 1041), *P. citrinum* (FCBP 508), *P. expansum* (FCBP 1101) and *P. digitatum* (FCBP 726) with maximum inhibition zones of 7.3 cm, 5.1 cm, 6.9 cm and 5.0 cm, respectively. *P. viridicatum* (FCBP 025) showed minimum inhibition zone with 2.2 cm against this bacteria (Table 2). Several metabolites have been purified from many *Penicillium* species (Larsen and Kno_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). *P. verrucosum* is a terverticillate species,

which produces a number of secondary metabolites, such as griseofulvin and penitrem A (Frisvad and Samson, 2004). Present results showed that culture filtrates of *P. verrucosum* (FCBP 025), *P. citrinum* (FCBP 580) and *P. expansum* (FCBP 1101) were weakly effective to control the growth of *A. xylinum* (FCBP 239) as compared to *P. viridicatum* (FCBP 025) and *P. digitatum* (FCBP 726) which exhibited maximum inhibitory activity against this bacterial species. However, culture filtrates of all tested *Penicillium* species were found the most effective to control *S. gallinarum* (FCBP 038). Several *Penicillium* species have also been reported to suppress the bacterial growth (Samane *et al.*, 1991; 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; Vey *et al.*, 2001; Fravel, 1988). *P. verrucosum* (FCBP 1041) and *P. expansum* (FCBP 1101) demonstrated moderate antibacterial activity against *C. mobile* (FCBP 245) with inhibition zone diameters of 3.4 cm and 3.1cm, respectively. Among tested *Penicillium* species *P. viridicatum*, *P. citrinum* and *P. digitatum* did not showed any satisfactory results against *C. mobile*. *X. autotrophicus* (FCBP 201) exhibited maximum inhibitory zone of 5.4 cm and minimum inhibitory zone of 1.3 cm in the presences of *P. viridicatum* (FCBP 025) and *P. citrinum* (FCBP 580) culture filtrate, respectively.

Penicillium species colonize plant's rhizosphere, waste and food materials world wide. According to Gaden (2000) and Malmstrom *et al.* (2000), the expression and production of secondary metabolites in fungi is influenced by medium composition, nutrients availability and culture conditions. In present investigation, preliminary data enable us to conclude that test *Penicillium* strains isolated from food and industrial effluents have remarkable antibacterial potential. These locally isolated *Penicillium* strains can be used for effective fungal natural products in biocontrol and industrial applications.

Table 1: List of *Penicillium* species and pathogenic bacterial strains.

FCBP #	<i>Penicillium</i> species	Sources	FCBP #	Bacterial strains	Sources
1041	<i>P. verrucosum</i>	Industrial effluent	038	<i>Salmonella gallinarum</i>	Sugarcane rhizospheric soil
580	<i>P. citrinum</i>	Khoya	119	<i>Xenorhabdus luminescens</i>	Citrus rhizospheric soil
025	<i>P. viridicatum</i>	Grapes rhizospheric soil	201	<i>Xanthobacter autotrophicus</i>	Wheat rhizospheric soil
1101	<i>P. expansum</i>	Apple fruit	239	<i>Acetobacter xylinum</i>	Vegetable rhizospheric soil
726	<i>P. digitatum</i>	Air	245	<i>Carnobacterium mobile</i>	Vegetable rhizospheric soil

Table 2: Antibacterial activity of culture extracts of *Penicillium* species

Bacterial Strains	Control	Zone of inhibition (cm)				
		<i>P. verrucosum</i>	<i>P. citrinum</i>	<i>P. viridicatum</i>	<i>P. expansum</i>	<i>P. digitatum</i>
<i>Salmonella gallinarum</i>	1.2±0.0 e	5.5±0.02 b	4.9±0.05 c	4.7±0.11cd	4.6±0.08d	6.6±0.06a
<i>Xenorhabdus luminescens</i>	2.2±0.0 d	7.3±0.08 a	5.1±0.05 c	2.2±0.11d	6.9±0.05b	5.0±0.06 c
<i>Xanthobacter autotrophicus</i>	0.5±0.0 e	2.7±0.06 c	1.3±0.08d	5.4±0.06 a	3.3±0.11b	2.4±0.02 c
<i>Acetobacter xylinum</i>	1.5±0.0 e	1.3±0.08 e	3.1±0.05 c	4.8±0.05 b	2.1±0.05d	6.2±0.13 a
<i>Carnobacterim mobile</i>	1.5±0.0 d	3.4±0.05 a	2.2±0.11 c	2.1±0.05 c	3.1±0.05b	1.0±0.06 e

Values with different letters show significant difference (P0.05) as determined by Duncan's Multiple Range Test.

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