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The antimicrobial spectra of selected Penicillia against some pathogenic bacteria and yeasts

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The antagonistic activity of eight isolates of penicillia has been studied against 13 pathogenic organisms, which included 6 Gram-positive bacteria, 4 Gram-negative bacteria and 3 yeasts.

INTRODUCTION

The phenomenon of antagonism which is common among microorganisms is one of the most important factors in the study of natural environments, where a number of relationships exist between individual microbial species and individual cells. Since the discovery of penicillin by Fleming (1929), a large number of antibiotics have been reported from different species of *Penicillium* (Darken, Sjolander 1951; Jeffers et al. 1953; Brian et al. 1957; Betina et al. 1962; Shimi, Imam, Saad 1966). The eight isolates of penicillia i.e. *P. chrysogenum* Thom strain 1, *P. chrysogenum* strain 2, *P. citrinum* Thom, *P. claviforme* Bainier, *P. griseo-fulvum* Dierckx, *P. notatum* Wstl. strain 1, *P. notatum* Westl. strain 2 and *P. urticeae* Bainier which were found to be very active against Gram-positive and Gram-negative bacteria and a fungus *Curvularia lunata* in the previous studies by Chauhan and Saksena (1979) were selected for further studies. These isolates were more rigorously tested against a set of 13 pathogenic organisms including 10 bacteria and 3 yeasts, a procedure which has come to be known as antimicrobial spectrum for a species. These test organisms were selected on a variety of considerations with a view of getting as correct and efficient a picture as may be possible about the nature and activity of these antagonists.

MATERIALS AND METHODS

The pathogenic organisms comprised both Gram-positive (*Bacillus anthracis*, which causes anthrax of cattle, sheep and swine; *B. megaterium*, cause of antihemolysis in goats; *Corynebacterium diphtheriae*, causative agent of diphtheria in man; *C. equi* cause of spontaneous pneumonia in foals; *Streptococcus pneumoniae*, cause of lobar pneumonia; *S. pyogenes*, causing pus or even fatal septicemias in the human body) as well as Gram-negative bacteria (*Salomonella paratyphi*, *S. typhi*, *Escherichia coli* and *Vibrio cholerae* causing paratyphoid fever, typhoid fever, gasteroenteritis and cholera respectively in man) and three pathogenic yeasts i.e. *Candida albicans*, *Cryptococcus neoformans* and *Trichosporon cutaneum* causing moniliasis, torula menongitis and skin infections respectively in man.

Pilot tests

Before these selected antagonists were confronted with the test organisms, they were subjected to pilot tests to determine if any genetic change has taken place due to storage, to effect their activity.

Preparation of the inoculum

Subcultures of antagonists and test organisms were made by inoculating them on Czapek's solution agar (R a p e r and T h o m, 1949) and nutrient agar (D i f c o, 1953) in case of fungi and bacteria respectively. Their spore or cell suspension was prepared in 2 ml of starile water containing 1:5000 Tween 80 solution. A continuous spread of uniform density of inoculum was made with the help of 3 mm chromium 100.

Test methods

The tests were done on Emerson's agar (W a k s m a n 1961) and Wickerham's Antibiotic Test medium (R a p e r, T h o m 1949). For testing the cross-streak method was employed in which the penicillia to be screened were grown as streaks near periphery for 5 to 7 days at 28°C on both the media. These plates were then cross-streaked with pathogenic bacterial test organisms and yeasts and reincubated at 37°C. The extent of inhibition and type of organisms against which this is expressed served to give an assessment of the inhibitor, as compared with that of control plates in which the test organisms were grown alone (Table 1).

Table 1

Antimicrobial activity of Penicillia against some pathogenic organisms

Test organisms	Inhibition zone /mm./															
	P. chrysogenum				P.citrinum		P.clavi-forme		P. griseo--fulvum		P. notatum				P.urticeae	
	strain 1		strain 2								strain 1		strain 2			
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Gram-positive bacteria:																
Bacillus anthracis	28	25	27	26	14	12	30	28	24	20	30	25	28	27	26	24
B. megaterium	12	11	12	10	10	10	16	10	20	18	7	6	3	+	20	18
Corynebacterium equi	17	15	19	10	11	10	22	15	15	18	12	10	12	8	19	14
C. diphtheriae	16	14	15	14	10	10	28	26	9	10	17	15	15	15	20	16
Streptococcus pneumoniae	14	10	12	10	10	10	35	25	-	-	13	11	10	10	35	29
S. pyogenes	12	11	12	12	17	17	26	25	-	-	-	-	-	-	28	28
Gram-negative bacteria:																
Escherichia coli	-	-	-	-	-	-	30	25	-	-	16	14	18	16	24	20
Salmonella paratyphi	-	+	-	-	17	15	30	25	-	-	-	-	+	-	35	35
S. typhi	-	-	+	+	-	-	35	32	32	27	8	7	7	6	33	30
Vibrio cholerae	-	-	-	-	-	-	20	18	-	-	-	-	-	-	20	20
Yeasts:																
Candida albicans	-	+	-	-	19	15	20	16	20	19	-	-	-	+	+	+
Cryptococcus neoformans	13	10	10	8	12	12	37	16	34	30	-	+	5	5	20	15
Trichosporon cutaneum	28	26	25	25	-	-	32	32	25	22	15	15	12	10	30	26

A - Activity on Wickerham's Antibiotic test medium

B - Activity on Emerson's medium

\+ Slight inhibition of test organism

\- No inhibition at all

RESULTS

If an antibiotic was produced by the antagonist, the growth of those test organisms which were sensitive to it was inhibited in the neighbourhood of the primary streak to a distance depending on the sensitivity of the test organism and on the amount of the diffusibility of the antibiotic produced. The inhibition zone was recorded as a clear zone between antagonist and the test organism and was measured in mm. with the help of a divider or a plastic millimeter scale.

During these studies it was observed that all the tested species of *Penicillium* were antagonistic against bacteria and yeasts and the activity was higher on Wickerham's Antibiotic test medium. *P. claviforme* was one of the most active antagonists and strongly inhibited all the three groups of test organisms, followed by *P. urticeae* which was also strongly active against all the organisms except *C. albicans* which was very slightly inhibited. All the three yeasts were fairly inhibited by *P. claviforme* and *P. griseo-fulvum*. All these isolates of penicillia have already been tested against fungal human pathogens (C h a u h a n 1980). A b e (1956) has also reported the antagonistic activity of penicillia against *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, *C. albicans*, *Mycobacterium*, *Saccharomyces cerevisae* and *Aspergillus oryzae*. Since the present studies reveal that penicillia could also produce substancial amounts of inhibitory effect to the Gram-negative bacteria, it can be concluded that there is a great possibility for the synthesis of antibiotics from this group of fungi which can also be effective against Gram-negative bacteria.

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