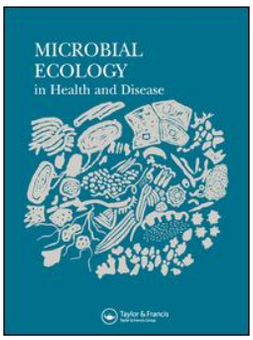

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Bacterial inhibition of fungal growth and pathogenicity

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Antifungal activity has been detected in many bacterial genera, both saprophytes and human pathogens, including *Actinomadura*, *Actinoplanes*, *Arthrobacter*, *Micromonospora*, *Streptomyces*, *Nocardia*, *Mycobacterium*, *Aureobacterium*, *Bacillus*, *Brevibacterium*, *Lactobacillus*, *Rhodococcus*, *Micrococcus*, *Streptococcus*, *Enterococcus*, *Escherichia*, *Proteus*, *Klebsiella*, *Enterobacter*, *Serratia*, *Pseudomonas*, *Burkholderia*, *Stenotrophomonas*, *Agrobacterium*, *Alcaligenes*, *Azotobacter*, *Clostridium* and *Fusobacterium*. A variety of methods have been used to detect this activity *in vitro*. Presumably, this activity confers an ecological advantage on a bacterial population which competes with other species in a particular habitat. The significance of this activity includes the following. First, development of therapeutic antifungal drugs. Second, development of plant protection agents. Third, fungal growth inhibition within the human body in sites with a normal flora with effects on the pathogenesis and course of human infection. Fourth, inhibition of pathogenic fungi in human clinical specimens, reducing the likelihood of *in vitro* culture of fungi.

INTRODUCTION

Antifungal activity is a relatively common characteristic among bacteria (Table 1), conferring an ecological advantage in environments which support the growth of a mixed bacterial and fungal flora. This activity has been detected using a variety of *in vitro* methods, and although the chemical basis for this activity has been elucidated in many cases, in some it has not, even though antifungal activity has been demonstrated. This activity has significance in four areas; development of therapeutic antifungal drugs, development of plant protection agents, suppression of fungal colonisation/proliferation within the human body resulting in modification of the pattern of certain human clinical infections, and reduction in the efficiency of isolation of fungal pathogens from clinical specimens.

A variety of mechanisms have been put forward to explain growth inhibition of one organism by another. For example, competition for a limited supply of nutrients, production of siderophores, antibiotics, enzymes, and volatile substances. It is the purpose of this review to document those bacteria, and their antifungal products, when known, and to review the significance of this activity in terms of the available literature.

BACTERIA WHICH INHIBIT FUNGAL GROWTH OR PATHOGENICITY

Actinomadura

Actinomadura spp. are aerobic actinomycetes forming waxy pigmented colonies with a branched aerial mycelium which are found in soil. This genus is one which may be associated with Actinomycetoma (Madura foot), a chronic suppurative disease of tissue and bone with multiple discharging sinuses. *Actinomadura hibisca*, isolated from soil samples in the Fiji Islands and India, produces the red pigmented pradimicins A, B and C (1). The pradimicins represent a new class of antifungal drugs undergoing development with broad-spectrum fungicidal activity against *Candida* spp., *Aspergillus* spp., and other fungi (2).

Actinoplanes

Actinoplanes spp. are motile aerobic actinomycetes, occurring widely in soil and not associated with human infection. Antifungal metabolites have been identified in various species. *Actinoplanes ianthinogenes* produces purpuromycin which has activity against *Trichophyton mentagrophytes* (3). *Actinoplanes* sp. SCC1906 produces Sch 42137, a novel polycyclic xanthone which exhibited MIC values < 0.125 μg/ml against yeasts and dermatophytes

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Table 1

Bacteria and their products which exhibit antifungal activity

Bacterial genus	Bacterial species	Antifungal molecule	Reference(s)
Actinomadura	*A. hibisca*	Pradimicins	1, 2
Actinoplanes	*A. ianthinogenes*	Purpuromycin	3
	SCC1906	Sch42137	4
	A. ianthinogenes subsp. octamycini	Octamycin	5
Arthrobacter	*A. citreus*	-	6
	A. nicotianae	-	6
	A. oxydans	-	6
	A. viscosus	-	6
Micromonospora	ATCC 53803	Spartanomycin B	7
	M. Neiheumicin	Neihumicin	8
	SCC1792	Sch 37137	9
	SF-1917	Dapiramicins A & B	10
	M. echinospora SCC1411	Hazimicin complex	11
	M. globosa	-	12
Streptomyces	*Streptomyces* spp.	Amphotericin B	13
		Nystatin	14
		Phoslactomycins	15
		UK-2A, B, C, D	16
		Kanchanamycins	17
		NP-101A	18
		Phthoxazolin A	19
		Phthoxazolins B, C, D	20
		AKD-2A, B, C, D	21
		Dorrigocins A & B	22
		Faeriefungin	23
		S-632	24
		Polyene A121	25
		Butyrolactols A & B	26
		Stendomycin	27
		Sultriecin	28
		TAN-950 complex	29
		44-homo-oligomycins A & B	30
		Dunaimycins	31
		Phosmidosine	32
		FR 109615	33
		Polyoxin	34
	S. lydicus	-	35
	S. galbus	-	36
Nocardia	*N. globerula*	-	6
Actinomycetes		PD 114, 720, PD 114, 721	37
		Pradimicins T1 & T2	38
		2-methylazalomycins	39
		Parvulomycin	40
		Polyenes AB023a, AB023b	41
Mycobacterium	*Mycobacterium* spp.	-	42
Aureobacterium	*A. barkeri*	-	6
Bacillus	*B.anthracis,cereus,subtilis*	Iturin A	43
		Bacillomycin L	44
		Bacillomycin Lc	45
		Bacillomycin D	46
		Bacillopeptins	47
		Rhizocticin A	48
		Mycosubtilin	49
		Fengycin	50
	B. cereus	Azoxybacilin	51
		Bacereutin	52
		Cispentacin	53
		Mycocerein	54
	B. licheniformis	Fungicidin M-4	55
		Peptide A12-C	56

Table 1 (Continued)

Bacterial genus	Bacterial species	Antifungal molecule	Reference(s)
	YM-03709B	YM-47522	57
	B. amyloliquefaciens	-	6
	B. chitinosporus	-	6
	B. longisporus	-	6
	B. maroccanus	-	6
	B. mycoides	-	6
	B. pabuli	-	6
	B. pasteurii	-	6
	B. polymyxa	-	6
	B. pumilis	-	6
Brevibacterium	*B. linens*	?Methanethiol	58
Lactobacillus	*L. acidophilus*	-	59, 60
	Lactobacillus spp.	-	61, 62
Rhodococcus	*R. equi*	-	6
Micrococcus	*M. luteus*	-	6, 63, 64
Streptococcus	*S. viridans group*	-	65
Enterococcus	*E. faecalis*	Peptides	65, 66
Escherichia	*E. coli*	-	65, 67–69
Proteus	*P. vulgaris*	-	63, 64
	P. mirabilis	-	69
Klebsiella	*Klebsiella* sp.	-	69
Enterobacter	*E. agglomerans*	Pyrrolnitrin	70, 71
		Herbicolins A & B	72, 73
	E. aerogenes	-	64
Serratia	*S. plymuthica*	CB-25-1	74
	S. liquefaciens	-	75
	S. rubideae	-	75
	S. marcescens	-	64
Pseudomonas	*P. aeruginosa*	Dihydroaeruginoic acid	76
		Pyocyanin, 1-hydroxyphenazine	78
	P. fluorescens	Pyrrolnitrin, chitinase, cyanide	79
	P. aureofaciens	Pyrrolnitrin, 2,4-diacetophluoroglucinol	80
	P. syringae	Pseudomycin family	82
	P. caryophylii	Caryoynencins	83
	P. alcaligenes	Cyclic hydroxamic acid, G1549	84
	P. antimicrobica	-	85
	P. putida	-	6
	P. chloroaphis	-	6
	P. marginalis	-	6
Burkholderia	*B. cepacia*	Cepacidine A	87
		Cepalycin	88
		Pyrrolnitrin	89
		Siderophores	90
		Xylocandins	91
		Heptyl-methyl-quinolinone	92
	B. pickettii	-	6
Stenotrophomonas	*S. maltophillia*	Maltophilin	97
Agrobacterium	*A. radiobacter*	-	63, 64
	A. Rhizogenes	-	63, 64
Alcaligenes	*A. faecalis*	-	69
	Alcaligenes sp. UC9152	-	100
Azotobacter	*A. Chroococcum*	-	101
Clostridium	*Clostridium sp.*	-	69
Fusobacterium	*F. varium*	-	69

(4). *Actinoplanes ianthinogenes subsp. octamycini* produces octamycin, a polyene with low level activity against fungi and yeasts (5).

Arthrobacter

Arthrobacter spp. are aerobic actinomycetes, occurring widely in the environment and not associated with human

infection. *Arthrobacter citreus*, *A. nicotianae*, *A. oxydans*, *A. viscosus* and an unidentified *Arthrobacter* sp. have been shown to possess activity against phytopathogens, *Fusarium oxysporum*, *Cylindrocarpon destructans* and *Pythium* group G (6), but the responsible factor/s have not been identified.

Micromonospora

Micromonospora spp. are aerobic actinomycetes, occurring widely in soil and not associated with human infection. Antifungal metabolites have been identified in various species. *Micromonospora* sp. ATCC 53803 produces spartanomycin B with MICs for *Candida albicans*, *Aspergillus*, *Cladosporium* and *Cryptococcus* from 1–2 μg/ml (7). *M. neiheumicin* produces neihumicin, a cytotoxic antibiotic which is active against *Saccharomyces cerevisiae* (8). *Micromonospora* sp. SCC 1792 produces Sch 37137, a dipeptide with activity against *Candida* spp. and dermatophytes (9). *Micromonospora* sp. SF-1917 produces nucleoside antibiotics dapiramicins A and B; dapiramicin B inhibits *Rhizoctonia solani* of rice plants in a green-house test (10). *M. echinospora* SCC 1411 produces the hazimicin complex, of which components 5 and 6 show *in vitro* activity against yeasts and dermatophytes (11). *M. globosa* also produces an antifungal antibiotic (12).

Streptomyces

Streptomyces spp. are aerobic Gram-positive branching bacilli, the majority of which are soil saprophytes. Although *Streptomyces* species may be isolated from clinical specimens, they are rarely significant pathogens. *S. somaliensis*, however, may be associated with actinomycetoma (Madura foot). Antifungal factors produced by *Streptomyces* spp. include Amphotericin B (13), nystatin (14), phoslactomycins (15), UK-2A, B, C, D (16), kanchanamycins (17), NP-101A (18), phthoxazolin A (19), phthoxazolins B, C, D (20), AKD-2A, B, C, D (21), dorrigocins A and B (22), faeriefungin (23), S-632 (24), polyene A121 (25), butyrolactols A and B (26), stendomycin (27), sultriecin (28), TAN-950 complex (29), 44-homo-oligomycins A and B (30), dunaimycins (31), phosmidosine (32), FR 109615 (33) and polyoxin (34). *S. lydicus* (35) and *S. galbus* (36) have been shown to possess antifungal activity although the active factors have not been identified.

Nocardia

Nocardia spp. are aerobic Gram-positive branching bacilli, the majority of which are soil saprophytes, but a few are pathogenic to man. For example, *N. asteroides* causes pulmonary nocardiosis occasionally with brain abscess in immunocompromised patients. *Nocardia globerula* has been shown to inhibit growth of the phytopathogen, *Cylindrocarpon destructans* (6), but active factor/s have not been reported. *N. globerula* is not a recognised human pathogen.

Other Actinomycetes

Actinomycetes are Gram-positive branching bacilli, most of which live in the soil. They may be aerobic, microaerophilic or anaerobic. Various antifungal antibiotics are produced by actinomycetes; anti-tumour antibiotics PD 114,720 and PD 114,721 which have antibacterial and antifungal activity (37), pradimicins T1 and T2 (38), 2-methylazalomycins with broad-spectrum antifungal activity (39), parvulomycin, a polyglycoside (40), and polyenes, AB023a and AB023b, with activity against phytopathogens (41). Antifungal activity has not been documented among the species pathogenic for humans.

Mycobacterium

Mycobacterium spp. are acid-fast bacilli and are widely distributed in nature. The various species cause tuberculosis, leprosy, lymphadenopathy, pulmonary infection, diarrhoea and skin ulcers. A report of antifungal activity from Russia refers to the genus as a whole (42).

Aureobacterium

Aureobacterium spp. are aerobic Gram-positive bacilli, widely distributed in the environment, and only rarely associated with human infection. *Aureobacterium barkeri* has been shown to inhibit growth of the phytopathogen, *Pythium* group G (6), but active factor/s have not been reported.

Bacillus

Bacillus spp. are aerobic, sporing, Gram-positive, chaining bacilli which are ubiquitous saprophytes. Species pathogenic for humans include *Bacillus anthracis*, *B. cereus* and *B. subtilis*. *B. subtilis* produces iturin A, a cyclic lipopeptide active against phytopathogens (43), lipopeptide bacillomycins L (44), Lc (45), D (46), bacillopeptins (47), rhizocticin A, a hydrophilic phosphono-oligopeptide active against *Candida* spp., *Aspergillus* spp., and phytopathogenic fungi (48), mycosubtilin, an iturin, active against *Saccharomyces cerevisiae* (49), and fengycin, a lipopeptide complex active against *Aspergillus* spp., but not *Candida* spp. (50). *B. cereus* produces azoxybacilin, an amino acid with an azoxy moiety with activity against *Aspergillus* spp. (51), bacereutin, active against *Saccharomyces*, *Rhizomucor*, *Fusarium* and *Paecilomyces* (52), cispentacin, active against *C. albicans in vitro* and *in vivo* in mice (53), and mycocerein, an iturin antifungal, which inhibits growth of *Saccharomyces cerevisiae* (54). *B. licheniformis* produces fungicidin M-4, a peptide with activity against *Microsporum canis*, *Mucor* spp. and *Sporothrix schenckii* (55) and peptide A12-C, active against *Trichophyton mentagrophytes*, *Microsporum canis*, *Mucor* spp., *Sporothrix schenckii* (56). *Bacillus* sp. YM-03709B produces YM-

47522, active against *Rhodotorula acuta* and *Pichia angusta* (57). *Bacillus amyloliquefaciens*, *B. chitinosporus*, *B. longisporus*, *B. maroccanus*, *B. mycoides*, *B. pabuli*, *B. pasteurii*, *B. polymyxa* and *B. pumilis* have been shown to possess antifungal activity against the phytopathogens, *Fusarium oxysporum*, *Cylindrocarpon destructans* and *Pythium* group G (6), but active factor/s have not been reported.

Brevibacterium

Brevibacterium spp. are aerobic Gram-positive bacilli. *Brevibacterium linens*, constitutes the predominant surface flora of aged surface-ripened cheeses and shows activity against a wide variety of moulds and yeasts (58). The active factor was thought to be methanethiol, a product of *B. linens* utilisation of methionine. Methanethiol is a major component of aged surface-ripened cheeses, and may play a contributory role in the inhibition of food spoilage fungi. Members of this genus are rarely associated with human infection.

Lactobacillus

Lactobacillus spp. are non-sporing, Gram-positive bacilli which form lactic acid from glucose. Lactobacilli are ubiquitous in nature and in humans, inhabiting the mouth, alimentary tract and vagina. Lactobacilli are a rare cause of septicaemia, endocarditis and meningitis. *L. acidophilus* inhibits growth of *C. albicans* (59, 60). In addition, vaginal (61) and oral (62) lactobacilli have been shown to inhibit yeast growth.

Rhodococcus

Rhodococcus spp. are aerobic actinomycetes which are widely distributed in soil, causing human infection by the respiratory route. *Rhodococcus equi* is a rare cause of pulmonary infection in immunosuppressed patients. An unidentified environmental *Rhodococcus* species was shown to inhibit growth of the phytopathogen, *Cylindrocarpon destructans* (6), but the active factor/s were not identified.

Micrococcus

Micrococcus spp. are often strict aerobes which may colonise human skin, but only rarely cause human infection. The yellow pigmented *Micrococcus luteus* (*Sarcina lutea*) has been shown to inhibit growth of *Fusarium oxysporum* (6, 63, 64), *Pythium* group G (6), *Gelasinospora cerealis* (63, 64), *Penicillium viridicatum* (63, 64), *Trichoderma viride* (63, 64) and *Zygorhynchus vuilleminii* (63, 64), although active factors were not described.

Streptococcus

Streptococcus spp. are Gram-positive cocci which are ubiquitous among humans and animals. Members of the *Streptococcus viridans* group, commensals of the mouth, were shown to inhibit yeast-mycelial transformation in *C. albicans* (65).

Enterococcus

Enterococcus spp. are aerobic Gram-positive cocci. *Enterococcus faecalis*, a ubiquitous member of the normal human gut flora, was shown to inhibit yeast-mycelial transformation in *C. albicans* (65). This inhibition was later shown to be due to production of various peptides, the activity of which was abolished by heating to 100°C for 10 min (66).

Escherichia

Escherichia spp. are aerobic and facultatively anaerobic, Gram-negative bacilli which inhabit the human and animal intestine. *Escherichia coli* causes urinary tract infection, wound infection, abscesses and septicaemia. *E. coli* has been shown to inhibit growth of (67–69) and yeast-mycelial transformation in *C. albicans* (65), however, active factors were not identified.

Proteus

Proteus spp. are aerobic and facultatively anaerobic, motile Gram-negative bacilli which inhabit the human and animal intestine. *Proteus* species may cause a variety of human infections, including urinary tract infection and septicaemia. *P. vulgaris* inhibits growth and sporulation of phytopathogens, *Fusarium oxysporum*, *Gelasinospora cerealis*, *Penicillium viridicatum*, *Trichoderma viride* and *Zygorhynchus vuilleminii* (63, 64). *P. mirabilis* (69) and unidentified *Proteus* spp. (69) inhibit growth of *C. albicans*, although active factors were not identified.

Klebsiella

Klebsiella spp. are aerobic and facultatively anaerobic, motile Gram-negative bacilli which inhabit the human and animal intestine. *Klebsiella* species may cause a variety of human infections, including urinary tract infection, respiratory infection and septicaemia. One report documents inhibition of growth of *C. albicans* in an unidentified *Klebsiella* sp. (69).

Enterobacter

Enterobacter spp. are aerobic and facultatively anaerobic, motile Gram-negative bacilli which inhabit the human and animal intestine. *Enterobacter* species may cause a variety of human infections, including urinary tract infection, wound infection and septicaemia. *Enterobacter agglomerans* produces pyrrolnitrin (70) which is active against *C. albicans*, *Aspergillus niger*, dermatophytes (71) and phytopathogenic fungi (70); herbicolins A and B, acylated peptides which are active against yeasts and filamentous fungi (72, 73). *Enterobacter aerogenes* inhibits growth and sporulation of phytopathogens, *Fusarium oxysporum*, *Gelasinospora cerealis*, *Penicillium viridicatum*, *Trichoderma viride* and *Zygorhynchus vuilleminii* (64). *E. agglomerans* and *E. aerogenes* are both human pathogens.

Serratia

Serratia spp. are aerobic and facultatively anaerobic, motile Gram-negative bacilli which inhabit the human and animal intestine. *Serratia* species may cause a variety of human infections, including urinary tract infection, wound infection, pulmonary infection and septicaemia. *Serratia plymuthica* produces CB-25-1, a water soluble dipeptide which inhibits growth of *C. albicans* (74). *S. liquefaciens* and *S. rubidaea* were shown to inhibit growth of phytopathogenic fungi (75). *S. marcescens* inhibits growth and sporulation of phytopathogens, *Fusarium oxysporum*, *Gelasinospora cerealis*, *Penicillium viridicatum*, *Trichoderma viride* and *Zygorhynchus vuilleminii* (64). *S. plymuthica*, *S. liquefaciens*, *S. rubidaea* and *S. marcescens* are all known to be pathogenic for humans.

Pseudomonas

Pseudomonas spp. are aerobic motile Gram-negative bacilli, widely distributed in water, soil and sewage. The genus includes pathogens for humans, animals and plants. The most significant human pathogen is *P. aeruginosa* which may be carried in the gut of normal persons and causes infection at a variety of sites including pulmonary infection in patients with cystic fibrosis and bronchiectasis. Many pseudomonads inhibit fungal growth. *P. aeruginosa* produces three antifungal factors; dihydroaeruginoic acid (76), which inhibits phytopathogenic fungi (77), and pyocyanin and 1-hydroxyphenazine which inhibit growth of various *Candida* spp. and *Aspergillus fumigatus* (78); pyocyanin may also inhibit yeast-mycelial transformation in *C. albicans* (78). *P. fluorescens* produces pyrrolnitrin, chitinase and cyanide (79) and dihydroaeruginoic acid (77), which all inhibit growth of phytopathogenic fungi. *P. aureofaciens* produces pyrrolnitrin (80) and 2, 4-diacetophluoroglucinol, which inhibits growth of phytopathogens, *Gaeumannomyces graminis var tritici*, *Pythium ultimum* and *Rhizoctonia solani* (81). *P. syringae*, a plant pathogen, produces the peptide pseudomycin family, which inhibit *C. albicans*, *Cryptococcus neoformans*, and phytopathogens including *R. solani* and *F. oxysporum* (82). *P. caryophylli*, a plant pathogen, produces caryoynencins which inhibit fungal growth (83). *P. alcaligenes* produces the cyclic hydroxamic acid, G1549, which has marked activity against *Microsporum canis* (84). *P. antimicrobica* inhibits the phytopathogen, *Botrytis cinerea* (85). *P. putida*, *P. chloroaphis* and *P. marginalis* inhibit growth of phytopathogens, *Fusarium oxysporum*, *Cylindrocarpon destructans* and *Pythium* group G (6).

Burkholderia

Burkholderia spp. are ubiquitous Gram-negative bacilli which are oxidase-positive, causing infection in immunocompromised persons, including CF patients (86). *B. cepacia* produces cepacidine A, a cyclic peptide which inhibits growth of various animal and plant fungi (87); cepalycin (88), a haemolytic and antifungal substance; pyrrolnitrin, which inhibits growth of *C. albicans*, *Aspergillus niger* and phytopathogenic fungi (89); siderophores, red and yellow pigments which inhibit growth of phytopathogenic fungi (90); xylocandins, a family of peptides which are active against *C. albicans* and dermatophytes (91); and heptylmethyl-quinolinone, active against *Trichophyton mentagrophytes*, *Phytophthora capsici*, the cause of red pepper blight, and other phytopathogens, *P. ultimum*, *R. solani*, *F. oxysporum* (92). *B. cepacia* also inhibits growth of 8 *Candida* spp. in addition to *C. albicans*, *S. cerevisiae* and *Aspergillus fumigatus* (93). *B. pickettii* inhibits growth of *P. ultimum* (6).

Stenotrophomonas

Stenotrophomonas spp. are ubiquitous Gram-negative, oxidase-negative bacilli. *S. maltophilia* has been isolated from water, milk, frozen fish, raw sewage, and rabbit and human faeces, and causes hospital-acquired infection with increasing frequency especially in immunocompromised patients (86). *S. maltophilia* may also colonise the respiratory tract of CF patients (94, 95), however, this significance of this remains unknown. Clinical strains of *S. maltophilia* were shown to inhibit growth of a variety of *Candida* spp., *S. cerevisiae* and Aspergillus fumigatus (96). Rhizosphere strains of *S. maltophilia* were shown to produce maltophilin, a macrocyclic lactam which inhibits growth of fungi pathogenic for humans and plants (97).

Agrobacterium

Agrobacterium spp. are nonfermentative Gram-negative bacilli which oxidise glucose, and are oxidase-positive. *A. radiobacter* is the only antifungal member to be associated, albeit rarely, with human infections; these infections typically occur in immunocompromised patients with intravenous lines or peritoneal dialysis catheters (98). *A. radiobacter* and *A. rhizogenes* have been shown to inhibit growth of phytopathogens, *Fusarium oxysporum*, *Gelasinospora cerealis*, *Penicillium viridicatum*, *Trichoderma viride* and *Zygorhynchus vuilleminii* (63, 64), although active factors were not described.

Alcaligenes

Alcaligenes spp. are aerobic motile oxidase-positive Gram-negative bacilli which are widely distributed in nature. This genus is rarely cultured from clinical specimens, but is known to cause opportunistic infection in immunocompromised persons including cystic fibrosis patients (99). *A. faecalis* inhibits growth of *C. albicans* (69) and *Alcaligenes* sp. UC9152 inhibits growth of a broad variety of pathogenic fungi (100).

Azotobacter

Azotobacter spp. are aerobic, nitrogen-fixing, Gram-negative bacilli occurring widely in the environment and not

associated with human infection. *A. chroococcum* produces an antifungal antibiotic which is active against phytopathogenic fungi (101).

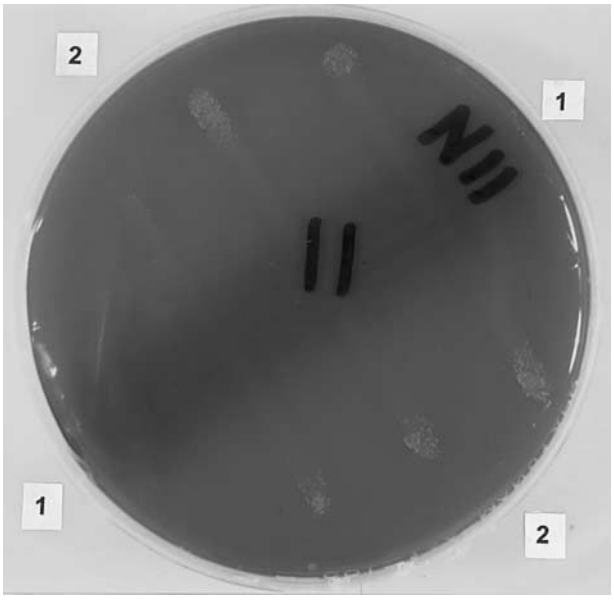

Fig. 1. Cross-streak assay on a blood agar plate. An antifungal strain of *Pseudomonas aeruginosa* was seeded in a band across the plate (Axis 1) and incubated for 18–24 h aerobically at 37°C. This growth was removed and the microscopic remnants killed with chloroform vapour. The indicator strain of *Candida albicans* was then inoculated in triplicate at 90° to the bacterial inoculum and incubated for 18–24 h aerobically at 37°C. The figure shows complete inhibition of the indicator yeast over and beyond the line of original inoculum of *Pseudomonas aeruginosa* (Reference (78)).

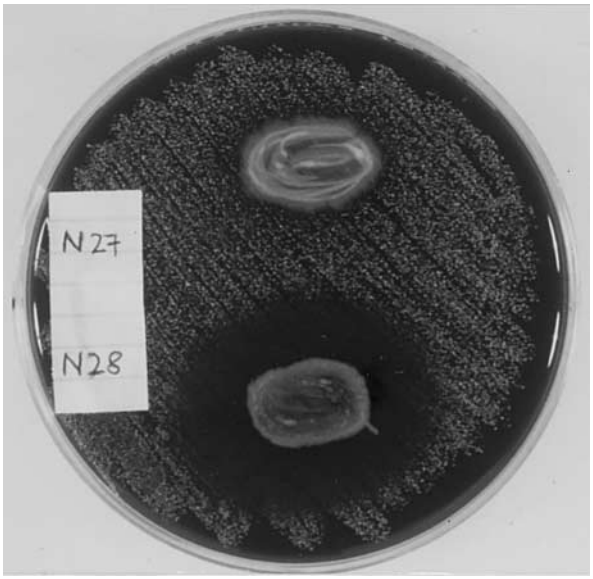

Fig. 2. Dual culture plate assay. The blood agar plate was surface-seeded with *Candida albicans*, spot inoculated with 2 strains of *Pseudomonas aeruginosa*, and incubated for 24 h aerobically at 37°C. Inhibition of yeast growth by *P. aeruginosa* strains N27 and N28 is minimal and marked, respectively.

Clostridium

Clostridium spp. are anaerobic, sporing, Gram-positive bacilli, which inhabit the soil, water and human and animal intestine. One report describes the growth inhibition of *C. albicans* by an unidentified *Clostridium* sp. (69).

Fusobacterium

Fusobacterium spp. are anaerobic Gram-negative bacilli. *F. varium* (formerly *Sphaerophorus varius*, *Bacteroides varius*), a member of the normal flora of the mouth, inhibits growth of *C. albicans* (69).

METHODS FOR DETECTION OF FUNGAL GROWTH INHIBITION

Cross-Streak Assay

The potential producer bacterial strain is streaked diametrically across an agar plate in such a way that the width of the inoculum is approximately 2 cm. Plates are then incubated for 24 h at an appropriate temperature, after which macroscopic growth is removed with a glass slide, and microscopic remnants killed with chloroform vapour. The indicator fungal strain is then streaked on to the medium at right angles to the line of the original inoculum and plates incubated for a further 24 h. Antifungal activity may be observed over the line of the original bacterial inoculum (Figure 1) (78).

Agar Block Assay

An agar plate seeded with the potential producer strain is incubated, after which the macroscopic growth is removed with a glass slide and microscopic remnants killed with chloroform vapour. A block of this agar is then set onto a fresh agar plate which has been seeded with the fungal indicator strain. Antifungal activity may be observed as a zone of inhibition around the agar block.

Dual Culture Plate Assays

The essence of this method is that both the potential producer bacterium and the indicator fungus are cultured simultaneously. There are a number of variations. First, suspensions of potential producer bacterium and indicator fungus are made and an agar plate is seeded with a drop of each, so that the drops mix. This is then examined after incubation for fungal growth inhibition (102). Second, a concentrated suspension of the potential producer bacterium is spot-inoculated onto the centre of an agar plate, while the indicator fungus is radially streaked from the margin of the plate into the centre of the bacterial inoculum (58). Third, molten agar (45°C) is inoculated with a concentrated suspention of the potential producer bacterium to give a final concentration of, for example, 1×10^6 cells per ml. The seeded agar is then poured and after solidification, the indicator fungus is streaked across the

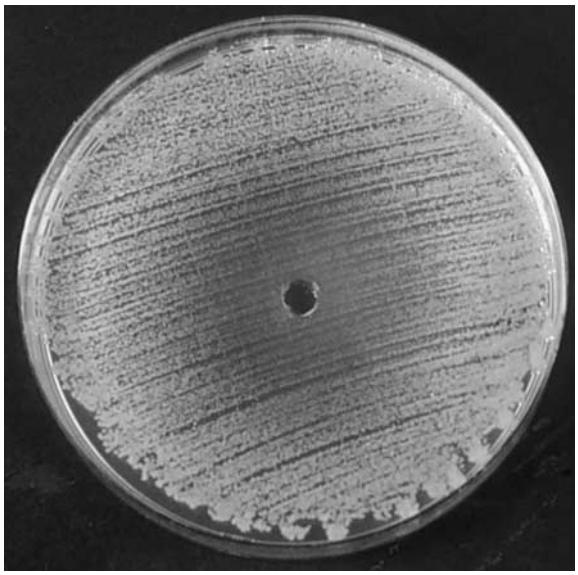

Fig. 3. Well-plate assay showing the activity of pyocyanin, 37.5 μg placed in the well, against the indicator strain of *Candida albicans* (Reference (78)).

agar surface after appropriate bacterial incubation (58). Fourth, an agar plate is seeded with the indicator fungus, and then the potential producer bacterium is spot-inoculated onto an area of the plate (Figure 2).

Dual Culture Broth Assay

Estimated concentrations of both potential producer and indicator organisms are prepared separately in broth and viable counts performed. These are then mixed in broth and incubated for 24 h, after which viable counts of both organisms are again performed. Antifungal activity is assessed on the basis of the 24 h fungal count in the mixed culture, compared with that in the fungal control culture (103, 104).

Agar Overlay Assay

Agar plates are inoculated with a suspension of the potential producer bacterial strains; this may conveniently be performed using a multipoint inoculator. The plate is then incubated for a variable period depending on the rate of growth of the potential producer bacterium, for example, 6–12 h for *P. aeruginosa*. Bacteria are then killed with chloroform vapour. The fungal indicator strain is then applied as a suspension incorporated in molten semi-solid agar, allowed to set, and incubated for a further 24 h. Bacterial antifungal activity may then be seen as circular zones of fungal inhibition (105).

Well-Plate Assay

A 7 mm well is cut in an agar plate which has been surface-seeded with the indicator fungal strain. An extract or product of the potential producer bacterium is then added to the well and the plate incubated for 24 h. Inhibition of the indicator fungal strain may be observed as a zone of inhibition around the well (Figure 3) (78).

Paper Disc Assay

Filter paper discs are loaded with an extract, product or culture supernatant of the potential producer bacterium and placed onto agar plates previously surface-seeded with the indicator fungus. After incubation, inhibition of fungal growth is seen as a zone of inhibition around the paper disc (58).

Agar Disc-Broth Method

The indicator organism is incorporated in an agar disc which is incubated in broth inoculated with the potential producer bacterial strain. After incubation, the agar disc is examined by light microscopy and an assessment of growth inhibition made in comparison with a control disc (106). Using subculture of the fungus, this method may also be used to assess whether a particular antifungal activity is bacteriostatic or bactericidal.

Dialysis Tube Assembly

This apparatus consists of one or more dialysis tubes, each sealed at its lower end and filled with culture broth, placed inside a large glass test tube which is also filled with culture broth. The system enables the culture of 2 or more separate fluid compartments, each inoculated with a micro-organism, such that molecules below a certain size can pass freely between the compartments. Therefore, although the microbes do not mix, they compete for nutrients and their metabolites can effect the growth of other organisms within the system. Viable counts are performed at fixed time intervals and comparison of these with control counts enables an assessment of the growth inhibition of one organism by another (69).

It is important to remember that all *in vitro* tests of microbial interaction inevitably simplify the environmental conditions that are relevant to a particular ecological niche. Fungal growth inhibition detected by the above tests may be due to one or more of a variety of mechanisms; competition for a limited supply of nutrients, production of siderophores, antibiotics, enzymes, and volatile substances. Therefore, these methods provide only circumstantial evidence of *in vivo* activity.

SIGNIFICANCE OF BACTERIAL ANTIFUNGAL ACTIVITY

Development of Therapeutic Antifungal Drugs

In general, three methods have been employed in the development of new antimicrobial drugs. First, the discovery that a particular micro-organism inhibits the growth of fungi with subsequent purification of the active factor. Screening of soil-derived actinomycetes led to the discovery of amphotericin B, a product of *Streptomyces nodosus*

(13). Similarly, nystatin produced by *Streptomyces noursei* (14) and pyrrolnitrin produced by *Pseudomonas pyrrocinia* (107). Second, modification of the chemical composition of an active natural microbial product to produce a semi-synthetic drug with an altered spectrum of activity. For example, the polyoxins and their semisynthetic derivatives are analogues of UDP-N-acetyl-D-glucosamine which is produced by *Streptomyces cacaoi var asoensis* (34); these compounds inhibit chitin synthase activity in mixed membrane preparations of *C. albicans* (108). Third, screening of synthetic compounds for antimicrobial activity. This approach led to the discovery of clotrimazole, an imidazole (109). As therapeutic options for antifungal chemotherapy are rather limited, worldwide interest in fungal growth inhibition by bacteria has been maintained with the goal of antifungal drug development.

Development of Plant Protection Agents

Certain fungi inhabiting the rhizosphere are pathogenic for plants causing reduced crop yields (110). Pesticides were once used extensively to reduce crop diseases. However, due to concerns regarding pesticide residues in foods and the environment, pathogen resistance, increasing requirements for toxicological safety and therefore increasing costs involved in pesticide development, the scope of pesticide-based control has diminished (111). One particular approach for control of these plant diseases has been to inoculate (or "bacterise") the seeds with particular strains of bacteria which produce factor/s inhibitory to the particular pathogenic fungi. These bacteria then proliferate on the plant root systems and, hopefully, prevent fungal disease. Such "biological control" has been claimed to be a realistic alternative to the problems of chemical pesticides. In certain cases this has been associated with improved plant growth and crop yields. For example, *Rhizoctonia solani*-induced damping-off of cotton was shown to be controlled by pyrrolnitrin produced by a soil isolate of *Pseudomonas fluorescens* (112). Take-all disease of wheat is caused by *Gaeumannomyces graminis var tritici*, and has been controlled by phenazine-1-carboxylic acid produced by a strain of *P. fluorescens* (113); this strain is also active against *R. solani* and *Pythium ultimum*, causes of damping-off of cotton (114). Sunflower wilt is caused by *Sclerotinia sclerotiorum*; sunflower emergence is increased in the presence of *S. sclerotiorum* by *B. cepacia* which produces pyrrolnitrin (115). Crown rot of groundnut is caused by *Aspergillus niger*, which may be controlled using an antifungal strain of *Bacillus subtilis* (116).

Fungal Growth Inhibition within the Human Body

Although various bacteria have been shown to inhibit fungal growth or pathogenicity *in vitro*, this does not necessarily mean that they will also do so *in vivo*. It is generally accepted that bacteria growing *in vivo* and *in vitro* have very different metabolic states, with gene expression tailored to the particular environment. However, from the available evidence it would seem that certain bacteria do indeed inhibit fungal growth *in vivo* within a mixed culture, and this evidence is presented below.

The Mouth

In mice and rats, *C. albicans* more easily colonises germ-free animals (117–120), and the oral anaerobic flora of mice was shown to inhibit growth of *C. albicans in vitro* (121). *C. albicans* commonly colonises the human mouth (122) and members of the *Streptococcus viridans* group were shown to inhibit yeast-mycelial transformation in *C. albicans in vitro* (65) and yeast growth *in vivo* in mice (118). In addition, oral lactobacilli have been shown to inhibit yeast growth (62). Antibiotic therapy increases the incidence and severity of oral candidosis, possibly by reducing fungal inhibition mediated by the oral bacterial flora.

The Digestive Tract

C. albicans is a ubiquitous member of the normal gastrointestinal flora of humans and systemic candidiasis is thought to develop as a result of haematogenous spread from the gut. Factors which predispose to candidiasis are those associated, either directly or indirectly, with alteration of bowel flora; namely, malnutrition, debility, chronic disease, and prolonged use of broad-spectrum antibiotics (122). Bacteria which may be carried in the gut and which have been reported to possess antifungal properties are Actinomycetes, *Mycobacterium* spp., lactobacilli, enterococci, *Escherichia coli*, *Proteus* spp., *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Salmonella* spp., fluorescent pseudomonads including *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Alcaligenes* spp., *Clostridium* spp. and *Fusobacterium* spp. (references listed previously). Therefore, within the gut and in the absence of fungal overgrowth, a combination of these antifungal organisms may be important in fungal growth suppression. However, in the event of a reduction in the number of antifungal organisms, for example due to antibiotic therapy, fungal growth inhibition may decrease, possibly allowing fungal proliferation and increasing the likelihood of fungal infection.

The Lung

Opportunistic pulmonary infection with fungi such as *C. albicans* is predisposed to by chronic pulmonary disease, prolonged use of broad-spectrum antibiotics, malnutrition and debility (122). However, despite having all of these risk factors, pulmonary candidiasis is not a recognised phenomenon in cystic fibrosis patients (123, 124), despite the presence of *C. albicans* as part of the normal flora of cystic fibrosis patients (124). Most cystic fibrosis patients become chronically colonised by *P. aeruginosa* (123). In one study of cystic fibrosis patients harbouring *P. aeruginosa*, only 10% had positive *C. albicans* skin tests, com-

pared with 30% positivity in those free of *P. aeruginosa* (124), suggesting an *in vivo* interaction between *P. aeruginosa* and *C. albicans*.

P. aeruginosa secretes pyocyanin and 1-hydroxyphenazine *in vivo* (125). The MICs of pyocyanin for both *C. albicans* and *A. fumigatus* are >64 μg/ml (78), which is over twice the maximum concentration of pyocyanin found in airway secretions of patients colonised with *P. aeruginosa* (125). The MICs of 1-hydroxyphenazine for *C. albicans* and *A. fumigatus* are 25 μg/ml and 50 μg/ml respectively (78), concentrations 5–10 fold those in colonised lung. Pyocyanin may also inhibit *C. albicans* hypha formation, known to be associated with yeast adherence to and invasion of various human tissues (78). These results may explain the relative rarity of respiratory colonisation by *C. albicans* when compared with that of *A. fumigatus* in cystic fibrosis patients (78). Cystic fibrosis patients may also acquire *B. cepacia* and, less frequently, *S. maltophilia* (94, 95), *A. faecalis* (99), *Proteus* spp. (94), *E. coli* (126), *Enterobacter* spp. (126) and *Serratia* spp. (94), all of which exhibit antifungal activity (references listed previously).

In another study, three surgery patients were followed post-op with particular reference to lung infection. The same pattern was observed in each patient. Initially, each received ventilation in an intensive care unit and broad spectrum antibiotics, at which stage *C. albicans* alone was isolated from sputum. Then, when sputum colonisation with *P. aeruginosa* occurred, *C. albicans* could not be isolated from sputum. However, when antipseudomonal therapy eradicated the *P. aeruginosa*, the yeast was re-cultured. For each of the 3 patients, the *P. aeruginosa* strain isolated completely inhibited growth of the corresponding *C. albicans* strain in an *in vitro* cross-streak assay (127).

The Vagina

The normal vaginal flora consists of lactobacilli (predominantly *L. acidophilus*), *Bacteroides* spp., *Enterococcus faecalis*, corynebacteria, *Gardnerella vaginalis* and yeasts. Factors thought to favour development of vaginal candidiasis include pregnancy, diabetes, antibiotic therapy and changes in vaginal pH (128). Alteration in vaginal flora may be important in the pathogenesis of vaginosis in general (128). Lactobacilli (59–62) and enterococci (66) have been shown to inhibit the growth of fungi and reduction in their numbers may allow yeasts to proliferate.

The Ear

The rare condition, fungal osteomyelitis of the temporal bone may be caused by *Cryptococcus* spp., *Candida* spp., *Blastomyces* spp., *Mucor* spp. or *Aspergillus* spp. (129). In this review of 9 cases, almost all patients had received broad-spectrum antibiotics for prolonged periods for presumed malignant external otitis which is caused by *P. aeruginosa*, and almost half the patients had a previous history of chronic suppurative otitis media, a condition from which both fungi and various antifungal organisms have been isolated; *P. aeruginosa*, *Alcaligenes* spp., *Proteus* spp., Mycobacteria, and *Bacteroides* spp. (130, 131). As antibiotics are frequently used to treat chronic suppurative otitis media, eradication of antifungal organisms from the external and middle ear may play a role in the pathogenesis of fungal osteomyelitis of the temporal bone (132).

Growth Inhibition of Pathogenic Fungi From Clinical Specimens

Two studies have shown that in blood culture medium inoculated with *P. aeruginosa* and one of a variety of yeast species (*C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *Cryptococcus neoformans*), *P. aeruginosa* consistently inhibited yeast growth. The degree of inhibition depended on yeast genus, bacterial inoculum, and method of subculture (133, 134). In a rabbit model of concomitant fungaemia with *C. albicans* and bacteraemia with *P. aeruginosa*, no yeasts were recovered from blood cultures despite 100% detection of *P. aeruginosa* (134). Such fungal growth inhibition may preclude yeast recovery from blood cultures in mixed infections consisting of *P. aeruginosa* and yeasts.

Conclusion

Fungal growth inhibition is common among bacteria, including those pathogenic for man. This activity may be detected *in vitro* using a variety of methods. Bacterial antifungal activity has implications for bacterial survival in the environment, development of therapeutic antifungal antibiotics, development of antifungal plant protection agents, the pathogenesis of human fungal infection, and culture of fungi from polymicrobial human infections.

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