SHORT ARTICLE

Inhibition of growth of fungi pathogenic to man by *Stenotrophomonas maltophilia*

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Ten strains of *Stenotrophomonas maltophilia* were tested by a cross-streaking method for in-vitro inhibition of 10 strains of fungi known to infect man; these were *Candida krusei*, *C. keyfr*, *C. guilliermondii*, *C. tropicalis*, *C. lusitaniae*, *C. parapsilosis*, *C. pseudotropicalis*, *C. albicans*, *C. glabrata* and *Aspergillus fumigatus*. Significant inhibition of fungal growth was observed with all 10 strains of *S. maltophilia*, but not with *Escherichia coli* NCTC 10418, *Staphylococcus aureus* NCTC 6571 and *Haemophilus influenzae* NCTC 11931.

Introduction

Originally named *Pseudomonas maltophilia*, then *Xanthomonas maltophilia*, *Stenotrophomonas maltophilia* is a free-living, ubiquitous bacterium that has been isolated from well- and river-water, raw milk, frozen fish, raw sewage, soil, rabbit and human faeces and the hospital environment [1]. *S. maltophilia* is a cause of bacteraemia, ecthyma gangrenosum, endocarditis associated with open heart surgery and intravenous drug abuse, wound infections following trauma or surgery, urinary tract infection, pneumonia, meningitis, epididymitis, eye infection and mastoiditis [1]. Both its isolation rate and clinical impact have increased in recent years, probably reflecting the increasing number of debilitated patients and use of broad-spectrum antibiotics in hospitals. In one study, 97% of *S. maltophilia* isolates were hospital-acquired, and 92% of colonised or infected patients had received prior antibiotic therapy [2].

The present study was prompted by the recent demonstration of antifungal activity in clinical isolates of *Pseudomonas aeruginosa* [3, 4] and *Burkholderia cepacia* [4], and the rarity of yeast infections in cystic fibrosis patients colonised by *P. aeruginosa*, *B. cepacia* and *S. maltophilia* and treated with prolonged courses of broad-spectrum antibiotics.

Materials and methods

Bacterial cultures

Ten *S. maltophilia* isolates were collected from routine cultures of blood and sputum. Their identity was confirmed by Gram's stain, oxidase production and the API20NE system (bioMérieux, La Balme les Grottes, France); each isolate used in the study had a unique API20NE profile. The isolates were stored at room temperature on nutrient agar slopes (Technical Service Consultants, UK) until the time of the study, when they were subcultured to blood agar plates and checked for purity.

Fungal cultures

Yeast isolates used as indicators were obtained from the Mycology Reference Laboratory, Department of Microbiology and Immunobiology, Royal Victoria Hospital, Belfast. These were *Candida krusei*, *C. keyfr*, *C. guilliermondii*, *C. tropicalis*, *C. lusitaniae*, *C. parapsilosis*, *C. pseudotropicalis*, *C. albicans* and *C. glabrata*. One strain of *Aspergillus fumigatus*, cultured from sputum and not associated with clinical infection, was also used; this was identified on the basis of macroscopic and microscopic morphology. All fungal strains were stored at 4°C on Sabouraud's dextrose agar plates until study, when they were subcultured to fresh identical medium and checked for purity.

Testing for antifungal activity

The method used was similar to that for pyocin typing of *P. aeruginosa* [5]. The 10 cultures of *S. maltophilia* and three control organisms, *Escherichia coli* NCTC 10418, *Staphylococcus aureus* NCTC 6571 and *Haem-*

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ophillus influenzae NCTC 11931, were each tested against each of the 10 fungal indicator strains. A 24-h culture of each bacterial strain was used to prepare a suspension of 10^8 cfu/ml in normal saline. For the *S. maltophilia* strains 30 μ l of this inoculum was transferred to plates of Sabouraud's dextrose and blood agar; for *E. coli* and *S. aureus*, 30 μ l of inoculum was transferred to plates of nutrient and blood agar; for *H. influenzae*, 50 μ l of inoculum was transferred to chocolate agar. The inoculum was spread across the diameter of each agar plate with a sterile swab to a width of c. 1 cm. Sabouraud's agar was used in addition to blood agar for *S. maltophilia*, as it enhanced fungal growth, and all the cultures of *S. maltophilia* grew well on it. The cultures of *S. maltophilia*, *E. coli* and *S. aureus* were incubated at 37°C in air for 24 h, whereas plates of *H. influenzae* were incubated at 37°C in air with CO₂ 10% for 24 h. Subsequently, macroscopic growth was removed with a glass slide and the plates were then inverted over 5-cm diameter filter paper disks soaked in chloroform and laid on a metal tray in a safety cabinet. After 30 min the plates were removed from the cabinet and traces of chloroform eliminated by exposure to air for a few minutes.

For the *Candida* spp., fresh 24-h plate cultures were used to prepare suspensions of c. 10^6 cells/ml, with a counting chamber to enumerate the yeast cells. For the strain of *A. fumigatus*, a 3–5-day plate culture was used to prepare a suspension of c. 10^6 conidiospores/ml with a counting chamber to count the conidia. Each fungal suspension was streaked on to the bacterial culture plates at a right angle to the original inoculum. The plates were then incubated for 24 h at 30°C, after which the results were read.

Results

Table 1 shows the antifungal activity demonstrated by the panel of 10 *S. maltophilia* isolates and the three control strains (*E. coli* NCTC 10418, *S. aureus* NCTC 6571 and *H. influenzae* NCTC 11931) against each of the 10 fungal strains. Each of the *S. maltophilia*

isolates reduced or inhibited growth of some or all of the fungal species (Table 1). Of the fungi, *C. guilliermondii*, *C. lusitaniae*, *C. pseudotropicalis* and *C. albicans* were the most sensitive to inhibition by *S. maltophilia*; *A. fumigatus* was the least sensitive. The control organisms showed no inhibition of fungal growth.

Discussion

To my knowledge, this is the first report of antifungal activity by *S. maltophilia*. Previously, it has been reported that human chorionic gonadotropin (CG)-like proteins from *S. maltophilia* increase the rate of transition from blastospores to hyphal forms in *C. albicans*, potentially affecting fungal pathogenicity [6] but these experiments were performed with purified proteins, not with whole-cell extracts or living organisms.

Antifungal activity has been observed for *P. fluorescens* [7], *P. multivorans* [7], *P. chloraphis* [7], *P. aureofaciens* [8], *P. pyrrocinia* [9], *P. aeruginosa* [3, 4] and *B. cepacia* [4]. This activity is due, at least in part, to production of pyrrolnitrin (3-chloro-4-(3-chloro-2-nitrophenyl) pyrrole) in *P. fluorescens* [7], *P. multivorans* [7], *P. pyrrocinia* [9], *P. chloraphis* [7], *P. aureofaciens* [7, 8] and *B. cepacia* [7]. Pyrrolnitrin is harvested from *P. pyrrocinia* and used in Europe and Japan for the topical therapy of dermatophyte infections. It has been shown to possess activity against a wide variety of fungi, including *Saccharomyces cerevisiae*, *Penicillium* spp., *C. albicans*, *Trichophyton* spp., *A. niger*, dermatophytes, *Cryptococcus neoformans*, *Blastomyces dermatitidis*, *Sporotrichium schenckii* and *Histoplasma capsulatum* [3]. Pyrrolnitrin must, therefore, be a candidate for the antifungal activity of *S. maltophilia*.

Production of antifungals by pseudomonas may, potentially, be significant at several levels. *C. albicans* [10], *P. aeruginosa* [11] and *S. maltophilia* [1] may be carried in human faeces. As most human candida infection is endogenous, eradication of co-existent

Table 1. Antifungal activity of 10 *S. maltophilia* isolates and control organisms

Fungal strains	Inhibition by <i>S. maltophilia</i> isolates										Inhibition by		
	1	2	3	4	5	6	7	8	9	10	<i>E. coli</i> NCTC 10418	<i>S. aureus</i> NCTC 6571	<i>H. influenzae</i> NCTC 11931
<i>C. krusei</i>	—	±	+	±	+	+	+	+	—	±	—	—	—
<i>C. keyfr</i>	+	+	+	+	±	±	+	±	±	+	—	—	—
<i>C. guilliermondii</i>	+	±	+	+	+	+	+	+	+	+	—	—	—
<i>C. tropicalis</i>	±	±	±	±	+	+	—	+	±	+	—	—	—
<i>C. lusitaniae</i>	+	+	+	+	+	+	±	+	±	+	—	—	—
<i>C. parapsilosis</i>	+	+	+	±	+	+	+	±	±	+	—	—	—
<i>C. pseudotropicalis</i>	+	+	+	+	+	±	±	+	+	+	—	—	—
<i>C. albicans</i>	+	+	±	+	+	+	+	+	±	+	—	—	—
<i>C. glabrata</i>	±	±	±	+	+	—	—	+	±	+	—	—	—
<i>A. fumigatus</i>	±	±	—	±	—	—	—	±	—	—	—	—	—

+, complete inhibition of growth; ±, partial inhibition; —, no inhibition.

antifungal *P. aeruginosa* and *S. maltophilia* strains with antibiotics may predispose to fungal proliferation. CF patients are prone to recurrent and chronic pulmonary infections with *P. aeruginosa* [12] and *B. cepacia* [12], and may be colonised infrequently by *S. maltophilia* [1]; the antifungal activity of these organisms may account for the infrequency of candidiasis in CF patients despite their extensive exposure to broad-spectrum antibiotics. *A. fumigatus* would seem to be the most important fungus causing disease in CF patients, and, therefore, it is noteworthy that this was the most resistant of the fungi tested to inhibition by *S. maltophilia* (Table 1), *P. aeruginosa* [3, 4] and *B. cepacia* [4]. In one study, minimum inhibitory concentrations (MICs) of pyrrolnitrin for *C. albicans* and *A. fumigatus* were 3.12 mg/L and 25 mg/L, respectively [13]. As topical application of pyrrolnitrin frequently causes contact dermatitis [14], its production in the CF lung may contribute to airway inflammation, the pathogenesis of which may suggest additional therapeutic interventions.

Nevertheless, the hypothesis that organisms which display antifungal activity *in vitro* suppress fungal growth *in vivo* remains speculative; further work is being done to clarify the significance of these observations.

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