Suppression of Fungal Growth Exhibited by *Pseudomonas aeruginosa*

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Three surgery patients were monitored postoperatively, with particular reference to lung infection. In each case there was a clinical impression that *Pseudomonas aeruginosa* suppressed the growth of *Candida albicans* in patients with clinically significant lung infections from whom both of these organisms were isolated from serial sputum samples. Regrowth of *C. albicans* after *P. aeruginosa* eradication occurred in two patients, despite fluconazole therapy, to which both *C. albicans* isolates were susceptible. In all three patients, the strain of *P. aeruginosa* was found to inhibit the growth of the corresponding *C. albicans* strain in vitro. Further in vitro susceptibility studies revealed significant inhibition by 10 strains of *P. aeruginosa* of 11 strains of fungi known to infect humans; these were *Candida krusei*, *Candida keyfr*, *Candida guillermondii*, *Candida tropicalis*, *Candida lusitaniae*, *Candida parapsilosis*, *Candida pseudotropicalis*, *Candida albicans*, *Torulopsis glabrata*, *Saccharomyces cerevisiae*, and *Aspergillus fumigatus*.

Pseudomonads represent the major group of nondifferentiating microorganisms that produce antibiotics (13). *Pseudomonas* species implicated in antibiotic production are *Pseudomonas aeruginosa* (13), *Pseudomonas fluorescens* (5, 13), *Pseudomonas multivorans* (5), *Pseudomonas aureofaciens* (19), *Pseudomonas pyrrocinia* (1), and *Pseudomonas cepacia* (12, 18). The antibiotic substances produced by this group of organisms are numerous, and of these, pyocyanin, pyrrolnitrin, and pseudomonic acid have been produced on a commercial basis (13). It has been demonstrated that some secondary metabolites produced by pseudomonads confer obvious selective advantages to the producer organisms in their natural environment. For example, the production of phytotoxins enables colonization of specific host plants (15), slime production by *P. aeruginosa* enables chest colonization in patients with cystic fibrosis (6, 11), and production of an antifungal compound by *P. cepacia* enables colonization of the grass *Tripsacum dactyloides* (12). *P. aeruginosa*, unlike other pseudomonads (1, 5, 12, 18, 19), has not been shown to possess antifungal properties. The present study was undertaken because of a clinical and laboratory impression that *P. aeruginosa* suppresses the growth of *Candida* species in clinically significant chest infections in compromised patients from whom both of these organisms were isolated from serial sputum samples.

Both *P. aeruginosa* and *Candida albicans* were isolated from serial sputum samples of three patients. The following information was noted about each of these patients: clinical details, the time that each sputum sample was obtained, relative numbers of each organism isolated from each sputum sample, and time and duration of administration of each antibiotic used in therapy. Only those important clinical details needed for understanding of and affecting the course of infection in each patient were recorded. In each case, the pseudomonads and candidae were identified to the species levels (see below), and susceptibility testing by the method of Stokes and Waterworth (16) was performed on the pseudomonads. The isolated pseudomonads were stored at room temperature on nutrient agar slopes (Technical Service Consultants, Lancaster, United Kingdom) until the time of

the study. The *Candida* isolates were stored at 4°C on Sabouraud dextrose agar plates until the time of the study.

To obtain a panel of *P. aeruginosa* isolates for use in screening for antifungal activity, 10 isolates of *P. aeruginosa* were collected from routine cultures of sputum. The identities of these and the *P. aeruginosa* isolates obtained from the three study patients (see above) were confirmed by Gram staining reaction, oxidase production, and the API 20NE system (BioMerieux, la Balme les Grottes, France). All pseudomonal strains were stored at room temperature on nutrient agar slopes (Technical Service Consultants) until the time of the study. At the time of the study, isolates were subcultured to blood agar plates and were checked for purity.

To obtain a panel of fungal strains for use as indicator organisms in the demonstration of antifungal activity by *P. aeruginosa*, a number of fungi were obtained from the Department of Microbiology and Immunobiology, Mycology Reference Laboratory, Royal Victoria Hospital, Belfast. These were *Candida krusei*, *Candida keyfr*, *Candida guillermondii*, *Candida tropicalis*, *Candida lusitaniae*, *Candida parapsilosis*, *Candida pseudotropicalis*, *C. albicans*, *Torulopsis glabrata*, and *Saccharomyces cerevisiae*. In addition, one strain of *Aspergillus fumigatus* was also used; this was identified as such by macroscopic and microscopic morphologies. All fungal strains were stored at 4°C on Sabouraud dextrose agar plates until the time of the study. At the time of the study, isolates were subcultured onto fresh Sabouraud dextrose agar plates and were checked for purity.

The method used to test for the antifungal activities of the pseudomonads was similar to that used for pyocin typing of *P. aeruginosa* (7). A fresh 24-h plate culture of each *Pseudomonas* strain to be tested for antifungal activity (i.e., the potential producer strain) was used to prepare an inoculum of 10^8 CFU/ml in normal saline. Thirty microliters of this inoculum was then streaked diametrically across plates of Sabouraud dextrose agar and blood agar in such a way that the width of the inoculum was approximately 1 cm. Sabouraud dextrose agar was used because it enhances fungal growth and all strains of *Pseudomonas* tested grew well on it. Plates were then incubated at 30°C for 24 h. The macro-

scopic growth was then removed with a glass slide. Filter paper disks of 5 cm in diameter were soaked in chloroform and were laid on a metal tray in a safety cabinet. Each plate was then placed face down without its lid on top of a chloroform-containing filter paper disk and was left for 30 min so that the microscopic remnants of the culture were killed. The plates were then removed from the cabinet, and traces of chloroform were eliminated by exposure to air for a few minutes. A fresh 24-h plate culture of each fungal strain was used to prepare an inoculum of 10^6 CFU/ml. This fungal suspension was streaked onto the chloroform-treated medium at right angles to the line of the original inoculum; plates were then incubated for 24 h at 30°C. Each *Pseudomonas* strain from the study patients was tested only against the corresponding *Candida* strain isolated from the same patient. However, isolates from the *Pseudomonas* panel were each tested against each of the 11 fungal indicator strains. Plates were read as follows: total inhibition of fungal growth was recorded as +, partial inhibition of fungal growth was recorded as ±, and no inhibition of fungal growth was recorded as −.

Three surgery patients were monitored postoperatively, with particular reference to lung infection. The same pattern was observed in each patient. Initially, each patient received ventilation in an intensive care unit and broad-spectrum antibiotics. At this stage, *C. albicans* alone was isolated from sputum. Then, when sputum colonization with *P. aeruginosa* occurred, *C. albicans* could not be isolated from sputum. However, when antipseudomonal therapy eradicated the pseudomonad, *C. albicans* was recultured. For each sputum sample, Gram staining was performed prior to culture; *C. albicans* isolates were not seen in any sample from which they were not also cultured.

Isolates of *C. albicans* from all three patients were susceptible to fluconazole. Isolates of *C. albicans* from patients 1 and 2 were susceptible to fluconazole both before and after the start of fluconazole treatment, yet sputum cultures remained positive for the growth of *C. albicans*. *C. albicans* was not isolated from patient 3 after the start of fluconazole treatment. Patient 2 died on day 29, and patients 1 and 3 survived beyond the study period; *C. albicans* was not isolated from the sputum of patients 1 and 3 after the time scale of the study. So, in these patients, fluconazole therapy was associated with the eventual clearance of *C. albicans* from the sputum. However, in each case this process took a longer amount of time than was expected for a drug to which the target organism was susceptible.

The *P. aeruginosa* strains isolated from each of the three patients completely inhibited growth of the corresponding *C. albicans* strain. Table 1 shows the antifungal activities demonstrated by the panel of 10 *P. aeruginosa* strains against each of the panel of 11 fungi. *P. aeruginosa* 1, 2, 3, 5, 7, and 8 completely inhibited all the fungi except *A. fumigatus*, which they partially inhibited. *P. aeruginosa* 4 showed complete inhibition of *C. krusei*, *C. keyfr*, *C. guillermondii*, *C. lusitaniae*, *C. albicans*, *T. glabrata*, and *S. cerevisiae*; partial inhibition of *C. tropicalis*, *C. parapsilosis*, and *C. pseudotropicalis*; and no inhibition of *A. fumigatus*. *P. aeruginosa* 6 showed complete inhibition of *C. keyfr*, *C. guillermondii*, *C. lusitaniae*, *C. parapsilosis*, *C. pseudotropicalis*, and *C. albicans*; partial inhibition of *C. tropicalis* and *S. cerevisiae*; and no inhibition of *C. krusei*, *T. glabrata* or *A. fumigatus*. *P. aeruginosa* 9 showed complete inhibition of *C. krusei*, *C. keyfr*, *C. guillermondii*, *C. lusitaniae*, *C. parapsilosis*, *C. albicans*, and *T. glabrata*; partial inhibition of *C. tropicalis*, *C. pseudotropicalis*, and *S. cerevisiae*; and

TABLE 1. Antifungal activities demonstrated by 10 *P. aeruginosa* strains against 11 fungi

Fungal strain	Activity of *P. aeruginosa* strain[a]:									
	1	2	3	4	5	6	7	8	9	10
C. krusei	+	+	+	+	+	−	+	+	+	−
C. keyfr	+	+	+	+	+	+	+	+	+	+
C. guillermondii	+	+	+	+	+	+	+	+	+	−
C. tropicalis	+	+	+	±	+	±	+	+	±	±
C. lusitaniae	+	+	+	+	+	+	+	+	+	+
C. parapsilosis	+	+	+	±	+	+	+	+	+	±
C. pseudotropicalis	+	+	+	±	+	+	+	+	±	±
C. albicans	+	+	+	+	+	+	+	+	+	+
T. glabrata	+	+	+	+	+	−	+	+	+	+
S. cerevisiae	+	+	+	+	+	±	+	+	±	+
A. fumigatus	±	±	±	−	±	−	±	±	−	−

[a] +, complete inhibition; ±, partial inhibition; −, no inhibition.

no inhibition of *A. fumigatus*. *P. aeruginosa* 10 showed complete inhibition of *C. keyfr*, *C. lusitaniae*, *C. albicans*, *T. glabrata*, and *S. cerevisiae*; partial inhibition of *C. tropicalis*, *C. parapsilosis*, and *C. pseudotropicalis*; and no inhibition of *C. krusei*, *C. guillermondii*, or *A. fumigatus*. Of the fungi, *C. keyfr*, *C. lusitaniae*, and *C. albicans* were the most susceptible to growth inhibition by the *P. aeruginosa* strains, each being completely inhibited by all 10 pseudomonads. *C. guillermondii* and *T. glabrata* were each completely and partially inhibited by nine and one pseudomonad, respectively. *C. parapsilosis* and *S. cerevisiae* were each completely and partially inhibited by eight and two pseudomonads, respectively. *C. krusei* was completely and not inhibited by eight and two pseudomonads, respectively. *C. pseudotropicalis* was completely and partially inhibited by seven and three pseudomonads, respectively. *C. tropicalis* was completely and partially inhibited by six and four pseudomonads, respectively. *A. fumigatus* was partially and not inhibited by six and four pseudomonads, respectively.

In vitro susceptibility tests demonstrate significant inhibition of fungi by the *Pseudomonas* strains tested, and findings from the three case reports present evidence for the in vivo suppression of growth of *C. albicans* by *P. aeruginosa*.

This is the first report of antifungal activity exhibited by *P. aeruginosa*. Additional work is required to elucidate the compound responsible for this activity. Of the known secondary metabolites of *Pseudomonas* species, pyrrolnitrin would seem the most likely candidate, because this metabolite has been shown to possess activity against a wide variety of fungi, including *S. cerevisiae* (17), *Penicillium* species (2, 17), *C. albicans* (2, 8), *Trichophyton* species (2), *Aspergillus niger* (2), dermatophytes (8), *Cryptococcus neoformans* (9), *Blastomyces dermatitidis* (9), *Sporotrichum schenckii* (9), and *Histoplasma capsulatum* (9).

Invasion of deep organs by *Candida* species may complicate the use of broad-spectrum antibiotics (4, 14). It has been presumed that this is due to alteration of the host's normal flora (14), allowing the *Candida* organisms to proliferate. Both *C. albicans* and *P. aeruginosa* may be carried by healthy individuals (3, 10), and because most human infections with *Candida* species are endogenous, it may be that eradication of coexistent antifungal *P. aeruginosa* strains by antipseudomonal antibiotics allows fungal proliferation.

It is difficult to draw conclusions about the delayed clearance of *C. albicans* after the start of fluconazole therapy in patients 1 and 3, because the MICs of fluconazole for the appropriate organisms and fluconazole levels in blood were

not measured. However, it may be that a secondary metabolite of *P. aeruginosa*, whether or not it is the one responsible for antifungal activity, interferes in some way with the action of fluconazole against *C. albicans*. Additional work is required to establish the significance of these observations.

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