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Preliminary Studies of Fluorescent Pseudomonads Capable of Growth at 41 C in Swimming Pool Waters

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During the summer of 1973 cultures of *Pseudomonas aeruginosa* and other fluorescent pseudomonads capable of growth at 41 C were obtained from swimming pool waters at a training center for the mentally retarded. Isolates were subjected to selected physiological tests, pyocine typing, and immunotyping. High counts of *P. aeruginosa* or other fluorescent pseudomonads consisted mainly of single predominant types. Both *P. aeruginosa* strains and unidentified fluorescent *Pseudomonas* strains predominated in pool waters at different times.

Although it is not a normal inhabitant of healthy auditory canals, *Pseudomonas aeruginosa* frequently is isolated from infected outer ears, especially among swimmers (11). The isolation of this organism from swimming pool waters has been reported by several investigators (3, 4, 6, 8). The bacteria are not killed readily by chlorine and often can be isolated from pool waters disinfected with quaternary ammonium algicides or cyanuric acid-stabilized chlorine, and from waters containing less than 0.3 mg of total residual chlorine per liter (3, 10, 14).

The waters of two swimming pools located at an institution for the mentally handicapped were sampled at approximately weekly intervals during the summer of 1973. Preliminary studies were undertaken to enumerate and characterize fluorescent pseudomonads capable of growth at 41 C from pool waters and to assess the possible relationship of these organisms to outer ear infections among swimmers. Although cultures isolated from the pools and from swimmers and nonswimmers were too few to establish significant epidemiologic relationships, the data suggest that extensive studies of the kind described could yield meaningful results.

MATERIALS AND METHODS

The Sunland Training Center at Gainesville, Fla., is a facility of the Division of Retardation of the Florida State Department of Health and Rehabilitation Services. Housed in cottages at the training center are over 1,700 male and female residents of all ages and all levels of retardation. Located on the grounds of the facility are two outdoor swimming pools (60 by 40 ft [ca. 18.3 by 12.2 m]) built in 1960 and 1962. During the study the pools were emptied for cleaning and refilled weekly.

Sampling of swimming pool waters. Samples of water from the two pools, designated Violet and Mimosa, were obtained between 21 June and 16 August 1973. Samples were taken in sterile 150-ml bottles containing sodium thiosulfate (1). Ten-, 1-, and 0.1-ml volumes were inoculated immediately into five replicate tubes of Drake medium 10 (7). At the same time, the total available residual was determined employing the orthotolidine technique and a Hach color comparator (Hach Chemical Co., Ames, Iowa). Inoculated media were returned immediately to the laboratory where they were incubated at 39 C for up to 5 days.

Sampling of outer ears and rectums. Swabs were obtained from the infected outer ears of 13 patients, eight of whom were swimmers and five of whom were nonswimmers. Rectal swabs were obtained from each patient to judge the possibility of self-contamination. Ear and rectal swabs were obtained also from healthy children. Swabs were placed in tubes of Drake medium 10 which are incubated at 39 C for up to 5 days.

Enumeration and isolation procedures. Most probable numbers (MPNs) of *P. aeruginosa* and other fluorescent pseudomonads capable of growth at 41 C were determined as described by Hoadley and Ajello (12). Cells from fluorescent tubes of Drake medium 10 inoculated with pool waters were streaked on plates of the cetrimide medium (Pseudosel agar, BBL) of Brown and Lowbury (5) and incubated for 24 h at 37 C. Isolated colonies were picked to nutrient agar slants. Strains not forming pyocyanin on cetrimide agar were inoculated on slants of King A medium (15) to confirm the absence of pyocyanin production or to indicate production of carotenoid pigments.

Characterization of isolates. Fluorescent isolates which produced pyocyanin were designated as *P. aeruginosa*. Apyocyanogenic strains were subjected to selected tests employed previously (12) to distinguish between apyocyanogenic *P. aeruginosa* and

other apyocyanogenic fluorescent pseudomonads capable of growth at 41 C. In this paper, strains differing significantly from *P. aeruginosa* will be referred to as unidentified fluorescent *Pseudomonas* (UFP).

All isolates were examined further to determine the pyocine types, immunotypes, and antibiotic susceptibility patterns. Pyocine typing was performed employing the 18 indicator strains of Jones et al. (13). The procedure is highly sensitive, and some variations in typing patterns of isolates were found to occur which disappear upon repetition of the test. The overall typing pattern also varied slightly in tests performed on different days.

Immunotyping was performed with seven antisera (9) provided by H. B. Devlin according to accompanying instructions. Representative strains untypable by use of the seven antisera were typed with Habs and other antisera at the Parke, Davis & Co. laboratories.

Susceptibility to seven antibiotics was determined by the method of Bauer et al. (2). Antibiotics included tetracycline (30 μg), chloramphenicol (30 μg), streptomycin (10 μg), kanamycin (30 μg), neomycin (30 μg), gentamycin (10 μg), and carbenicillin (50 μg). Results were recorded as sensitive or resistant. Strains exhibiting zones of inhibition falling in the intermediate and resistant categories were classified as resistant. Strains were designated as resistant to carbenicillin only if the zone of inhibition was less than 15 mm in diameter.

RESULTS AND DISCUSSION

Population of fluorescent pseudomonads in pool waters. Populations of fluorescent pseudomonads capable of growth at 41 C varied from day to day and from pool to pool (Table 1). Organized swimming was permitted in the morning until noon and in the afternoon after 2:00 p.m. All samples were obtained during afternoons. Attendants at the pools reported that satisfactory total available chlorine residual concentrations always were present in each pool when they were opened each morning, but that they were dissipated rapidly after swimming began. On only one occasion, 11 August in the Violet pool, did the total residual chlorine approach 0.3 mg/liter at the time of sampling.

In only one of eight samples from the Violet pool were fluorescent pseudomonads isolated. In contrast, fluorescent pseudomonads were isolated from four of six samples obtained at the Mimosa pool prior to August. After 30 July, calcium hypochlorite (high test hypochlorite) was added to the Mimosa pool water over the noon hour to reduce counts of pseudomonads which accumulated during the morning swimming. Although the residual chlorine concentration remaining at the time of sampling did not increase, fluorescent pseudomonads were not isolated from the subsequent three samples taken in August.

TABLE 1. *Most probable numbers of fluorescent pseudomonads capable of growth at 41 C in swimming pool waters at Sunland Training Center during the summer of 1973*

Swimming pool	Date	Residual chlorine (mg/liter)	MPN per 100 ml	
			Total fluorescent pseudomonads	*P. aeruginosa*
Mimosa	6-21	Trace	13	7.8
	6-28	0.0	350	11
	7-5	0.15	ND[a]	ND
	7-11	0.15	350	350
	7-20	0.1	ND	ND
	7-26	Trace	2	2
	8-4	Trace	ND	ND
	8-11	0.0	ND	ND
	8-16	Trace	ND	ND
Violet	6-21	0.1	ND	ND
	6-28	Trace	ND	ND
	7-5	E[b]	*	*
	7-11	Trace	ND	ND
	7-20	0.0	350	11
	7-26	0.0	ND	ND
	8-4	Trace	ND	ND
	8-11	0.3	ND	ND
	8-16	Trace	ND	ND

[a] ND, Not detected.
[b] E, Pool empty.

On 20 July, the MPN of total fluorescent pseudomonads capable of growth at 41 C was 350 per 100 ml in the Violet pool water. At the same time, the MPN of *P. aeruginosa* was 11 per 100 ml. Similarly, in the Mimosa pool water, MPNs of total fluorescent pseudomonads were higher than those of *P. aeruginosa* in two of four samples yielding fluorescent pseudomonads. On 11 July, the total population of 350 fluorescent pseudomonads per 100 ml consisted of *P. aeruginosa*. *P. aeruginosa* was isolated from 25% of the 17 samples examined, which is consistent with the observations of Black et al. (3) in Florida. The incidence of isolations of *P. aeruginosa* was higher than observed by Brodsky and Nixon (4), however, as was the relative abundance of isolates not identified as *P. aeruginosa*. The frequent isolation of *P. aeruginosa* from pool waters must be regarded with concern in view of the suggestion of Favero et al. (8) that "the isolation of *P. aeruginosa* in 100 ml or less of swimming pool water would warrant closure of the pool until an adequate chlorine residual could be maintained."

Characteristics of isolates from pools. Samples of swimming pool waters were obtained weekly at Sunland Training Center and, therefore, do not provide a complete picture of the nature of *Pseudomonas* populations which

TABLE 2. *Characteristics of apyocyanogenic fluorescent pseudomonads capable of growth at 41 C isolated from pool waters at Sunland Training Center during the summer of 1973*[a]

Culture[b]	Gelatin liquefaction	Denitrification	Utilization of:							Pyocine type	Immunotype	Resistance to:							Remarks
			Sebacate	Saccharate	Mannitol	Glycollate	Gluconate	Geraniol	Acetamide			Gentamicin	Carbenicillin	Chloramphenicol	Streptomycin	Tetracycline	Neomycin	Kanamycin	
621-3	+	−	+	−	−	−	−	+	P/M	NT	NT	S	R	R	S	R	S	S	UFP
628-2	+	−	+	−	−	−	−	+	M	NT	NT	S	R	R	S	R	S	S	UFP
628-4	+	−	+	−	−	−	−	+	M	NT	NT	S	R	R	S	R	S	S	UFP
628-6	+	−	+	−	−	−	−	M	M	NT	NT	S	R	R	S	R	R	S	UFP
628-7	+	−	+	−	−	−	−	M	M	NT	NT	S	R	R	S	R	R	S	UFP
628-9	+	−	+	−	−	−	−	M	P	NT	NT	S	R	R	S	R	S	S	UFP
628-10	+	−	+	−	−	−	−	M	P	NT	NT	S	R	R	S	R	S	S	UFP
720-4	+	+	+	−	−	−	−	+	P/M	NT	NT	S	R	R	S	R	S	S	UFP
720-5	+	+	+	−	−	−	−	+	P	NT	NT	S	R	R	S	R	S	S	UFP
720-6	+	+	+	−	−	−	−	+	P	NT	NT	S	R	R	S	R	R	S	UFP
720-7	+	+	+	−	−	−	−	+	P	NT	NT	S	R	R	S	R	S	S	UFP
720-8	+	+	+	−	−	−	−	M	P	NT	NT	S	R	R	S	R	S	S	UFP
720-9	+	+	+	−	−	−	−	+	+	NT	NT	S	R	R	S	R	S	S	UFP
720-10	+	+	+	−	−	−	−	+	+	NT	NT	S	R	R	S	R	S	S	UFP
628-5	+	+	+	−	+	P	−	+	+	788878	4	S	S	R	R	R	R	R	Apyocyanogenic *P. aeruginosa*
711-M1	+	+	+	−	+	−	+	M	+	111124	6	S	S	R	R	R	R	R	Apyocyanogenic *P. aeruginosa*
711-M2	+	+	+	−	+	−	+	M	+	111124	6	S	S	R	R	R	R	R	Apyocyanogenic *P. aeruginosa*
711-M3	+	+	+	−	+	−	+	+	+	111124	6	S	S	R	R	R	R	R	Apyocyanogenic *P. aeruginosa*
711-M4	+	+	+	−	+	−	+	M	+	111124	6	S	S	R	R	R	R	R	Apyocyanogenic *P. aeruginosa*
711-M5	+	+	+	−	+	−	+	M	+	111124	6	S	S	R	R	R	R	R	Apyocyanogenic *P. aeruginosa*
711-M6	+	+	+	−	+	−	+	P	+	111124	6	S	S	R	R	R	R	R	Apyocyanogenic *P. aeruginosa*
711-M7	+	+	+	−	+	−	+	M	+	111124	6	S	S	R	R	R	R	R	Apyocyanogenic *P. aeruginosa*
711-M8	+	+	+	−	+	−	+	+	+	111124	6	S	S	R	R	R	R	R	Apyocyanogenic *P. aeruginosa*
711-M9	+	+	+	−	+	−	+	M	+	111124	6	*	*	*	*	*	*	*	Apyocyanogenic *P. aeruginosa*
711-M10	+	+	+	−	+	−	+	M	+	111124	6	*	*	*	*	*	*	*	Apyocyanogenic *P. aeruginosa*
711-M11	+	+	+	−	+	−	+	M	+	111124	6	S	S	R	R	R	R	R	Apyocyanogenic *P. aeruginosa*
711-M12	+	+	+	−	+	−	+	M	+	111124	6	S	S	R	R	R	R	R	Apyocyanogenic *P. aeruginosa*

[a] All strains fluorescent, gram-negative rods possessing single polar flagella, oxidase positive, exhibiting oxidative glucose metabolism, capable of growth at 41 C, and producing neither phenazine nor carotenoid pigments. +, Positive good growth; −, negative; NT, not typable; S, sensitive; M, moderate growth; P, poor growth; R, resistant; *, not tested.

[b] Cultures were isolated from pool waters during the month indicated by the first digit and on the day indicated by the second and third digits of the strain number.

may occur in swimming pools. Among fluorescent pseudomonads capable of growth at 41 C were pyocyanogenic strains of *P. aeruginosa*, apyocyanogenic strains of *P. aeruginosa*, and strains which resembled isolates from surface waters which are believed to differ significantly from *P. aeruginosa* (12).

Characteristics of all apyocyanogenic isolates are presented in Table 2. Pyocyanogenic *P. aeruginosa* strains were typed by pyocine typing, serological testing, and their resistance to seven antibiotics. They were not subjected to the remaining tests listed in the table, however, and are not included.

Fourteen apyocyanogenic strains were judged to differ significantly from *P. aeruginosa*, whereas the remaining 13 strains were easily identifiable based upon all tests employed. All apyocyanogenic strains of *P. aeruginosa* utilized mannitol and all but two strains utilized gluconate. None of the 14 UFP (non-*P. aeruginosa*) strains utilized either mannitol or gluconate. Furthermore, none of the UFP strains isolated during the month of June exhibited the ability to denitrify, although strains isolated on 20 July did exhibit this ability. All apyocyanogenic strains of *P. aeruginosa* were typable by pyocine typing and immunotyping, whereas none of the UFP strains were typable by these methods. Finally, patterns of resistance and susceptibility to seven antibiotics proved useful in distinguishing apyocyanogenic strains of *P. aeruginosa* from UFP strains. Both pyocyanogenic and apyocyanogenic strains of *P. aeruginosa* were sensitive to gentamicin and carbenicillin and resistant to the remaining five antibiotics. In contrast, the UFP strains isolated during the present study were consistently resistant to carbenicillin and sensitive to streptomycin and kanamycin.

When high counts occurred in pool waters, single strains predominated. On 28 June, the MPN of fluorescent pseudomonads capable of growth at 41 C was 350 per 100 ml, and that of *P. aeruginosa* was only 11 per 100 ml. The large component of the population consisted of UFP strains unable to denitrify. Identical strains had been isolated from the pool water on 21 June and were isolated from the healthy auditory canals of a swimmer on 27 June and from the infected outer ear of another swimmer on 6 July.

Two weeks later, on 11 July, also at the Mimosa pool, the population of fluorescent pseudomonads was again 350 per 100 ml, all of which consisted of an apyocyanogenic *P. aeruginosa* (immunotype 6, pyocine type 111124). On a third occasion (20 July), the population of fluorescent pseudomonads in the sample from the Violet pool was 350 per 100 ml, of which 11 per 100 were *P. aeruginosa*. The remainder of the population consisted of a UFP strain which differed from the UFP strains isolated earlier in the summer from Mimosa pool water in its ability to denitrify.

The predominance of a single type in each of the three samples exhibiting high populations of fluorescent pseudomonads may be explained in either of two ways. First, if the sample of pool water contained mucous carrying a single type, individual cells would be dispersed upon shaking of the sample and would cause both an excessively high count and a predominance of the type. Second, gross contamination of pool waters by children with urinary infections is probable. In the latter event, high populations of a single type might be expected, at least locally, in pool waters.

It is of interest to note that, in addition to the UFP strain isolated from pool waters and from outer ears, *P. aeruginosa* (immunotype 3, pyocine type 621424) was isolated from Mimosa pool water on two occasions (21 and 28 June), from the infected outer ears of two swimmers, and from both the infected outer ear and rectum of a third swimmer.

The present work demonstrates the value of pyocine typing and immunotyping of *P. aeruginosa* isolated in studies of the dynamics of populations of fluorescent pseudomonads in swimming pool waters. It also suggests benefits from more extensive studies of populations of swimmers and pool waters to which they have access to determine the relationship between swimming water quality and bather health and to establish criteria for bathing water quality.

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