APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1982, p. 1086–1095
0099-2240/82/111086-10$02.00/0

Vol. 44, No. 5

# Growth of *Pseudomonas aeruginosa* in Tap Water in Relation to Utilization of Substrates at Concentrations of a Few Micrograms per Liter

D. VAN DER KOOIJ,* J. P. ORANJE, AND W. A. M. HIJNEN

*The Netherlands Waterworks' Testing and Research Institute, KIWA Ltd, 2280 AB Rijswijk, The Netherlands*

Received 6 April 1982/Accepted 26 July 1982

Five *Pseudomonas aeruginosa* strains were tested for the utilization of 47 low-molecular-weight compounds as their sole sources of carbon and energy for growth at a concentration of 2.5 g/liter. Of these compounds, 31 to 35 were consumed. Growth experiments in tap water at 15°C were carried out with one particular strain (P1525) isolated from drinking water. This strain was tested for the utilization of 30 compounds supplied at a concentration of 25 μg of C per liter. The growth rate (number of generations per hour) of strain P1525 in this tap water was approximately 0.005 $h^{-1}$, and with 10 compounds it was larger than 0.03 $h^{-1}$. An average yield of $6.2 \times 10^9$ colony-forming units per mg of C was obtained from the maximum colony counts (colony-forming units per milliliter). The average yield and maximum colony count of strain P1525 grown in tap water supplied with a mixture of 45 compounds, each at a concentration of 1 μg of C per liter, enabled us to calculate that 28 compounds were utilized. Growth rates of two *P. aeruginosa* strains (including P1525) in various types of water at 15°C were half of those of a fluorescent pseudomonad. The concentrations of assimilable organic carbon calculated from maximum colony counts and average yield values amounted to 0.1 to 0.7% of the total organic carbon concentrations in five types of tap water. The assimilable organic carbon percentages were about 10 times larger in river water and in water after ozonation.

The presence of *Pseudomonas aeruginosa* in surface water is generally associated with fecal pollution by raw or treated domestic sewage (3, 10, 29). Much attention is paid to the occurrence of the organism in tap water because of its opportunistic-pathogenic character. *P. aeruginosa* is usually not present in 100-ml volumes of piped or stored drinking water (4, 7, 14, 22, 25, 30). The organism has frequently been isolated from contaminated drinking water and from drinking water in hot climates (15, 20, 23), but quality standards have rarely been defined (10). The ability of *P. aeruginosa* to grow in water at low concentrations of organic substrates has been studied in relation to its presence in water used in hospitals (1, 2, 5, 6). The present paper describes the effect of various low-molecular-weight compounds on the growth of *P. aeruginosa* in tap water supplied with very low amounts of these compounds.

## MATERIALS AND METHODS

**Isolates.** Five strains of *P. aeruginosa* isolated from different habitats and belonging to different serological, pyocine, and phage types were tested (Table 1). For comparison, a representative strain (P17) of *P. fluorescens*, obtained from tap water and belonging to biotype 7.2 (26) was included in a number of experiments. A detailed description of this strain has been given previously (28).

**Replica test.** Colonies of the isolates grown on a peptone beef-extract agar (Lab-Lemco; Oxoid Ltd.) were replicated onto plates of mineral salts agar supplied with separately sterilized carbon compounds at a concentration of 2.5 g/liter. A metal device with 12 inoculation pins was used for replication. The mineral salts agar consisted of the basal salts solution (27) solidified with 12 g of agar per liter. The replica plates were examined for growth after 2 and 7 days of incubation at 25°C.

**Growth in tap water.** Growth in tap water was tested in 600-ml samples contained in rigorously cleaned 1-liter glass-stoppered Erlenmeyer flasks of Pyrex glass (27). Drinking water originating from the Municipal Dune Waterworks of The Hague was used for measuring the effect of low concentrations of added substrates on the growth of *P. aeruginosa*. This water had been prepared from dune-infiltrated river (Meuse) water by the addition of powdered activated carbon followed by rapid and slow sand filtration. It contained 3.6 mg of organic carbon (TOC) and 7.3 mg of nitrate per liter. The autochthonous bacteria were eliminated by heating the sampled water at 60°C for 1 h. Compounds to be tested as carbon sources were added from similarly treated, freshly prepared solutions in

TABLE 1. Type and origin of the *P. aeruginosa* strains[a]

| Strain | Serotype | Pyocine type | | Reacting phages | Origin |
|---|---|---|---|---|---|
| | | Active | Passive | | |
| P1525 | (5)C | 15C | NT | 21; 44; 73; 119x; 1214++; 16; 31±; F7; F8; F10 | Tap water |
| 4A[b] | NT[c] | 15C | 34D | 21; 44; 73; 109; 119x± | River Lek[b] |
| 6A[b] | 6 | 15C | 24D | 21++; 119x+ | River Lek[b] |
| M14[b] | 1 | NT | NT | NT | River Lek[b] |
| 6324[b] | L | 15C | 54A | 21; 119x+; F8± | Wound pus[b] |

[a] Serotyping, pyocine typing and phage reactions were performed by J. Borst, National Institute of Public Health (RIV), Bilthoven, The Netherlands.
[b] Supplied by A. H. Havelaar, RIV.
[c] NT, Nontypable.

tap water. A nitrogen source was not added because the tap water contained sufficient nitrate.

In a few experiments, the water was supplied with mixtures of substrates. The amino acids (AA) mixture included glycine, L-alanine, L-valine, L-leucine, L-isoleucine, DL-serine, L-threonine, L-lysine, L-arginine, L-asparagine, L-aspartate, L-glutamine, L-glutamate, L-tyrosine, L-proline, DL-tryptophan, L-histidine, and DL-phenylalanine. The aliphatic carboxylic acids (CA) mixture included sodium formate, acetate, glycolate, glyoxylate, oxalate, propionate, DL-lactate, pyruvate, malonate, fumarate, succinate, L-tartrate, citrate, and adipate. The carbohydrate (CHA) mixture included L-arabinose, D-glucose, D-maltose, and the polyalcohols ethanol, glycerol, and D-mannitol. The aromatic acids (AR) mixture included sodium benzoate, *p*-hydroxybenzoate, anthranilate, vanillate, phtalate, nicotinate, and DL-mandelate. Stock solutions of the mixtures were prepared by dissolving the individual compounds in tap water at a concentration of 15 mg of C per liter, except glutamate, which was used in a double concentration. After neutralization, the mixtures were sterilized (120°C, 16 min) in Pyrex glass bottles.

To prepare the inoculum, a small amount of cells from a 24-h slant culture on Lab-Lemco agar was suspended in 9 ml of sterile tap water. A 0.1-ml volume of a decimal dilution of this suspension was added to 100-ml infusion bottles containing 50 ml of autoclaved tap water with 1 mg of glucose C per liter. The initial number of cells was less than $10^3$ colony-forming units (CFU) per ml. Maximum colony counts ($N_{max}$) of $4 \times 10^6$ to $6 \times 10^6$ CFU/ml in the infusion bottles were reached within a few days of incubation at 25°C. Thereafter, the colony counts decreased very slowly, and during a period of several months these cultures were used to inoculate the Erlenmeyer flasks (initial colony counts, $<10^3$ CFU/ml). The growth of the bacteria in the experimental solutions was assessed by periodic colony counts in triplicate, using the spread plate technique on Lab-Lemco agar plates (27). The generation times (G, in hours) were calculated for the period in which growth was exponential. The growth experiments in water were conducted in duplicate at 15 ± 0.5°C unless otherwise stated.

## RESULTS

**Utilization of substrates at a concentration of 2.5 g/liter.** Out of 47 compounds, 31 to 35 were utilized by the *P. aeruginosa* isolates and *P. fluorescens* P17 when tested by the replica procedure (Table 2). Strain P17 and the strains of *P. aeruginosa* differed only in the utilization of a few of the substrates tested. Differences among the various *P. aeruginosa* isolates regarding the utilization of a number of aliphatic amino acids, as well as the production of fluorescent pigments and the appearance of the colonies (results not shown), confirmed the heterogeneity of the strains as revealed by serotyping and pyocine and phage typing (Table 1).

**Utilization of substrates at very low concentrations.** With the exception of strain 6324, the *P. aeruginosa* isolates did not grow in the tap water from the Municipal Dune Waterworks of The Hague. Strain 6324 gave a G value of approximately 60 h and an $N_{max}$ value of approximately $2 \times 10^4$ CFU/ml. These values approached those of strain P17 (Table 3). Addition of a mixture of 45 different compounds (total concentration added amounted to 46 μg of C per liter) enhanced growth of all strains, including strain P17.

Further tests were done with strain P1525, isolated from drinking water (Table 1), and with strain 6324 because of its relatively rapid growth in drinking water (Table 3). A total of 30 different low-molecular-weight compounds at a concentration of 25 μg of C per liter were tested as sources of carbon and energy for growth of strain P1525. The growth rate ($G^{-1}$) of strain P1525 was greater than 0.03 $h^{-1}$ for 10 of the compounds tested (Fig. 1). These compounds were also tested at a concentration of 10 μg of C per liter. At that concentration, $G^{-1}$ was greater than 0.03 $h^{-1}$ only for arginine. An average yield ($Y_a$) of $6.2 \times 10^9$ CFU/mg of C was calculated from the $N_{max}$ values observed with these 10 substrates (Table 4). Strain P1525 grew at a very low rate in tap water supplied with amino acids (25 μg of C per liter) which were not utilized by the organism at a concentration of 2.5 g/liter (viz. glycine, serine, threonine; Table 2), and $N_{max}$ values ($<4 \times 10^3$ CFU/ml) did not exceed those of the blanks. However, very low rates of

TABLE 2. Utilization of substrates at a concentration of 2.5 g/liter by five *P. aeruginosa* strains and *P. fluorescens* strain P17 determined with the replica test

| Substrate | Utilization by strain:[a] | | | | | |
|---|---|---|---|---|---|---|
| | P17 | P1525 | 4A | 6A | M14 | 6324 |
| Amino acids | | | | | | |
| Glycine | − | − | − | − | − | − |
| L-Alanine | + | + | + | + | + | + |
| L-Valine | + | + | + | (+) | + | − |
| L-Isoleucine | + | + | + | (+) | + | − |
| L-Leucine | + | + | + | − | + | − |
| DL-Serine | + | − | − | − | − | − |
| L-Threonine | (+) | − | − | − | − | − |
| L-Lysine | + | (+) | (+) | (+) | + | + |
| L-Arginine | + | + | + | + | + | + |
| L-Aspartate | + | + | + | + | + | + |
| L-Asparagine | + | + | + | + | + | + |
| L-Glutamate | + | + | + | + | + | + |
| L-Glutamine | + | + | + | + | + | + |
| L-Proline | + | + | + | + | + | + |
| L-Histidine | + | + | + | + | + | + |
| L-Tyrosine | + | + | + | + | + | + |
| DL-Phenylalanine | + | + | + | + | + | + |
| DL-Tryptophan | + | + | + | + | + | + |
| L-Citrulline | + | + | + | + | + | + |
| L-Ornithine | + | + | + | + | + | + |
| Carboxylic acids | | | | | | |
| Formate | − | − | − | − | − | − |
| Acetate | + | + | + | + | + | + |
| Glycolate | − | − | − | − | − | − |
| Glyoxylate | − | − | − | − | − | − |
| Oxalate | − | − | − | − | − | − |
| Propionate | + | + | + | + | + | + |
| DL-Lactate | + | + | + | + | + | + |
| Pyruvate | + | + | + | + | + | + |
| Malonate | + | + | + | + | + | + |
| Fumarate | + | + | + | + | + | + |
| Succinate | + | + | + | + | + | + |
| L-Tartrate | − | − | − | − | − | − |
| Citrate | + | + | + | + | + | + |
| Adipate | + | + | + | + | + | + |
| Carbohydrates and polyalcohols | | | | | | |
| L-Arabinose | − | − | − | − | − | − |
| D-Glucose | + | + | + | + | + | + |
| D-Maltose | − | − | − | − | − | − |
| Ethanol | + | + | + | + | + | + |
| Glycerol | + | + | + | + | + | + |
| D-Mannitol | (+) | (+) | (+) | (+) | (+) | (+) |
| Aromatic acids | | | | | | |
| Benzoate | + | + | + | + | + | + |
| *p*-Hydroxybenzoate | + | + | + | + | + | + |
| Anthranilate | + | + | + | + | + | + |
| Vanillate | − | + | + | + | + | + |
| Phtalate | − | − | − | − | − | − |
| Nicotinate | − | − | − | − | − | − |
| DL-Mandelate | − | + | + | + | + | + |

[a] +, Good growth; (+), weak growth; −, no growth.

growth were also observed at concentrations of 25 μg of C per liter with a number of amino acids which were utilized at a concentration of 2.5 g/liter. Moreover, the colony counts of strain P1525 in the presence of these compounds did not exceed $10^4$ CFU/ml after 15 days of incubation at 15°C. Very low rates of growth (Fig. 1) and low colony counts also were observed after 15 days of incubation with propionate, fumarate, D-glucose, glycerol, and malonate.

Isolate 6324 was supplied with fewer compounds than strain P1525 (Fig. 1). The more rapid growth of this strain as compared to strain P1525 at 10 μg of C per liter may be explained by its ability to multiply in tap water without added substrate (Table 3).

Generation times of strain P1525 with the AA mixture were nearly equal to those with the total mixture, indicating that the amino acids were particularly growth promoting (Table 5). Of the other mixtures, only the carboxylic acids enhanced growth, but the rate of growth was very low, and after 77 days $N_{max}$ values were not yet reached. The $N_{max}$ values shown in Table 5 reveal that compounds included in the CA, AR, and CHA mixtures were more effectively utilized when incorporated in the total mixture than when present in separate mixtures.

**Utilization by strain P1525 of aspartate and acetate supplied at different concentrations.** A linear relationship between the $N_{max}$ values of strain P1525 and the concentration of added substrate ($\Delta S$) was obtained by growth of strain P1525 in tap water supplied with different amounts of either aspartate or acetate (Fig. 2). From these results, a Y value of $5.3 \times 10^9$ CFU per mg of acetate C and of aspartate C, was calculated. Plotting G against $\Delta S^{-1}$ revealed different linear relationships between G and $\Delta S^{-1}$ (Fig. 3). Such relationships may be expressed by the following Lineweaver-Burk equation:

$$G = G_{min} + (G_{min} \cdot K_s/\Delta S)$$

in which $G_{min}$ is minimal G and $K_s$ is the substrate saturation constant, the substrate concentration at which G is equal to $2G_{min}$. The equations for growth with aspartate and with acetate are presented in Table 6 together with the $K_s$ values.

**Growth of *P. aeruginosa* isolates P1525 and 6324 and *P. fluorescens* P17 in various types of water.** Table 7 gives generation times and $N_{max}$ values of strains P1525, 6324, and P17 in a few types of tap water, in ozonated water, and in river water. In these water types, $NO_3$ nitrogen was present in concentrations of between 1.2 and 3.6 mg/liter and therefore was not growth limiting. From the average yield of strain P1525 on a number of substrates (cf., Table 4) and the

TABLE 3. Growth of five *P. aeruginosa* strains and *P. fluorescens* P17 at 15°C in the filtrate of slow sand filters, with and without a mixture of 45 substrates[a]

| Strain | $N_o$ (CFU/ml)[b] | Growth[c] | | | |
|---|---|---|---|---|---|
| | | No substrates added | | Substrate mixture added | |
| | | G (h) | $N_{max}$ (CFU/ml) | G (h) | $N_{max}$ (CFU/ml) |
| P17 | 80 | 42, 46 | $2.4 \times 10^4$, $2.7 \times 10^4$ | ≤9, ≤9 | $2.4 \times 10^5$, $2.5 \times 10^5$ |
| P1525 | 170 | 342, 264 | —[d], $5.6 \times 10^2$ | 12.7, 11.3 | $1.7 \times 10^5$, $2.0 \times 10^5$ |
| 4A | 320 | 244, 232 | $1.4 \times 10^3$, $6.3 \times 10^2$ | 19.1, 18.9 | $1.4 \times 10^5$, $1.4 \times 10^5$ |
| 6A | 180 | 188, 1,022 | $8.2 \times 10^2$, $2.9 \times 10^2$ | 14.0, 14.2 | $1.7 \times 10^5$, $1.8 \times 10^5$ |
| M14 | 140 | 192, 113 | $4.2 \times 10^3$, $8.2 \times 10^2$ | 21.5, 20.8 | $1.3 \times 10^5$, $1.3 \times 10^5$ |
| 6324 | 180 | 65, 53 | $1.7 \times 10^4$, $2.1 \times 10^4$ | 17.3, 14.9 | —, — |

[a] The concentration of each individual substrate was 1 μg of C per liter; glutamate was present at twice this concentration.
[b] $N_o$, Initial colony count (inoculum).
[c] Both values of duplicate measurements are shown.
[d] —, Not determined.

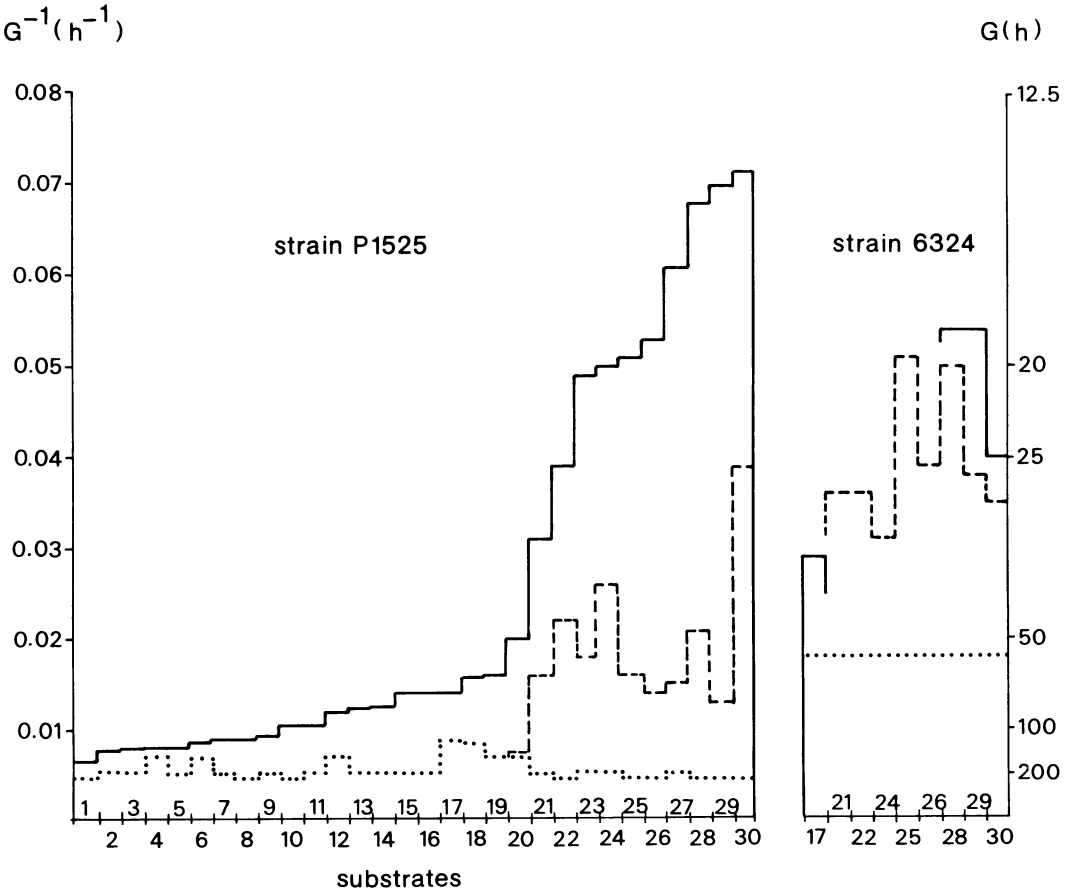


FIG. 1. Growth rates ($G^{-1}$ [$h^{-1}$]) of *P. aeruginosa* P1525 and 6324 at 15°C in tap water supplied with 25 and 10 μg of substrate C per liter. Substrates: 1, *p*-Hydroxybenzoate; 2, L-threonine; 3, L-citrulline, 4, DL-tryptophan; 5, L-leucine; 6, DL-phenylalanine; 7, L-lysine; 8, DL-serine; 9, L-tyrosine; 10, L-alanine; 11, L-ornithine; 12, glycine; 13, L-valine; 14, L-isoleucine; 15, propionate; 16, fumarate; 17, D-glucose; 18, glycerol; 19, malonate; 20, citrate; 21, succinate; 22, pyruvate; 23, L-aspargine; 24, L-proline; 25, L-aspartate; 26, L-glutamate; 27, L-glutamine; 28, acetate; 29, DL-lactate; 30, L-arginine. ——, 25 μg of C per liter; — — —, 10 μg of C per liter; . . . . . , blanks. Experiments not in duplicate except in blanks, for which the average $G^{-1}$ values are shown.

TABLE 4. $N_{max}$ values of *P. aeruginosa* P1525 grown at 15°C on different substrates added to tap water

| Substrate | $N_{max}$ (CFU/ml)[a] | |
|---|---|---|
| | 25 μg of C per liter | 10 μg of C per liter |
| Acetate | $1.1 \times 10^5$ | $5.7 \times 10^4$ |
| Pyruvate | $1.3 \times 10^5$ | $4.4 \times 10^4$ |
| DL-Lactate | $1.6 \times 10^5$ | $7.9 \times 10^4$ |
| Succinate | —[b] | $6.7 \times 10^4$ |
| L-Aspartate | $1.3 \times 10^5$ | — |
| L-Asparagine | $1.5 \times 10^5$ | $4.3 \times 10^4$ |
| L-Glutamate | $1.8 \times 10^5$ | $4.9 \times 10^4$ |
| L-Glutamine | $1.9 \times 10^5$ | $6.9 \times 10^4$ |
| L-Proline | $1.7 \times 10^5$ | $9.7 \times 10^4$ |
| L-Arginine | $1.6 \times 10^5$ | $6.6 \times 10^4$ |

[a] $Y_a$, $6.2 \times 10^9$ CFU per mg of C. $N_{max}$ of the blanks was less than $10^3$ CFU/ml and was therefore neglected in this determination.
[b] Single values.
[c] —, Not determined.

$N_{max}$ values presented, it may be concluded that the concentration of assimilable organic carbon (AOC) available for strain P1525 varied from less than 0.1 μg of C per liter (tap water 2) to 150 μg of C per liter (ozonated water). For strain P17, a yield of $4.2 \times 10^9$ CFU per mg of acetate C has been obtained (28). Hence, the AOC available for strain P17 varied from 1.4 (tap water 4) to 200 (ozonated water) μg of acetate C equivalents per liter.

In general, $N_{max}$ values for strains P1525 and 6324 did not differ much from those of strain P17 (cf. Tables 3 and 7), suggesting that the three organisms were utilizing similar substrates. This suggestion was supported by the observation that strain P17 did not grow in river water in which maximum numbers of strain P1525 had grown. Moreover, strain 6324 was unable to grow in river water in which strain P17 had reached $N_{max}$ (Fig. 4). In all types of water tested, strain P17 grew more rapidly than strains P1525 and 6324, the growth rate of these organisms being about half the growth rate of strain P17 (Fig. 5). Therefore, it is expected that at a temperature of 15°C, *P. aeruginosa* cannot attain large numbers in water where bacteria such as strain P17 are present.

Competition is further demonstrated by the effect of autochthonous bacteria, added to the heated tap water, on the growth of strain 6324. Even when the water had been supplied with the total mixture of substrates (46 μg of C per liter), growth of strain 6324 was effectively repressed by the more rapidly developing autochthonous bacteria. Further incubation resulted in a pronounced decrease of the colony counts of strain 6324, whereas the autochthonous bacteria remained present in larger numbers (Fig. 6). The similarity of the duplicate experiments clearly demonstrates the reproducibility of the processes which determine the growth of the various groups of bacteria.

The described experiments reveal that *P. aeruginosa* is able to grow at relatively low concentrations of substrates. In natural environments, however, the organism can not compete effectively with many aquatic bacteria, including fluorescent pseudomonads such as strain P17.

## DISCUSSION

**Utilization of amino acids at low concentrations by *P. aeruginosa*.** Utilization of substrates by *P. aeruginosa* at a concentration of 2.5 g/liter (Table 2) gives no information about the utilization of these substrates at a very low concentration. Similar observations have been reported for an *Aeromonas hydrophila* isolate (27). Arginine was found to be the most effective of the growth-promoting compounds tested at 10 and 25 μg of C per liter with strain P1525. In this respect, the organism resembles *P. fluorescens* P17 (28). A preference of *P. aeruginosa* for arginine has also been reported by Kay and Gronlund (12), who found that this amino acid was rapidly taken up by the cells when present at a concentration of 1 μM. Moreover, the greatest chemotactic re-

TABLE 5. Growth response of *P. aeruginosa* P1525 to mixtures of different substrates added to the filtrate of slow sand filters[a]

| Mixture added (no. compounds in mixture) | Amount of carbon supplied (μg of C per liter)[b] | Growth[c] | |
|---|---|---|---|
| | | G (h) | $N_{max}$ (CFU/ml) |
| None | | 401, 236 | $7.5 \times 10^2$, $8.3 \times 10^3$ |
| AR (7) | 5 | 270, 256 | $1.7 \times 10^3$, $4.7 \times 10^3$ |
| CHA (6) | 4 | 125, 144 | $1.2 \times 10^3$, $9.5 \times 10^2$ |
| CA (14) | 9 | 89, 77 | $3.5 \times 10^3$, $2.0 \times 10^4$ |
| AA (18) | 16 | 21.5, 21.7 | $1.1 \times 10^5$, $1.1 \times 10^5$ |
| Total mixture (45) | 34 | 16.1, 18.0 | $1.8 \times 10^5$, $1.7 \times 10^5$ |

[a] Incubation temperature, 15°C.
[b] Total concentration of the compounds utilized in the replica test (cf. Table 2).
[c] Both values of duplicate measurements are shown.

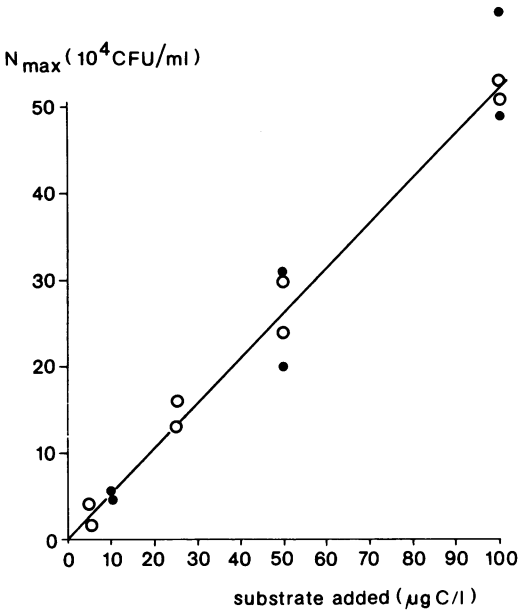


FIG. 2. $N_{max}$ of *P. aeruginosa* P1525 in tap water supplied with different amounts (µg of C per liter) of either acetate (●) or aspartate (○).

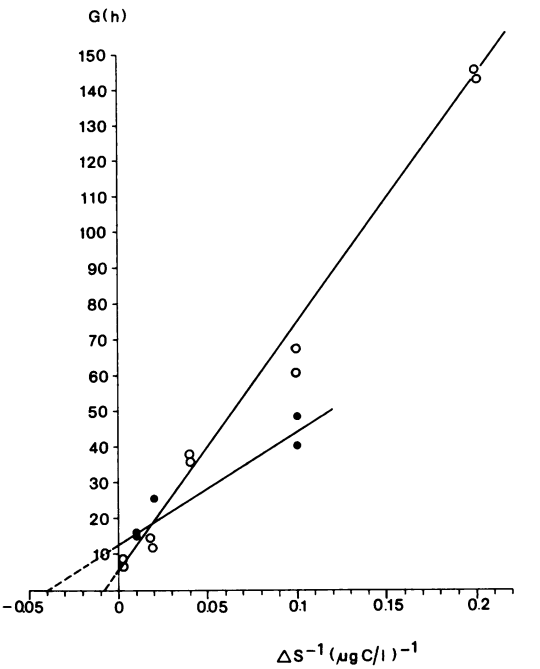


FIG. 3. Generation times of *P. aeruginosa* P1525 in tap water in relation to the reciprocal values ([µg of C per liter]$^{-1}$) of different concentrations (ΔS) of added acetate (●) and aspartate (○).

sponses of *P. aeruginosa* have been observed with amino acids of which arginine, with a threshold of 0.7 µM, was the best attractant (18, 19). Citrulline and ornithine, intermediates of the degradation of arginine by the arginine deiminase pathway in *P. aeruginosa*, did not favor growth of strain P1525 when present at a low concentration (Fig. 1). The arginine decarboxylase pathway seems to be the main degradation route in aerobically grown cells of *P. aeruginosa* (16). Hence, no relation may be expected between growth on arginine on the one hand and on citrulline and ornithine on the other.

The $N_{max}$ values of strain P1525 obtained with the AA mixture (Table 5) and the $Y_a$ value of 6.2 × $10^9$ CFU/mg of C (Table 4) revealed that 18 µg of C, i.e., approximately all of the amino acids present in the mixture, were utilized. Obviously many amino acids were more efficiently utilized at a concentration of 1 µg of C per liter when present in a mixture at a total concentration of 19 µg of C per liter than when present singly at a concentration of 25 µg of C per liter. The presence of constitutive transport systems in growing *P. aeruginosa* cells for the uptake of most naturally occurring amino acids (12) may have been responsible for this phenomenon. Even amino acids which did not serve as the sole source of carbon and energy may have contributed to the production of biomass during growth with the amino acids mixture.

Many substrate saturation constants for ami-

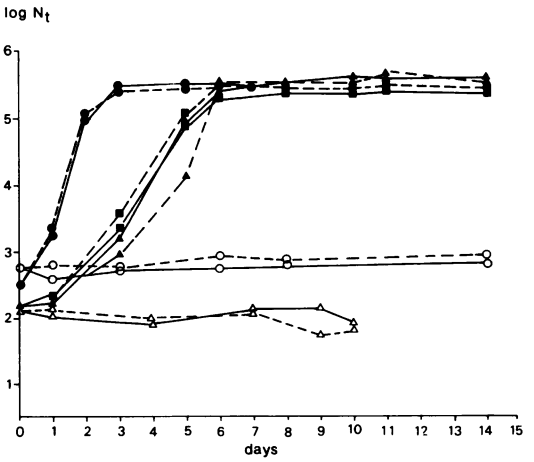


FIG. 4. Growth curves of *P. fluorescens* P17 (●), *P. aeruginosa* P1525 (■), and 6324 (▲) in pasteurized river (Lek) water at 15°C. Growth curves of *P. fluorescens* P17 (○) in river water in which strain P1525 had reached $N_{max}$ and growth of strain 6324 (△) in river water in which strain P17 had reached $N_{max}$. Colony counts ($N_t$) of P17 growing in the presence of P1525 were determined by incubation of the plates at 10°C. Colony counts of strain 6324 growing in the presence of strain P17 were determined by incubation of the plates at 37°C. Solid and dashed lines represent duplicate experiments.

TABLE 6. Kinetics of growth of *P. aeruginosa* P1525 with aspartate or acetate at 15°C

| Substrate | Adapted Lineweaver-Burk equation[a] | $K_s$ | |
|---|---|---|---|
| | | μg of C per liter | μM |
| L-Aspartate | $G = 6.0 + 676/\Delta S$ | 113 | 2.3 |
| Acetate | $G = 11.7 + 328/\Delta S$ | 28 | 1.2 |

[a] $G = G_{min} + (G_{min} \cdot K_s/\Delta S)$. This equation is described in the text.

no acid transport ($K_t$) by *P. aeruginosa* are below 1 μM (11–13). Assuming that $G_{min}$ of strain P1525 with arginine is similar to $G_{min}$ with aspartate, a $K_s$ value of approximately 0.4 μM of arginine may be calculated by using the growth rates of strain P1525 at 10 and 25 μg of arginine C per liter (Fig. 1). For aspartate, a clearly higher $K_s$ value (2.3 μM) was obtained (Table 6). To what extent $K_t$ and $K_s$ values are similar may be estimated by uptake experiments with radioactively labeled substrates combined with growth experiments as described in this paper. The $K_s$ values of strain P17 were 0.04 and 0.34 μM for arginine and aspartate, respectively (28). This organism seems much better adapted to growth at low concentrations of individual amino acids than strain P1525.

**Utilization of carboxylic acids, carbohydrates, polyalcohols, and aromatic acids at low concentrations by *P. aeruginosa*.** Low concentrations of lactate, pyruvate, succinate, and particularly acetate clearly promoted growth of *P. aeruginosa* P1525 and 6324 (Fig. 1). Yet the $K_s$ value of strain P1525 for acetate (1.2 μM) is high compared to that of strain P17 (0.17 μM) for this substrate (28). Glucose and glycerol uptake by *P. aeruginosa* are dependent on inducible transport systems with a $K_t$ value of about 8 μM (8, 17, 24). This value, which is 10 times above the $K_s$ value of strain P17 for glucose (28), may explain the low growth rates of strain P1525 with glucose and glycerol, each at a concentration of 25 μg of C per liter (Fig. 1). The aromatic acid *p*-hydroxybenzoate is an excellent substrate for *P. aeruginosa* (Table 2) and for nearly all fluorescent pseudomonads (26) when present at a high concentration. At 25 μg of *p*-hydroxybenzoate C per liter, growth of strain P1525 is very slow (Fig. 1). Therefore, the $K_s$ value of strain P1525 for this compound seems to be higher than the one obtained for strain P17 (0.76 μM) (28).

The poor growth of strain P1525 with the CA, CHA, and AR mixtures (Table 5) seemed only partly due to a lack of sufficient suitable substrates in these mixtures. The difference between $N_{max}$ values observed with the total mixture and the AA mixture indicates that about 10 compounds other than amino acids were taken up at 1 μg of C per liter during growth with the total mixture. The identity of these compounds

TABLE 7. Growth of *P. aeruginosa* P1525 and 6324 and *P. fluorescens* P17 at 15°C in four types of tap water, in ozonated water, and in river water

| Water source | TOC (mg/liter) | Growth of strain: | | | | | |
|---|---|---|---|---|---|---|---|
| | | P17 | | P1525 | | 6324 | |
| | | G (h) | $N_{max}$ (CFU/ml) | G (h) | $N_{max}$ (CFU/ml) | G (h) | $N_{max}$ (CFU/ml) |
| Tap water 1[a] | 2.1 | 17.9 | $1.2 \times 10^5$ | 47.8 | $9.3 \times 10^4$ | 29.1 | $1.2 \times 10^5$ |
| | | 14.6 | $9.9 \times 10^4$ | 42.7 | $9.1 \times 10^4$ | 22.1 | $1.2 \times 10^5$ |
| Tap water 2[b] | 2.8 | 18.8 | $8.4 \times 10^4$ | 388 | $3.7 \times 10^2$ | 28.6 | $9.7 \times 10^4$ |
| | | 19.5 | $6.9 \times 10^4$ | 155 | $5.7 \times 10^2$ | 35.9 | $7.1 \times 10^4$ |
| Tap water 3[c] | 5.8 | 8.7 | $1.9 \times 10^5$ | 15.9 | $1.1 \times 10^5$ | 26.2 | $1.5 \times 10^5$ |
| | | 9.9 | $1.7 \times 10^5$ | 22.3 | $1.3 \times 10^5$ | 22.5 | $1.4 \times 10^5$ |
| Tap water 4[d] | 2.3 | 25.5 | $5.9 \times 10^3$ | 33.3 | $1.1 \times 10^3$ | 72.0 | $4.7 \times 10^3$ |
| | | 24.2 | $5.5 \times 10^3$ | 39.1 | $9.7 \times 10^2$ | 60.1 | $6.7 \times 10^3$ |
| Ozonated water[e] | 2.9 | 5.3 | $8.4 \times 10^5$ | 7.8 | $9.7 \times 10^5$ | —[g] | — |
| | | 5.1 | $8.3 \times 10^5$ | 8.3 | $9.5 \times 10^5$ | — | — |
| River water[f] | 3.4 | 4.1 | $3.5 \times 10^5$ | 9.3 | $2.7 \times 10^5$ | 8.3 | $4.9 \times 10^5$ |
| | | 4.2 | $3.4 \times 10^5$ | 9.5 | $3.1 \times 10^5$ | 6.8 | $4.8 \times 10^5$ |

[a] Prepared from stored river (Meuse) water by coagulation/sedimentation, ozonation, dual-media filtration, activated-carbon filtration, and chlorination.

[b] Prepared from pretreated and dune-infiltrated river (Lek) water by rapid sand filtration, slow sand filtration, and chlorination.

[c] Prepared from polder water after precoagulation and storage in an open reservoir followed by rapid sand filtration, ozonation, coagulation/sedimentation, rapid sand filtration, slow sand filtration, and chlorination.

[d] Prepared from anaerobic ground water by aeration and rapid filtration.

[e] Stored river (Meuse) water, ozonated after coagulation.

[f] From the river Lek, which receives its water from the river Rhine.

[g] —, Not tested.

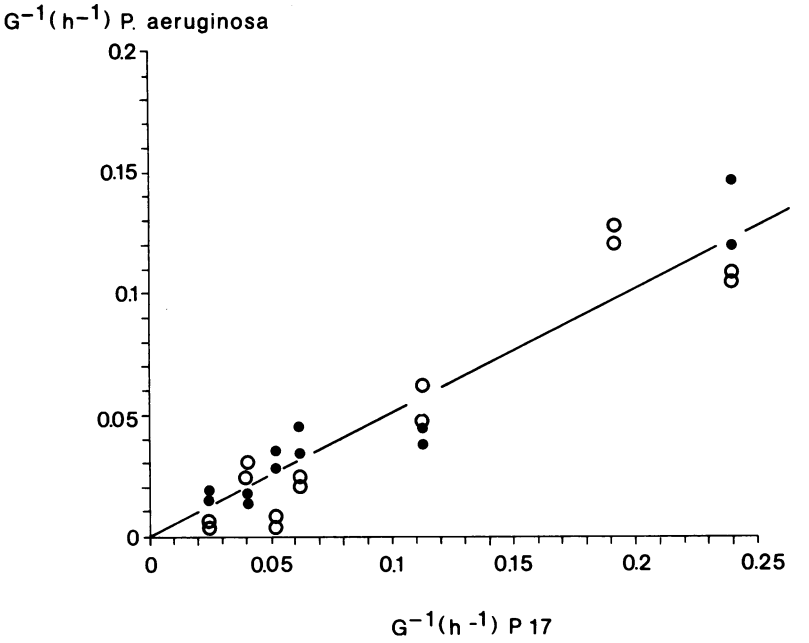


FIG. 5. Growth rates ($G^{-1}$ [$h^{-1}$]) at 15°C of *P. aeruginosa* P1525 (○) and 6324 (●) in various types of water (cf. Table 7) in relation to the growth rates of *P. fluorescens* P17 in these waters.

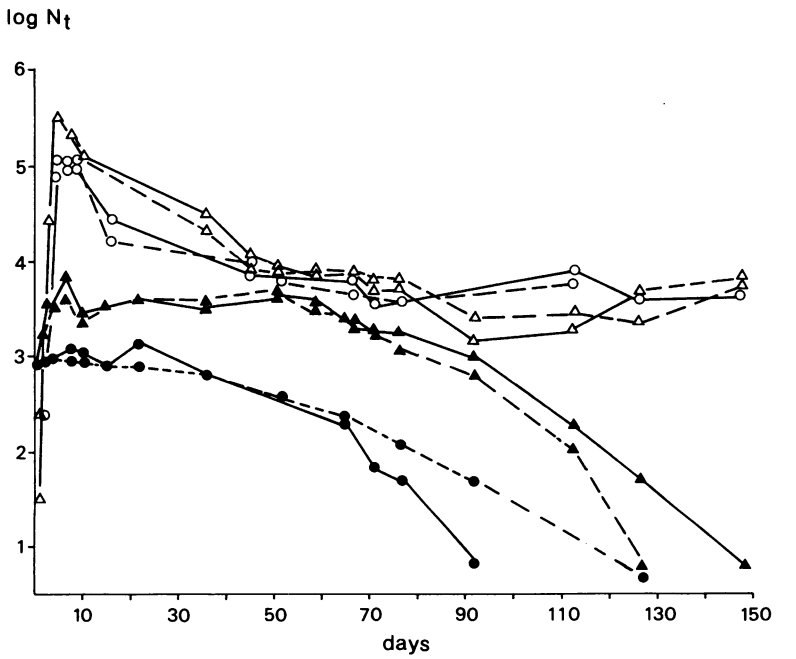


FIG. 6. Growth curves of *P. aeruginosa* 6324 in the presence of autochthonous bacteria in tap water and in tap water supplied with the total mixture of substrates (46 μg of C per liter); Symbols: (●) strain 6324 and (○) autochthonous bacteria in the tap water without substrates added; (▲) strain 6324 and (△) autochthonous bacteria in the tap water supplied with substrates. Solid and dashed lines represent duplicate experiments. For colony counts ($N_t$) of strain 6324, the agar plates were incubated at 37°C; colonies of the autochthonous bacteria were counted after incubation of the plates at 25°C for 10 days.

was not further determined, but the rapid growth with a number of carboxylic acids (Fig. 1) suggests that at least a few of these compounds were involved. However, half the number of potential substrates other than the amino acids were not utilized by *P. aeruginosa* cells growing with amino acids (total mixture).

**Growth of *P. aeruginosa* in water without added substrates.** Despite the ability of *P. aeruginosa* to utilize amino acids and a number of carboxylic acids at very low concentrations, the organism grew at a low rate in the types of tap water tested. The AOC concentration available for *P. aeruginosa* in these water types constituted only 0.1 to 0.7% of the TOC concentration. The identity of the utilized compounds remains unknown, and it cannot be excluded that the *P. aeruginosa* strains studied are able to grow with more compounds at low concentrations than those tested in this study (Fig. 1). Yet it is clear that dissolved free amino acids and carboxylic acids as used in the described experiments are a very minor part of the organic carbon in tap water. This may be the result of the various filtrations with biological activity, viz., rapid sand filtration, activated-carbon filtration, and slow sand filtration (Table 7). The AOC concentration in river water and ozonated water was 2.3 and 5.4% of the TOC concentration, respectively. In ozonated water, biodegradable low-molecular-weight compounds are present as a result of the effect of ozone on the large molecules of humic and fulvic acids.

The $N_{max}$ values for tap water obtained in this study were 10 to 100 times below those of other *P. aeruginosa* strains grown in distilled water, in inorganic salt solutions, and in tap water (1, 3, 6). The maximum colony counts reported in those papers ($10^6$ to $10^7$ CFU/ml) indicate that the waters examined were not really poor in organic substrates, because AOC concentrations calculated from these $N_{max}$ values and a $Y_a$ value of $6.2 \times 10^9$ CFU/mg of C (Table 4) varied from 0.16 to 1.6 mg of C per liter.

In most water types tested in this study, growth rates of *P. fluorescens* P17 were about twice as high as those of the *P. aeruginosa* isolates (Fig. 5). $G_{min}$ values of strain P17 (28) were about half those of strain P1525 on acetate and aspartate at 15°C (Table 6), possibly as a result of the mesophilic character of *P. aeruginosa*. Therefore, differences between growth rates of strain P17 and those of the *P. aeruginosa* strains in the various types of water may be due mainly to differences in $G_{min}$ values, suggesting that the average $K_s$ values of strains P17 and P1525 with substrates present in the water are similar. Such a similarity would be in contrast with the difference between $K_s$ values of strains P17 and P1525 as observed in this study for single substrates. This difference may be less pronounced when the organisms are growing with a number of substrates.

A large variety of *P. aeruginosa* serotypes have been observed in aquatic environments (9). To what extent specific serotypes differ in their adaptation to low substrate concentrations is not clear, but the differences observed between strains P1525 and 6324 (Fig. 1; Table 7) demonstrate some nonhomogeneity of the species in this respect. The presence of specific *P. aeruginosa* types in water may also be affected by other properties, e.g., slime production in chlorinated water (21). *P. fluorescens* P17 resembles the *P. aeruginosa* isolates with respect to their nutritional versatility (Table 1) and their ability to denitrify (26). Yet fluorescent pseudomonads belonging to the same biotype as strain P17 are found far more frequently in tap water and in surface water than *P. aeruginosa* (26). The ability of strain P17 to grow more rapidly than *P. aeruginosa* in water at relatively low temperatures while utilizing similar substrates (Fig. 4) explains this difference. At water temperatures where $G_{min}$ of *P. aeruginosa* is equal to or below $G_{min}$ of the psychrotrophic fluorescent pseudomonads, the latter organisms may become replaced by *P. aeruginosa*. Indications for such a temperature effect have been presented repeatedly (9, 14, 20, 23). The minor contribution of fluorescent pseudomonads to the bacterial flora of water (25) suggests that in these situations *P. aeruginosa* will also be only a minor component of the bacterial flora.

## ACKNOWLEDGMENTS

This investigation was part of the research program of KIWA Ltd., assigned and financed by the Netherlands Waterworks Association (VEWIN).

The authors are grateful to E. G. Mulder and M. H. Deinema for their critical reading of the manuscript.

The kind cooperation of the four waterworks involved in this study is gratefully acknowledged.

## LITERATURE CITED

1. **Botzenhart, K., and R. Kufferath.** 1976. On the growth of various *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Alcaligenes* spec. in distilled water, deionized water tap water and mineral salt solution. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **163**:470–485.
2. **Botzenhart, K., and S. Röpke.** 1971. Lebensfähigkeit und Vermehrung von *Pseudomonas aeruginosa* in anorganischen Salzlösungen. Arch. Hyg. **154**:509–516.
3. **Botzenhart, K., R. Wolf, and E. Thofern.** 1975. The behaviour of *Pseudomonas aeruginosa* in surface water, cooling water and waste water. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **161**:72–83.
4. **Clark, J. A.** 1980. The influence of increasing numbers of nonindicator organisms upon the detection of indicator organisms by the membrane filter and presence-absence test. Can. J. Microbiol. **26**:827–832.
5. **Dickgiesser, N., and F. Rittweger.** 1979. Examinations on the behaviour of Gram-positive and Gram-negative bacte-

ria in aqua bidest and tap water at different initial colony counts and different temperatures. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **169**:308–319.

6. **Favero, M. S., L. A. Carson, W. W. Bond, and N. J. Petersen.** 1971. *Pseudomonas aeruginosa*: growth in distilled water from hospitals. Science **177**:836–838.
7. **Geldreich, E. E., H. D. Nash, D. J. Reasoner, and R. H. Taylor.** 1975. The necessity of controlling bacterial populations in potable waters, bottled water and emergency water supplies. J. Am. Water Works Assoc. **67**:117–124.
8. **Guymon, L. F., and R. G. Eagon.** 1974. Transport of glucose, gluconate, and methyl α-D-glucoside by *Pseudomonas aeruginosa*. J. Bacteriol. **117**:1261–1269.
9. **Hoadley, A. W.** 1977. Potential health hazards associated with *Pseudomonas aeruginosa* in water, p. 80–114. *In* A. W. Hoadly and B. J. Dutka (ed.), Bacterial indicators/health hazards associated with water, ASTM STP 635. American Society for Testing and Materials, Philadelphia.
10. **Hoadley, A. W., E. McCoy, and G. A. Rohlich.** 1968. Untersuchungen über *Pseudomonas aeruginosa* in Oberflächengewässern. I. Quellen. Arch. Hyg. **152**:328–338.
11. **Hoshino, T.** 1979. Transport system for branched-chain amino acids in *Pseudomonas aeruginosa*. J. Bacteriol. **139**:705–712.
12. **Kay, W. W., and A. F. Gronlund.** 1969. Amino acids transport in *Pseudomonas aeruginosa*. J. Bacteriol. **97**:273–281.
13. **Kay, W. W., and A. F. Gronlund.** 1971. Transport of aromatic amino acids by *Pseudomonas aeruginosa*. J. Bacteriol. **105**:1039–1046.
14. **Labonde, J., and B. Festy.** 1979. Bilan d'une recherche systematique de *Pseudomonas aeruginosa* dans les eaux de consommation. Rev. Inst. Pasteur. Lyon **12**:507–517.
15. **Lantos, J., M. Kiss, B. Lanyi, and J. Völlgyesi.** 1969. Serological and phage typing of *Pseudomonas aeruginosa* invading a municipal water supply. Acta Microbiol. Acad. Sci. Hung. **16**:333–336.
16. **Mercenier, A., J. P. Simon, D. Haas, and V. Stalon.** 1980. Catabolism of L-arginine by *Pseudomonas aeruginosa*. J. Gen. Microbiol. **116**:381–389.
17. **Midgley, M., and E. A. Dawes.** 1973. The regulation of transport of glucose and methyl α-glucoside in *Pseudomonas aeruginosa*. Biochem. J. **132**:141–154.
18. **Moench, T. T., and W. A. Konetzka.** 1978. Chemotaxis in *Pseudomonas aeruginosa*. J. Bacteriol. **133**:427–429.
19. **Moulton, R. C., and T. C. Montie.** 1979. Chemotaxis by *Pseudomonas aeruginosa*. J. Bacteriol. **137**:274–280.
20. **Reitler, R., and R. Seligmann.** 1957. *Pseudomonas aeruginosa* in drinking water. J. Appl. Bacteriol. **20**:145–150.
21. **Seyfried, P. L., and D. J. Fraser.** 1980. Persistence of *Pseudomonas aeruginosa* in chlorinated swimming pools. Can. J. Microbiol. **26**:350–355.
22. **Schubert, R., and U. Blum.** 1974. Zur Frage der Erweiterung der hygienischen Wasserkontrolle auf den Nachweis von *Pseudomonas aeruginosa*. Gas Wasserfach **115**:224–226.
23. **Schubert, R., and P. Scheiber.** 1975. Das Vorkommen von *Pseudomonas aeruginosa* im Grundwasser, Oberflächenwasser und in Wasserversorgungsleitungen in tropischen Gebieten. Gas Wasserfach **116**:413–415.
24. **Tsay, S.-S., K. K. Brown, and E. T. Gaudy.** Transport of glycerol by *Pseudomonas aeruginosa*. J. Bacteriol. **108**:82–88.
25. **Van der Kooij, D.** 1977. The occurrence of *Pseudomonas* spp. in surface water and in tap water as determined on citrate media. Antonie van Leeuwenhoek. J. Microbiol. **43**:187–197.
26. **Van der Kooij, D.** 1979. Characterization and classification of fluorescent pseudomonads isolated from tap water and surface water. Antonie van Leeuwenhoek. J. Microbiol. **45**:225–240.
27. **Van der Kooij, D., A. Visser, and W. A. M. Hijnen.** 1980. Growth of *Aeromonas hydrophila* at low concentrations of substrates added to tap water. Appl. Environ. Microbiol. **39**:1198–1204.
28. **Van der Kooij, D., A. Visser, and J. P. Oranje.** 1982. Multiplication of fluorescent pseudomonads at low substrate concentrations in tap water. Antonie van Leeuwenhoek J. Microbiol. **48**:229–243.
29. **Wheater, D. W. F., D. D. Mara, L. Jawad, and J. Oragui.** 1980. *Pseudomonas aeruginosa* and *Escherichia coli* in sewage and fresh water. Water Res. **14**:713–721.
30. **Windle-Taylor, E.** 1971. Silt deposits in service reservoirs, p. 39–42. Forty-fourth report on the results of the bacteriological, chemical and biological examination of the London waters for the years 1969–1970. Metropolitan Water Board, London.