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Multiplication of fluorescent pseudomonads at low substrate concentrations in tap water

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Two fluorescent pseudomonads, strains P17 and P500, belonging to different biotypes were tested for growth in tap water supplied with different concentrations of acetate and glutamate, low concentrations (10 and 20 μg of C per liter) of various other substrates and mixtures of related substrates, the latter being present in amounts of 1 μg of C per liter each. Amino acids appeared to be excellent substrates for both isolates, but many other substrates were utilized at very low concentrations as well. Saturation constants (K_s) of P17 with acetate, arginine, aspartate, glutamate, lactate, succinate, malonate, *p*-hydroxybenzoate and glucose were all below 1 μM. The K_s values of strain P500 were about 5 times larger than those of P17. Since especially P17 is able to use a large number of different substrates at low concentrations, assessment of maximal colony counts of this organism by growth experiments in various types of tap water may give information about the concentrations of easily assimilable organic carbon.

INTRODUCTION

Despite their frequent isolation, fluorescent pseudomonads usually constitute small minorities (<1%) of the bacterial populations of surface and tap water (Van der Kooij, 1977), silt deposits in drinking water service reservoirs (Windle Taylor, 1971–73), raw and treated sewage (Hankin and Sands, 1974) and soil (Rovira and Sands, 1971). Characterization of fluorescent pseudo-

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monads obtained from surface water and various types of drinking water showed that these bacteria belonged to at least 45 different biotypes (Van der Kooij, 1979*a*), many of which were similar to those defined by Stanier et al. (1966). This characterization study further revealed distinct relationships between the various water types and the occurrence of representatives of specific biotypes. Unfortunately, most characters suited for classification have no ecological significance, since they do not give information about the ability of the fluorescent pseudomonads to multiply with very low concentrations of substrates. The growth of representatives of two clearly different biotypes in tap water supplied with low concentrations of substrates was studied to explain differences observed in the presence of these biotypes in various types of tap water as part of an investigation into the behaviour of bacteria in drinking water during distribution.

Materials and Methods

Isolates

The isolation of bacterial strains used in this study as well as their characterization have been described previously (Van der Kooij, 1977; 1979*a*). The following strains were used: *Pseudomonas fluorescens* P17, originating from tap water prepared from dune infiltrated river water and belonging to biotype 7.2 which corresponds to biotype C (Stanier et al., 1966), and *P. fluorescens* P500, obtained from tap water prepared from river water by physicochemical treatment and belonging to biotype 1.1, which corresponds to biotype G (Stanier et al., 1966). Both biotypes were generally observed in surface water and tap water prepared from surface water. Biotype 7.2 was also found in ground water. Both isolates are proteolytic and do not multiply at 37°C. Isolate P17 is able to produce gaseous nitrogen from nitrate under anaerobic conditions.

Replica test

Utilization of organic compounds as sources of carbon and energy for growth was tested by the replica technique using plates of basal salts agar with separately sterilized carbon compounds in a concentration of 2.5 g per liter (Van der Kooij, 1979*a*). The carbon compounds tested are listed in Table 1.

Growth experiments in liquid media

The growth experiments were performed in 1-liter, calibrated, glass-stoppered Erlenmeyer flasks of Pyrex glass. These flasks were cleaned with a 10% solution of $K_2Cr_2O_7$ in concentrated H_2SO_4, followed by rinsing with hot tap water, with 10% HNO_3 and with hot tap water again. Thereafter, they were heated overnight at 250–300°C. The pipettes (1 ml) were cleaned in the same way. The cleaned flasks were filled with 600 ml of tap water originating from

Table 1. Compounds tested as sources of carbon and energy for growth of the fluorescent pseudomonads P17 and P500.

	Amino acids (AA)	Carboxylic acids (CA)	Carbohydrates and (poly)alcohols (CHA)	Aromatic acids (AR)
Compounds utilized by P17 and P500	L-alanine, L-valine, L-leucine,L-isoleucine, L-lysine, L-arginine, L-aspartate, L-asparagine, L-glutamate, L-glutamine, L-tyrosine, L-proline.	acetate, propionate, DL-lactate, pyruvate, malonate, fumarate, succinate, citrate	D-glucose, D-mannitol, glycerol	*p-hydroxy*-benzoate
Compounds utilized by P17 only	DL-serine, L-threonine, L-histidine, DL-tryptophan	adipate	ethanol	benzoate anthranilate
Compounds utilized by P500 only	DL-phenylalanine		D-arabinose	
Not utilized	glycine	formate, glycolate, glyoxylate, oxalate, L-tartrate	D-maltose	vanillate, phtalate, nicotinate, DL-mandelate

the municipal Dune Waterworks of The Hague, where it is prepared from dune-infiltrated river water by the addition of powdered activated carbon, followed by rapid and slow sand filtration. This water contained 3.6 mg of dissolved organic carbon and 7.3 mg of nitrate per liter; the pH was 7.6 (average values).

For cultures in tap water, the water was not autoclaved but heated for 2 hours at 60 °C. This treatment was chosen to preserve the organic content of the water as much as possible in its original state. The counting plates done in the course of the experiments showed that bacteria originally present in the water were effectively removed by this treatment. Solutions of single organic compounds and of nitrogen compounds were also heated at 60 °C. Unless otherwise stated, NH_4Cl was added with the carbon compounds to obtain a C/N ratio similar to that of ammonium acetate. In a few experiments the water was supplied with mixtures of amino acids (AA), carboxylic acids (CA), carbohydrates and (poly)alcohols (CHA) and aromatic acids (AR), as listed in Table 1. Stock solutions of the 4 mixtures were prepared by dissolving the individual compounds in demineralized water in equal carbon concentrations, except glutamate which was used at twice this concentration. After neutralization with a sodium hydroxide solution, the mixtures were sterilized (120 °C, 16 min) in Pyrex glass bottles containing a screw cap with a teflon shield. Addition of

NH_4Cl was omitted in the experiments with the mixtures, as it had become clear from previous experiments that N was no limiting factor.

Inoculation and determination of bacterial growth in liquid media

Turbid suspensions of 24-h slant cultures of the isolates were used for inoculation of 50 ml of autoclaved tap water contained in 100-ml normally washed infusion bottles. The initial number of cells in the inoculated water amounted to about 100 to 500 colony-forming units (CFU) per ml. Incubation of the infusion bottles at 25°C gave maximal colony counts (N_{max}) varying from 10^5 to 2.10^5 CFU/ml which were reached within one or two weeks. Hereafter, the colony counts decreased very slowly during a period of several months. These cultures were used for inoculating the experimental solutions to provide an initial cell density of 50 to 300 CFU/ml.

Incubation was carried out at 15 ± 0.5°C. Growth curves were obtained by periodic determination of the number of viable cells (N_t, CFU/ml) in triplicate. This was done by spreading 0.05 ml of the culture either directly or from decimal dilutions on predried Lab-Lemco (Oxoid) agar plates. Counting was performed after 40–48 h of incubation at 25°C. The doubling time (G in hours) of the cultures was calculated with the equation:

$$G = (t' - t) \log 2/(\log N_{t'} - \log N_t) \quad (1)$$

in which $t' - t$ = the incubation time (hours) in which N_t increased to $N_{t'}$. These calculations were performed for the period in which the growth curve was linear with time in a half-logarithmic plot and in which $N_{t'} < 0.1\ N_{max}$. All experiments were performed in duplicate, unless stated otherwise.

Results

Utilization of organic compounds as sources of carbon and energy

Table 1 shows that at a concentration of 2.5 g of substrate per liter, 24 of the tested compounds were utilized by both isolates, 8 other substrates only promoted the growth of P17 and two substrates were only utilized by P500.

Utilization of acetate and glutamate at low concentrations

At concentrations varying from 1 mg to 2.5 μg of C per liter acetate clearly enhanced the multiplication of P500 in tap water (Fig. 1). Similar observations were obtained for P500 with glutamate and P17 with glutamate and acetate, respectively (results not shown). From the maximal colony counts (N_{max}) obtained for the applied substrate concentrations (Fig. 2) the following yield values (Y) were calculated: $Y(P500) = 4 \times 10^9$ CFU/mg acetate C and 4×10^9 CFU/mg of glutamate C and $Y(P17) = 4.2 \times 10^9$ CFU/mg of acetate C. N_{max} values for P17 with glutamate were not estimated.

Using these yield data and the N_{max} values in the blanks, the natural substrate concentration (S_n) available for the two pseudomonads in the heated tap

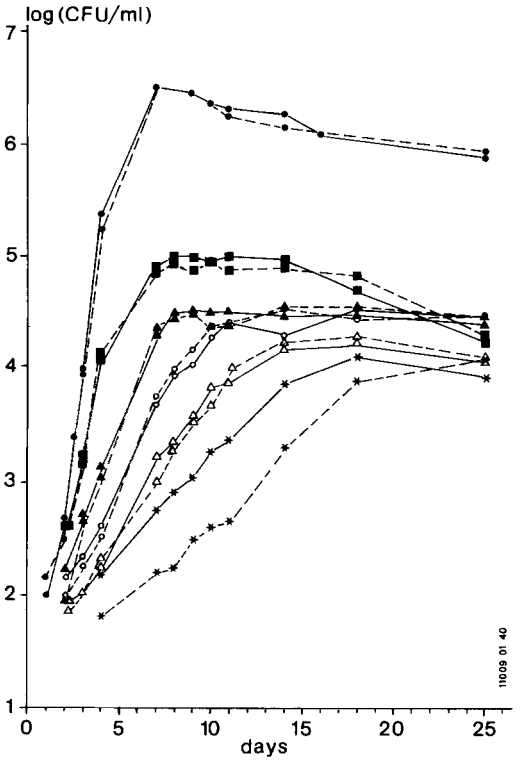

Fig. 1. Growth curves of *P. fluorescens* P500 at 15°C ± 0.5°C with different concentrations of acetate added to tap water: ●——● and●— —●, 1 mg; ■——■ and ■— —■, 25 µg; ▲——▲ and ▲— —▲, 10 µg; ○——○ and○— —○, 5 µg; △——△ and △— —△, 2.5 µg of acetate C per liter; ★——★ and ★— —★, blanks.

water may be expressed in equivalents of acetate C or glutamate C, giving $S_n(P500) = 0.7$ to 3.5 µg of acetate C or glutamate C equivalents per liter and $S_n(P17) = 4.5$ µg of acetate C equivalents per liter. These S_n values are only 0.1% of the concentration of dissolved organic carbon.

In Fig. 3, the doubling times of P17 and P500 (derived from Fig. 1) are plotted against the reciprocal values of the concentrations of added substrate. The linear part of the relationship is expressed by the following adapted Lineweaver-Burk equation:

$$G = G_{min} + G_{min} \cdot K_s/\Delta S \quad (2)$$

in which G is the observed doubling time (in hours) at the concentration of substrate added ΔS (in µg of C per liter); G_{min} = minimal G. K_s, the saturation constant, is the substrate concentration at which $G = 2G_{min}$. The non-linearity of the presented curves may be explained by concurrent utilization of the natu-

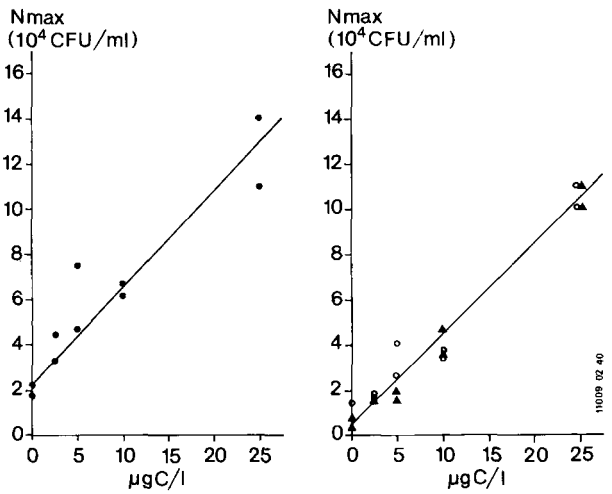

Fig. 2. Maximum colony counts of *P. fluorescens* P17 and P500 at different concentrations of acetate and glutamate added to tap water: •, P17 on acetate; ○, P500 on acetate; ▲, P500 on glutamate.

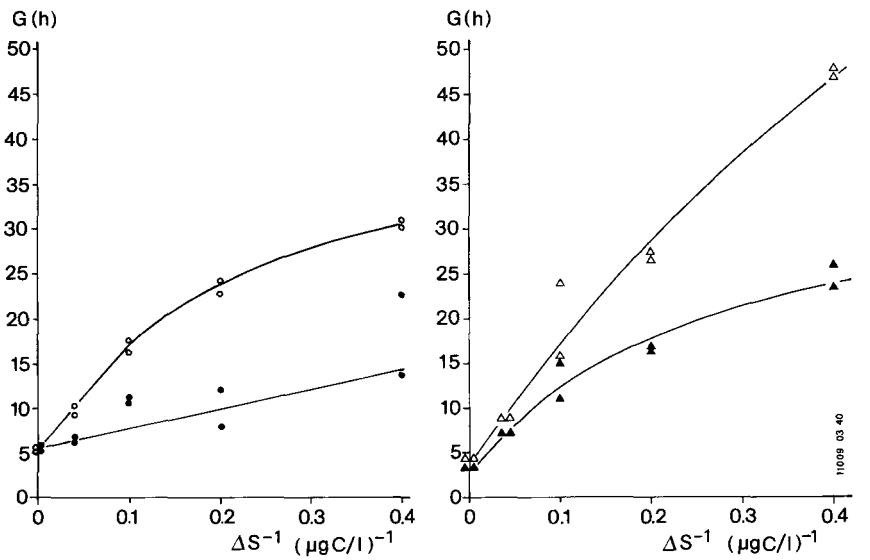

Fig. 3. Lineweaver-Burk plots of the growth of *P. fluorescens* P17 and P500 at low concentrations of acetate and glutamate added to tap water: •, P17 on acetate; ○, P500 on acetate; ▲,P17 on glutamate; △, P500 on glutamate.

Table 2. Growth constants of *P. fluorescens* P17 and *P. fluorescens* P500 with acetate and glutamate calculated from Fig. 3.

Isolate	Substrate	Lineweaver-Burk equation[1]	K_s		Growth in blanks G(h)	S_{max}[2] (µg C/l)
			(µg C/l)	(µM)		
P500	glutamate	$G = 3.7 + 130.2/\Delta s$	34.8	0.58	86.3; 60.5	1.6–2.3
P17	glutamate	$G = 3.3 + 97.6/\Delta s$	29.5	0.49	43.4; 27.5	2.4–4.0
P500	acetate	$G = 5.2 + 118.3\Delta/s$	22.8	0.95	48.5; 45.1	2.7–3.0
P17	acetate	$G = 5.5 + 21.8/\Delta s$	4.0	0.17	22.1 21.6	1.3–1.4

[1] $G = G_{min} + G_{min} \cdot K_s/\Delta s$
[2] Maximal natural concentration of the substrate involved.

ral substrates, which obviously was important when $\Delta S < 10$ µg of C/l, or when G according to eq.2 was above 20 to 30% of G of the blanks. From the plots in Fig. 3, values for G_{min} and $G_{min} \times K_s$ were derived as presented in the equations listed in Table 2, which permitted calculations of K_s. It appears from Table 2 that P17 is better adapted (lower K_s value) to low concentrations of acetate than P500.

Using the listed equations and the G values found in the blanks, estimations could be made of the maximal natural concentrations (S_{max}) of either acetate or glutamate that might have been present in the tap water used (Table 2). Comparison of the acetate C concentrations, calculated from the G values of P17, with S_n obtained from the N_{max} values indicates that acetate might have been at most 30% of the substrate available for isolate P17 in the tap water.

Utilization of various compounds at very low concentrations

Experiments with a number of substrates, representing different types of naturally occurring organic compounds of low molecular weight, revealed that the rates of growth (G^{-1}) of P17 and P500 in tap water were increased by the addition of 20 µg of substrate C per liter of all compounds tested except fumarate (Fig. 4). Fig. 5 indicates that fumarate was used by P17 after the exhaustion of S_n. P500 was unable to utilize fumarate when present in a concentration of 20 µg of C per liter. Strain P17 multiplied more rapidly with the compounds tested than isolate P500. A number of compounds promoted growth more clearly than others. The latter effect was even more pronounced at initial concentrations of 10 µg of C per liter (P17 only). P17 multiplied more rapidly at 10 µg of arginine C per liter than at 20 µg of C per liter in the form of all the other substrates tested.

The coefficients of the modified Lineweaver-Burk equations (2) for growth with the various substrates were calculated using the generation times at 1 mg of C per liter and those observed at 20 µg of substrate C per liter (Table 3). With arginine, the generation time at 10 µg of C was used. With a few compounds, especially malonate, *p*-hydroxybenzoate and glucose, natural sub-

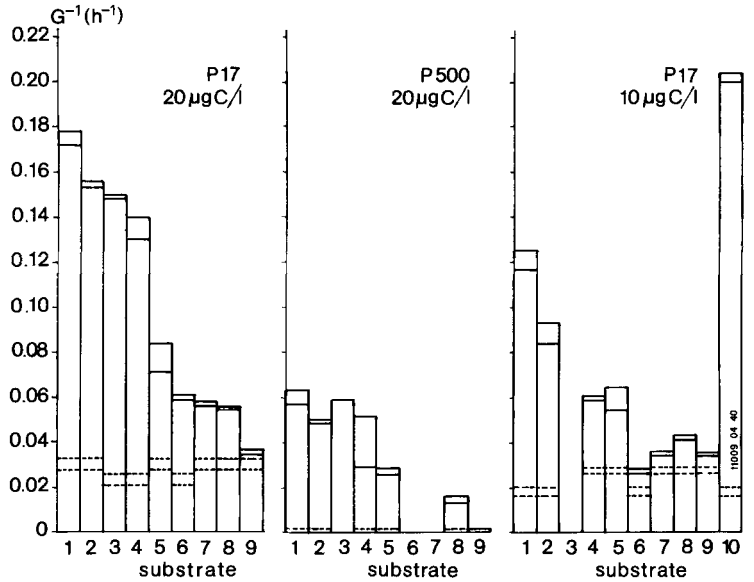

Fig. 4. Growth rates (G^{-1}) of *P. fluorescens* P17 and P500 at 15 ± 0.5°C with 20 and 10 µg C of different substrates added to tap water 1 = L-aspartate; 2, succinate; 3, acetate; 4, DL-lactate; 5, yeast extract; 6, malonate; 7,*p*-hydroxybenzoate; 8,D-glucose; 9, fumarate; 10, L-arginine. ——, G^{-1} observed for growth with the added substrate; – – – G^{-1} observed without substrate added; observations in duplicate. For P500 with 20 µg of acetate C/l, G^{-1} was calculated using the equation in Table 2.

strates may have significantly affected the generation times at low substrate concentrations. In these cases calculations were made with the assumption of complete preferential uptake of the added substrates. The substrate affinities of P500 were calculated assuming that this isolate has generation times similar to those of isolate P17 at 1 mg of substrate C per liter.

The K_s values obtained with P17 were all below 1 µM, and a few were extremely low, e.g. 0.04 µM with arginine and 0.16 µM with malonate (Table 3). The K_s for malonate C approximates the one for acetate (Table 2). The K_s values of P500 were clearly above those of P17. The slopes of the Lineweaver-Burk equations for P500 on aspartate, succinate, lactate and glucose were all about 5 times steeper than for isolate P17 on the same compounds. This factor which was also observed with acetate, but not with glutamate (Table 2), appears to be a characteristic difference between the strains and possibly between the biotypes to which the isolates belong.

Utilization of mixtures of substrates at very low concentrations

In natural habitats, including water, S_n is composed of low concentrations of a great variety of compounds. For this reason and to obtain rapid informa-

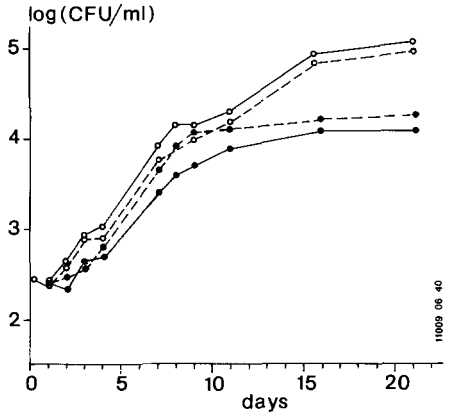

Fig. 5. Growth curves of *P. fluorescens* P17 at 15 ± 0.5°C in tap water supplied with a low concentration of fumarate: ●——● and ●– –●, tap water without substrate added; ○——○ and ○– –○, tap water with 20 µg of fumarate C per liter.

Table 3. Modified Lineweaver-Burk equations and K_s values of strains P17 and P500 with various substrates[1].

Isolate	Substrate	G(h) at 1 mg C/l	Lineweaver-Burk equation[2]	K_s (µg C/l)	K_s (µM)
P17	L-arginine	3.8; 3.9	$G = 3.9 + 11.0/\Delta s$	2.8	0.04
P17	L-aspartate	3.1; 3.3	$G = 3.1 + 51.0/\Delta s$	16.4	0.34
P17	succinate	3.1; 3.1	$G = 3.0 + 68.4/\Delta s$	22.8	0.48
P17	DL-lactate	3.5; 3.8	$G = 3.6 + 77.3/\Delta s$	21.4	0.59
P17	*p*-hydroxy-benzoate	4.4; 4.4	$G = 4.1 + 264.3/\Delta s$	64.4	0.76
P17	D-glucose	4.8; 5.0	$G = 4.6 + 264.3/\Delta s$	57.4	0.79
P17	malonate	11.6; 14.6	$G = 13.0 + 74.4/\Delta s$	7.5	0.16
P500	L-aspartate	as P17	$G = 2.9 + 274/\Delta s$	94	1.9
P500	succinate	as P17	$G = 2.8 + 347/\Delta s$	124	2.6
P500	DL-lactate	as P17	$G = 3.2 + 464/\Delta s$	145	4.0
P500	D-glucose	as P17	$G = 3.6 + 1341/\Delta s$	372	5.2

[1] In all cases preferential uptake of the added substrate is assumed.
[2] $= G_{min} + G_{min} \cdot K_s/\Delta s$.

tion about the ability of the isolates to utilize many different compounds at low concentration (1 µg of C per liter), growth experiments were performed in tap water supplied with the mixtures of 18 amino acids (AA), 6 carbohy-

Table 4. Maximal colony counts of the fluorescent pseudomonads P17 and P500 grown at 15°C in tap water supplied with mixtures of compounds

Mixture[1] added	Total concentration[2] (µg C/l)	Strain P17			Strain P500		
		Conc. of compounds used[3] (µg C/l)	N_{max} (10^4CFU/ml)	Y_a (10^9CFU/mg C)	Conc. of compounds used[3] (µg C/l)	N_{max} (10^4CFU/ml)	Y_a[4] (10^9CFU/mg C)
None	–	–	2.3; 2.3	–	–	2.9; 5.0	–
AA	19	17	15; 16	7.8	14	4.8; 8.1	3.0
CA	14	9	4.6; 5.8	3.2	8	3.8; 6.9	3.9
CHA	6	4	4.1; 4.8	5.4	4	2.5; 3.5	2.0
AR	7	3	3.5; 4.2	5.2	1	2.2; 2.2	< 1
TM	46	33	22; 23	6.1	27	6.2; 6.4	1.5

[1] AA = amino acids; CA = carboxylic acids; CHA = carbohydrates and (poly)alcohols; AR = aromatic acids; TM = total mixture.
[2] All compounds added in amounts of 1 µg of C per liter, except glutamate which was added in an amount of 2 µg of C per liter.
[3] Total concentration of compounds used as sources of carbon and energy for growth when present at 2.5 g/l (cf. Table 1).
[4] Due to unreliable N_{max} values in the blanks, Y_a of P 500 is calculated using N_{max} with the AR mixture as reference values for growth on S_n.

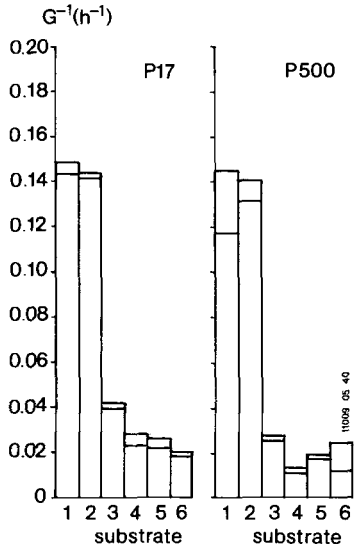

Fig. 6. Growth rates (G^{-1}) of *P. fluorescens* strains P17 and P500 at 15 ± 0.5°C in the presence of mixtures of substrates. The concentration of the individual compounds was 1 µg C per liter, except for glutamate of which the double amount was added. 1, total mixture (46 µg of C per liter); 2, amino acids, 19 µg of C per liter; 3, carboxylic acids, 14 µg of C per liter; 4, aromatic acids, 7 µg of C per liter; 5, carbohydrates and (poly)alcohols, 6 µg of C per liter; 6, without substrates added; observations in duplicate.

drates and (poly) alcohols (CHA), 14 carboxylic acids (CA), 7 aromatic acids (AR) and the total mixture (TM). Fig. 6 shows that the growth rates (G^{-1}) with the amino acid mixture equalled those with the total mixture, suggesting that amino acids were preferred substrates for strains P17 and P500. Furthermore it is shown that from the other mixtures growth was most rapid with the carboxylic acids.

Using the N_{max} values observed and the numbers of compounds which may serve as a source of carbon and energy for growth (Table 1), average yields (Y_a in CFU/mg of substrate C) were calculated (Table 4). Most Y_a values of P17 exceeded or approximated the yield observed with growth on acetate. This suggests that all substrates which served as a source of carbon and energy for growth of P17 when present in 2.5 g/l were also utilized when present in 1 µg of C per liter. As compared with P17, P500 had relatively low average yields on the mixtures, which is clearly demonstrated with the total mixture of compounds. These differences might result from the inability of P500 to utilize a number of compounds at very low concentrations. Evidence for such a possibility is obtained by the inability of strain P500 to multiply in the presence of 20 µg of fumarate C per liter.

Discussion

This study and previous ones (Van der Kooij et al., 1980; Van der Kooij and Hijnen, 1981) reveal that simple growth experiments in batch cultures provide information about bacterial growth at low substrate concentrations. In such experiments colony counts are used as a parameter for biomass concentration. From the linear relationship between maximal colony count (N_{max}) and initial substrate concentrations as observed in this study (Fig. 2) and those mentioned above, it is concluded that this procedure is justified in a number of cases. A decrease in yield at very low concentrations as observed with an *Aeromonas hydrophila* isolate growing on glucose (Van der Kooij et al., 1980) was not found with the isolates P17 and P500 growing on acetate or glutamate (Fig. 2), probably because of the presence of the natural substrates in the tap water used. The interpretation of the generation times observed at initial substrate concentrations below 10 µg C per liter was also complicated by the utilization of S_n. (Fig. 3). These observations indicate that for growth experiments at extremely low substrate concentrations, the natural substrate concentration of the water is of critical importance. Drinking water prepared by biological treatment (e.g. slow sand filtration) seems to be most suited for this purpose.

Fluorescent pseudomonads are able to utilize a wide variety of naturally occurring compounds as sources of carbon and energy (Den Dooren de Jong, 1926; Stanier et al., 1966; Table 1). Fumarate which was consumed at 2.5 g per liter could not be utilized by P500 when present at 20 µg of C per liter. Previous investigations revealed that an *Aeromonas hydrophila* isolate did not grow with acetate, glutamate or succinate when these compounds were present in an amount of 10 µg of C per liter though these substrates were utilized at 1 mg of C per liter (Van der Kooij et al., 1980). These findings stress the importance of investigating growth responses at concentrations of a few µg per liter.

The significance of the naturally occurring amino acids as sources of carbon and energy for strains P17 and P500 was clearly demonstrated (Fig. 6; Table 4). Amino acids are also suitable substrates for *P. aeruginosa* (Stanier et al., 1966), which organism possesses constitutive transport systems for the uptake of these compounds at very low concentrations (Kay and Gronlund, 1969; 1971). Therefore, utilization of low concentrations of amino acids seems a common characteristic of the mesophilic and psychrotrophic fluorescent pseudomonads. This may explain why fluorescent pseudomonads occur in the bacterial populations on fresh plant debris and in rhizospheres in larger percentages than in soil itself (Rovira and Sands, 1971). Despite high-affinity uptake systems for amino acids, fluorescent pseudomonads appear to be unable to compete with many other bacteria, as may be concluded from the low percentage in which they are usually found in natural bacterial populations. Very low concentrations of dissolved free amino acids in natural environments (Burnison and Morita, 1974) combined with the presence of biodegradable compounds for

which the fluorescent pseudomonads do not have saturation constants as low as other bacteria, e.g. carbohydrates which are utilized by flavobacteria (Van der Kooij and Hijnen, 1981), may explain these observations.

The occurrence of representatives of specific biotypes of the fluorescent pseudomonads in specific environments as demonstrated by Rovira and Sands (1971) and Van der Kooij (1979*a*) points to significant differences between the various psychrotrophic fluorescent pseudomonads. The distinct differences in substrate affinities as demonstrated in this study help explain that bacteria similar to strain P17 (biotype 7.2, Van der Kooij, 1979*a*) were isolated more frequently from drinking water prepared from ground water than bacteria similar to strain P500 (biotype 1.1, Van der Kooij, 1979*a*). The relatively frequent occurrence of biotype 7.2 in various waters may further be related to its ability of using nitrate as a hydrogen acceptor combined with a high affinity for acetate, a compound which occurs in oxygen-depleted environments. The frequent predomination of biotype 1.1 in surface water and in tap water derived from surface water (Van der Kooij, 1979*a*) cannot be explained with the obtained K_s values, but the similarity of the growth rates of P17 and P500 with the amino acids mixture, which is in contrast with the differing saturation constants, may be important in this respect.

Experiments with fluorescent pseudomonads, including *P. aeruginosa*, using labelled substrates have frequently revealed high affinity transport systems which are highly substrate specific, though groups of related compounds, e.g. dicarboxylic acids, aromatic amino acids or aliphatic amino acids, may be transported by one and the same system (Kay and Gronlund, 1969, 1971; Tsay et al., 1971; Duber et al., 1974; Eisenberg et al., 1974; Hoshino, 1979; Romano et al., 1980). Although transport constants (K_t, 0.1–1 μM) resemble K_s values obtained for P17, it remains uncertain whether K_s and K_t should have similar numerical values. Such a similarity would indicate that the transport of a substrate is the growth limiting step. The observed constant difference in K_s values of strains P17 and P500 for compounds requiring a number of totally different transport systems (Tables 2 and 3) suggests that a metabolic process was limiting the growth of P500 in the described experiments. Arguments in support of this suggestion have been given by Kay and Gronlund (1969, 1971), who observed that transport of amino acids into cells of *P. aeruginosa* rapidly declined after a few minutes as a result of saturation of the pool with the unchanged compounds. Further uptake was depending on incorporation of these amino acids from the pool into cellular proteins. The rate of C_4-acid transport into cells of *Escherichia coli* also appeared to be determined by the rate at which these substrates were metabolized (Kay and Kornberg, 1971).

The experiments reported in this paper (Table 2) revealed that the N_{max} value of a pure culture in a specific water does provide information about the concentration of compounds available to the organism as a substrate. In addition, using the observed generation time, maximally possible concentrations of spe-

cific compounds for which the coefficients of the Lineweaver-Burk equations (Tables 2 and 3) are known, may be calculated. Since P17 is able to utilize a large variety of compounds at very low concentrations, growth experiments with this organism may be valuable for the assessment of the level of easily assimilable organic carbon (AOC) in water. In recent investigations, such growth experiments are being used to study the quality of various types of drinking water and for measuring the effects of water treatment procedures on biodegradable compounds in water (Van der Kooij, 1979*b*).

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References

BURNISON, B. K. and MORITA, R. Y. 1973. Competitive inhibition for amino acid uptake by the indigenous microflora of Upper Klamath Lake. — Appl. Microbiol. **25**: 103–106.

DEN DOOREN DE JONG. L. E. 1926. Bijdrage tot de kennis van het mineralisatieproces — Thesis, Delft.

DUBLER, R. E., TOSCANO, W. A. and HARTLINE, R. A. 1975. Transport of succinate by *Pseudomonas putida*. — Arch. Biochem. Biophys. **160**: 422–429.

EISENBERG, R. C., BUTTERS, S. J., QUAY, S. C. and FRIEDMAN, S. B. 1974. Glucose uptake and phosphorylation in *Pseudomonas fluorescens*. — J. Bacteriol. **120**: 147–153.

HANKIN, L., and SANDS, D. C. 1974. Bacterial production of enzymes in activated sludge systems. — J. Water Poll. Control. Fed. **46**: 2015–2025.

HOSHINO, T. 1979. Transport systems for branched-chain amino acids in *Pseudomonas aeruginosa*. — J. Bacteriol. **139**: 705–712.

KAY, W. W. and GRONLUND, A. F. 1969. Amino acid transport in *Pseudomonas aeruginosa*. — J. Bacteriol. **97**: 273–281.

KAY, W. W. and GRONLUND, A. F. 1971. Transport of aromatic amino acids by *Pseudomonas aeruginosa*. — J. Bacteriol. **105**: 1039–1046.

KAY, W. W. and KORNBERG, H. L. 1971. The uptake of C_4-dicarboxylic acids by *Escherichia coli*. — Eur. J. Biochem. **18**: 274–281.

ROMANO, A. H., VOYTEK, A. and BRUSKIN, A. M. 1980. Energization of glucose transport by *Pseudomonas fluorescens*. — J. Bacteriol. **142**: 755–762.

ROVIRA, A. D. and SANDS D. A. 1971. Fluorescent pseudomonads; a residual component in the soil microflora. — J. Appl. Bacteriol. **34**: 253–259.

STANIER, R. Y., PALLERONI, N. J. and DOUDOROFF, M. 1966. The aerobic pseudomonads: a taxonomic study. — J. Gen. Microbiol. **43**: 159–271.

STINSON, M. W., COHEN, M. A. and MERRICK, J. M. 1976. Isolation of dicarboxylic acid and glucose-binding proteins from *Pseudomonas aeruginosa*. — J. Bacteriol. **28**: 573–579.

TSAY, S. S., BROWN, K. K. and GAUDY, A. T. 1971. Transport of glycerol by *Pseudomonas aeruginosa*. — J. Bacteriol. **108**: 82–88.

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 **43**: 187–197.

VAN DER KOOIJ, D. 1979*a*. Characterization and classification of fluorescent pseudomonads isolated from tap water and surface water. — Antonie van Leeuwenhoek **45**: 225–240.

VAN DER KOOIJ, D. 1979*b*. Ontwikkeling van een methode om de nagroeimogelijkheid van bacteriën in drinkwater te bepalen. — H_2O **12**: 164–167.

VAN DER KOOIJ, D. and HIJNEN, W. A. M. 1981. Utilization of low concentrations of starch by a *Flavobacterium* species isolated from tap water. — Appl. Environ. Microbiol. **41**: 216–221.

VAN DER KOOIJ, D. VISSER, A. and HIJNEN, W. A. M. 1980. Growth of *Aeromonas hydrophila* at low concentrations of substrates added to tap water. — Appl. Environ. Microbiol. **39**: 1198–1204.

WINDLE TAYLOR, E. 1971–73. Silt deposits in service reservoirs. Metropolitan Water Board, Report **45**: 26.