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Growth Kinetics of Coliform Bacteria under Conditions Relevant to Drinking Water Distribution Systems

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The growth of environmental and clinical coliform bacteria under conditions typical of drinking water distribution systems was examined. Four coliforms (*Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter aerogenes*, and *Enterobacter cloacae*) were isolated from an operating drinking water system for study; an enterotoxigenic *E. coli* strain and clinical isolates of *K. pneumoniae* and *E. coli* were also used. All but one of the coliforms tested were capable of growth in unsupplemented mineral salts medium; the environmental isolates had greater specific growth rates than did the clinical isolates. This trend was maintained when the organisms were grown with low levels (<1 mg liter^{-1}) of yeast extract. The environmental *K. pneumoniae* isolate had a greater yield, higher specific growth rates, and a lower K_s value than the other organisms. The environmental *E. coli* and the enterotoxigenic *E. coli* strains had comparable yield, growth rate, and K_s values to those of the environmental *K. pneumoniae* strain, and all three showed significantly more successful growth than the clinical isolates. The environmental coliforms also grew well at low temperatures on low concentrations of yeast extract. Unsupplemented distribution water from the collaborating utility supported the growth of the environmental isolates. Growth of the *K. pneumoniae* water isolate was stimulated by the addition of autoclaved biofilm but not by tubercle material. These findings indicate that growth of environmental coliforms is possible under the conditions found in operating municipal drinking water systems and that these bacteria could be used in tests to determine assimilable organic carbon in potable water.

Occurrences of coliforms in finished drinking water in the absence of known breaches of treatment barriers continue to be a major problem in the drinking water industry and have emerged as a critical regulatory issue. Coliforms may grow within the distribution system at the expense of organic carbon. Sufficient quantities of other nutrients (nitrogen, phosphorus) must also be available in the water to support the proliferation of suspended or biofilm coliforms. Levels of bacterial nutrients present in potable water are typically low, with total organic carbon concentrations in finished drinking water varying from 0.05 to 12.2 mg liter^{-1} (9, 10). The microbially assimilable fraction is even smaller, ranging from 3 to 500 μg liter^{-1} (2, 9). However, these concentrations of organic material in potable water have been shown to support the growth of various heterotrophic organisms and some coliforms (1, 9, 11–13). Since coliforms are traditionally viewed as copiotrophic, especially fecal biotypes, experiments were performed to determine whether coliform bacteria originating from a drinking water distribution system were capable of growth under typical temperature and nutrient concentrations. Prior research on coliform bacteria isolated from a drinking water distribution system demonstrated that no one phenotypic characteristic could differentiate between environmental and clinical isolates of the same species (5), but growth rates have not been previously determined.

In the experiments reported herein we used coliform bacteria isolated from the New Haven, Conn., drinking water distribution system, where unexplained coliform occurrences have been reported (3). Comparisons of growth rates and cell yields at various temperatures and nutrient concentrations were made with clinical isolates of the same species. These data were used to assess the specific capability of environmentally isolated coliforms for growth under conditions relevant to distribution systems. This information is important from a regulatory standpoint, since the presence of coliforms is used as an indicator of potable water quality. In addition, an enterotoxigenic *Escherichia coli* (ETEC) strain was used to determine whether a known pathogen was capable of replication under the low-nutrient and low-temperature conditions representative of those in drinking water. Coliforms isolated from and presumed to be proliferating in a drinking water distribution system may be considered by some to be unimportant from a public health standpoint, but growth of known enteric pathogens under these conditions cannot be disregarded. Experiments were also performed to evaluate potential nutrient sources in the New Haven water, including biofilm, pulverized tubercle, and unsupplemented drinking water. Determination of operational parameters leading to the proliferation of these organisms may assist utilities in selecting and implementing appropriate remedial actions.

MATERIALS AND METHODS

Organisms. Environmental strains of *E. coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, and *Enterobacter aerogenes* were isolated during routine monitoring of the municipal drinking water distribution system at New Haven. These organisms were associated with unexplained occurrences of coliforms in finished water. The ETEC strain H10407 (O78:H11) was obtained from R. A. Wilson, *E. coli* Reference Center, Pennsylvania State University, University Park. A clinical isolate of *K. pneumoniae* was procured from the Montana State University Department of Microbiology culture collection. The clinical *E. coli* isolate was obtained from a patient with a urinary tract infection and was provided by

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the Bozeman Deaconess Hospital, Bozeman, Mont. Identification of all strains was performed with API 20E rapid identification systems (Analytab Corp., Plainview, N.Y.). All cultures were stored at −70°C in 20% peptone–40% glycerol.

Total organic carbon determinations. Total organic carbon levels were determined for double-glass-distilled water, finished mineral salts (MS) medium, and yeast extract solutions prior to each experiment. The organic carbon contents of suspended tubercle material, dry tubercle, and the scraped biofilm used as substrates were also measured. All analyses were done with an Oceanography International Corp. Organic Carbon Analyzer (model 0524B-8A-3030). Sufficient replicate determinations were performed for each analysis to give acceptable precision in the measurements.

Preparation of conditioned cultures. All glassware was acid washed and oven sterilized at 180°C for 3 h. The acid-washing procedure consisted of soaking the glassware in chromic acid for at least 3 h and then rinsing it three times with tap water and three times with ultrapure water. MS medium, which consisted of K_2HPO_4 (7.0 g liter^{-1}), KH_2PO_4 (3.0 g liter^{-1}), $(NH_4)_2SO_4$ (1.0 g liter^{-1}), and $MgSO_4 \cdot 7H_2O$ (0.1 g liter^{-1}), was autoclaved prior to use. All media were prepared with fresh double-glass-distilled water which consistently contained under 0.8 mg of total organic carbon liter^{-1}. Aliquots of 100 ml of MS medium containing 10 mg of yeast extract (Difco Laboratories, Detroit, Mich.) liter^{-1} were added to 250-ml Erlenmeyer flasks. The flasks were inoculated with 0.1 ml of the frozen stock cell suspension. Each flask was incubated at 25°C for 24 h, after which 0.1 ml was transferred to a flask containing 5.0 mg of yeast extract liter^{-1} in MS medium. Following a 24-h incubation period at 25°C, this culture was diluted with sterile double-glass-distilled water to deliver a final concentration of 10^2 to 10^3 cells per ml in the growth experiment test flasks.

Growth experiments. A stock solution of yeast extract was prepared and diluted with double-glass-distilled water to provide final concentrations of 0.1, 0.25, 0.5, and 1.0 mg liter^{-1} in 100 ml of MS medium. The yeast extract contained 62% organic carbon by weight. The flasks were inoculated with the conditioned coliform cultures. A zero time sample was removed, and bacterial numbers were determined in triplicate by the spread plate technique on 10% plate count agar (1/10-strength Difco plate count agar, 1.5% total agar). Plates were incubated at 25°C for 24 h, and the colonies were counted. The flasks were incubated at 25°C and sampled every 24 h for plate counts until the stationary phase was reached. All experiments were performed with triplicate flasks and were conducted three times.

Similar procedures were used to assess the effect of temperature on the growth of coliforms in the presence of low nutrient concentrations. Triplicate flasks containing 0.1 or 1.0 mg of yeast extract liter^{-1} were inoculated to a final concentration of 10^2 to 10^3 coliforms per ml with preconditioned *K. pneumoniae*, *E. coli*, or *Enterobacter aerogenes*. The test flasks underwent stationary incubation at 10, 15, and 20°C. Growth was monitored as in the prior experiments until the stationary phase was reached.

The effect on coliform growth of iron tubercle material from drinking water distribution systems was examined. Three flasks containing 100 ml of MS medium plus 0.1 or 1.0 mg of yeast extract liter^{-1} and 20 mg of pulverized, autoclaved, and dried iron tubercle liter^{-1} were inoculated with *K. pneumoniae* as described above. The tubercle material was a composite of samples removed from the New Haven distribution system; it contained, on a dry weight basis, 56% iron, 0.02% manganese, and 0.004% calcium. Aluminum, magnesium, silicon, and total organic carbon concentrations were below the levels of detection. The flasks were incubated at 25°C for up to 3 weeks. Samples were removed routinely, and bacterial numbers were determined in triplicate by the spread plate technique. Prior to inoculation of the plates, the fluid samples were homogenized (Ultra-Turrax; Tekmar Co., Cincinnati, Ohio) at 4°C for 3 min to ensure an even cell distribution in the inoculum.

Experiments were also performed with biofilm material as the sole carbon source. The biofilm, consisting primarily of bacteria and their extracellular polymer, was removed from a simulated polyvinyl chloride distribution system operated with New Haven water (15). The substance was autoclaved and dispersed in quantities of 1.0 ml per 100 ml of MS medium. Measurements of organic carbon in the biofilm were also performed. Triplicate flasks were inoculated with *K. pneumoniae*, incubated, and enumerated as previously described.

The period of most rapid growth was used to determine the specific growth rate (μ). It was calculated by the formula $\mu = (\log_{10} n_2 - \log_{10} n_1)(2.303)/(t_2 - t_1)$, where n_2 is the cell number at time 2 (t_2) and n_1 is the cell number at time 1 (t_1).

Determination of growth in drinking water. Drinking water samples were collected at sites in the New Haven distribution system where coliforms had been recovered. The samples were frozen in acid-washed sterile 1-liter Nalgene bottles (Nalge Co., Rochester, N.Y.) and shipped to the laboratory. The water was thawed and pasteurized at 65°C for 30 min by the method for assimilable organic carbon described by van der Kooij et al. (14). Chlorine levels were measured colorimetrically (Hach Co., Loveland, Colo.) and found to be below the level of detection. Four aliquots of 100 ml were dispensed into 250-ml acid-washed and baked Erlenmeyer flasks and inoculated with each of the four conditioned coliform cultures which had received an additional passage in MS medium containing 1.0 mg of yeast extract liter^{-1}. The flasks were incubated at 25°C, and samples were removed daily for enumeration.

RESULTS

The organisms chosen for this study represent four coliform species reported as being most frequently isolated from the water of the cooperating drinking water system. *K. pneumoniae* made up 30.5% of the total coliforms identified and was the most prevalent species. *Enterobacter cloacae* was the second most common at 21.8%, *E. coli* was sixth at 5.3%, and *Enterobacter aerogenes* was seventh at 2.1%. All isolates except the *Enterobacter aerogenes* species used in these experiments exhibited a fecal biotype as determined by gas production in EC broth (Difco) at 44.5°C in 24 h.

The origin of the large numbers of coliforms detected during regrowth or aftergrowth events in distribution systems has not been clearly defined, although it is assumed that these coliforms are not the result of breaching the disinfection barrier, of cross-connections, or of main breaks. Current thinking is that they are replicating under distribution system conditions in biofilms on pipe wall surfaces. Experiments were designed to determine whether coliforms from the system that were adapted to the oligotrophic conditions typical of finished drinking water exhibited a higher growth rate than copiotrophic clinical isolates of the same species. Figure 1 illustrates four growth curves for the clinical and environmental *K. pneumoniae* strains grown in unsupple-

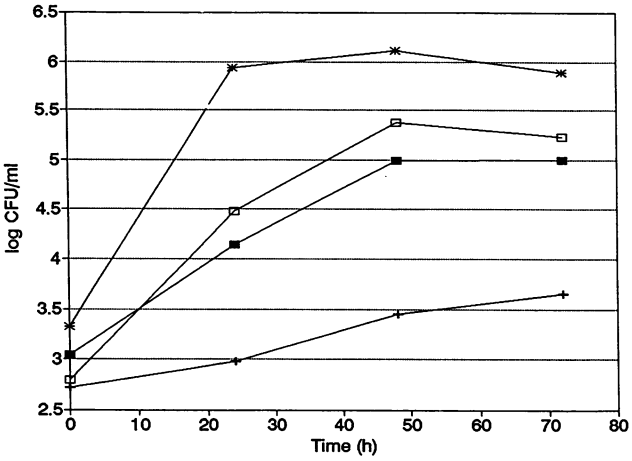

FIG. 1. Growth of environmental and clinical *K. pneumoniae* isolates in the presence of two concentrations of yeast extract at 25°C. Symbols: *, environmental strain, 1.0 mg liter^{-1}; □, clinical strain, 1.0 mg liter^{-1}; ■, environmental strain, 0 mg liter^{-1}; +, clinical strain 0 mg liter^{-1}.

mented MS medium and in the same medium with 1.0 mg of yeast extract liter^{-1}. The distilled water used in these experiments contained 0.4 mg of organic carbon liter^{-1}. The environmental isolate grown in MS medium without added carbon increased in number by 2 logs, whereas the conditioned clinical strain numbers increased by less than 1 log. When grown in the presence of 1.0 mg of yeast extract liter^{-1}, the drinking water isolate numbers increased by 3 logs in 23 h, whereas the clinical organism numbers increased by a maximum of 2 logs in 48 h.

Specific growth rates for the four environmental coliform isolates, their clinical counterparts, and the ETEC strain are presented in Table 1 for the various nutrient concentrations. All of the organisms from the distribution system were capable of measurable growth in MS medium made with double-glass-distilled water and no added yeast extract. The distribution system *K. pneumoniae* generally had the highest specific growth rate of the four water isolates in the presence of all yeast extract concentrations above zero. These rates were also not significantly different from those for *Enterobacter cloacae* grown at the three highest nutrient levels. At all yeast extract concentrations, the specific growth rate of the *K. pneumoniae* clinical isolate was approximately half that of the distribution system culture. The specific growth rate of the *E. coli* water isolate was slightly lower than that of the *K. pneumoniae* at all yeast extract levels. The clinical *E. coli* cell numbers did not increase when no yeast extract was added, but increased as the yeast extract levels increased. At 0.5 and 1.0 mg of yeast extract liter^{-1}, the clinical strain grew as rapidly as the environmental isolate. Unexpectedly, the highest specific growth rate for the ETEC strain was only slightly lower than that of the *K. pneumoniae* water isolate. The *Enterobacter aerogenes* strain had the lowest growth rate of all the environmental isolates at 0, 0.1, and 0.25 mg of yeast extract liter^{-1} and a comparable growth rate to the others at the two highest nutrient concentrations. At greater yeast extract concentrations, all four environmental isolates had similar specific growth rates (μ = 0.30 to 0.22 h^{-1}), with the *K. pneumoniae* and *Enterobacter cloacae* strains having the highest rates. Ranks of the growth rates for the four organisms under the low-nutrient conditions were similar to the relative abundances of the isolates in the distribution system. For example, the *K. pneumoniae* drinking water isolate had the highest specific growth rate at 0.1 mg liter^{-1} and was the most frequently isolated coliform, whereas the *Enterobacter aerogenes* isolate had the lowest specific growth rate and was the least frequently isolated coliform.

The maximal numbers of bacteria produced at each of the nutrient levels are presented in Table 2. Of interest is the difference in final cell concentrations at the lower yeast extract concentrations. When no yeast extract was present, all of the strains except the clinical *E. coli* isolate were capable of some growth. The environmental *K. pneumoniae* isolate attained greater numbers than the other species at very low nutrient concentrations (0 and 0.1 mg liter^{-1}). The ETEC strain had a cell yield identical with that of the environmental isolate at all concentrations except 0 mg liter^{-1}, when the yield was greater than with the environmental strain. At the higher yeast extract concentrations, all strains produced equivalent cell yields.

TABLE 1. Specific growth rates of coliforms in various concentrations of yeast extract at 25°C

Organism and isolate	Specific growth rate (μ [h^{-1}])[a] ± SE for yeast extract concn (mg liter^{-1}):				
	0	0.1	0.25	0.5	1.0
K. pneumoniae					
Water isolate	0.10 ± 0.007	0.18 ± 0.013	0.19 ± 0.008	0.29 ± 0.030	0.29 ± 0.011
Clinical isolate	0.07 ± 0.020	0.06 ± 0.006	0.08 ± 0.025	0.12 ± 0.001	0.14 ± 0.025
E. coli					
Water isolate	0.12 ± 0.015	0.13 ± 0.006	0.15 ± 0.018	0.16 ± 0.040	0.22 ± 0.036
Clinical isolate	NG[b]	0.08 ± 0.004	0.12 ± 0.009	0.17 ± 0.023	0.20 ± 0.003
ETEC	0.09 ± 0.014	0.15 ± 0.007	0.19 ± 0.010	0.21 ± 0.010	0.26 ± 0.044
E. aerogenes					
Water isolate	0.04 ± 0.002	0.10 ± 0.004	0.11 ± 0.005	0.18 ± 0.006	0.26 ± 0.007
E. cloacae					
Water isolate	0.08 ± 0.008	0.12 ± 0.031	0.20 ± 0.022	0.28 ± 0.034	0.30 ± 0.069

[a] Mean value for μ for three measurements from three replicate experiments. One-way analysis of variance on growth rates for all seven isolates at each substrate concentration demonstrated that the values shown are different at the 0.01 level.

[b] NG, no growth detected.

TABLE 2. Maximum cell production of coliforms in various concentrations of yeast extract at 25°C

Organism and isolate	Max cell production (no. per ml) in yeast extract concn (mg liter^{-1}):				
	0	0.1	0.25	0.5	1.0
K. pneumoniae					
Water isolate	9.5×10^4	1.6×10^5	3.0×10^5	5.8×10^5	1.1×10^6
Clinical isolate	4.9×10^3	1.1×10^4	2.3×10^4	6.2×10^4	1.5×10^5
E. coli					
Water isolate	2.6×10^4	1.6×10^5	3.1×10^5	6.4×10^5	1.2×10^6
Clinical isolate	-0.5×10^2[a]	6.0×10^2	1.8×10^4	1.3×10^5	4.9×10^5
ETEC	4.3×10^4	1.6×10^5	3.1×10^5	6.4×10^5	1.2×10^6
E. aerogenes					
Water isolate	4.0×10^4	8.6×10^4	2.1×10^5	5.0×10^5	1.2×10^6
E. cloacae					
Water isolate	1.4×10^3	5.3×10^4	8.6×10^4	2.4×10^5	5.6×10^5

[a] Decrease in cell number from inoculum.

The observation that the coliforms isolated from drinking water were able to proliferate under conditions of very low organic carbon motivated us to examine coliform growth in 12 unsupplemented drinking water samples. Of these, four samples contained enough assimilable organic carbon to support growth of at least one of the coliforms (Table 3). The two water samples with the lowest total organic carbon content supported the growth of one coliform each, the water sample with an intermediate level (3.02 mg liter^{-1}) permitted growth of two of the coliforms, and the water sample containing the highest organic carbon level (5.44 mg liter^{-1}) permitted growth of all three coliforms tested.

A potential source of nutrients for bacteria in drinking water distribution systems may be bacterial products associated with biofilm. This possibility was examined by using biofilm material which had been obtained from the surface of a polyvinyl chloride pipe system receiving treated drinking water from a filtration plant as the sole carbon source in growth experiments. A carbon-hydrogen-nitrogen elemental analysis of the autoclaved biofilm indicated that it contained 34.9% carbon, 5.4% hydrogen, and 5.4% nitrogen. A sufficient amount of this material was added to MS medium to give a total organic carbon concentration of 0.5 mg liter^{-1}. The environmental isolate of *K. pneumoniae* grown in this medium increased in numbers by approximately 1 log over the level for growth without the biofilm material.

When 20 mg of pulverized iron tubercle liter^{-1} was added to flasks containing 0, 0.1, and 1.0 mg of yeast extract liter^{-1}, there was very little enhancement of growth of the environmental *K. pneumoniae* strain when compared with incubation without tubercle. For example, after 48 h of incubation at 25°C, 1.6×10^6 bacteria per ml were enumerated from the test flask containing 1.0 mg of yeast extract liter^{-1} and 20 mg of tubercle and 1.4×10^6 bacteria were enumerated from a control flask without tubercle (mean values for triplicate readings from triplicate samples). Starting cell numbers were near 10^2/ml. Specific growth rates were also similar for cultures with and without tubercle (μ = 0.30 and 0.29 h^{-1}, respectively).

TABLE 3. Organic carbon content and number of environment coliform bacteria[a] produced in unsupplemented drinking water

Water sample	Total organic carbon concn (mg liter^{-1}) ± SE[b]	No. of cells ml^{-1} for:		
		K. pneumoniae	*E. aerogenes*	*E. coli*
1	1.77 ± 0.26	1.5×10^5	NG[c]	NG
2	1.41 ± 0.28	NG	7.1×10^3	NG
3	3.02 ± 0.77	7.4×10^5	1.9×10^5	NG
4	5.44 ± 0.74	1.0×10^4	1.5×10^5	2.0×10^5

[a] Mean value of viable bacteria as determined by plate count from three replicates; initial inocula were less than 10^2 ml^{-1}.
[b] Mean total organic carbon measured in triplicate before inoculation.
[c] NG, no growth detected.

The capability of the coliforms to grow at low levels of organic carbon suggested that they may also be capable of growth at water temperatures near those typically observed in distribution systems and treatment facilities. Temperatures in the New Haven distribution system ranged from 4.8°C in February to 21.9°C in August (monthly average temperatures). Three of the coliforms isolated from water (*K. pneumoniae*, *E. coli*, and *Enterobacter aerogenes*) were incubated at 10, 15, 20, and 25°C in MS medium plus 0.1 or 1.0 mg of yeast extract liter^{-1}. Specific growth rates (μ) for each of the three species at the four temperatures with 0.1 mg of yeast extract liter^{-1} are presented in Fig. 2. The environmental *E. coli* isolate demonstrated no increase in cell numbers after 3 weeks of incubation at 10°C. The other two coliforms were capable of proliferation at the low temperatures documented in a drinking water system. When 0.1 mg of yeast extract liter^{-1} was used, maximal cell counts of ca. 10^5 bacteria per ml were reached within 5 days for the *K. pneumoniae* isolate at 10°C and 10 days for *Enterobacter aerogenes*. The higher temperatures (15, 20, and 25°C) yielded ca. 10^5 bacteria per ml within 4 days for all three isolates. At the higher yeast extract concentration (1.0 mg liter^{-1}) and higher temperatures, ca. 10^6 bacteria per ml were found after 2 days of incubation. The same cell numbers were produced at 10°C but only after some delay (6 days for *K. pneumoniae*, 5 days for *E. coli*, and 8 days for *Enterobacter aerogenes*). The specific growth rates at the higher yeast extract concentration were similar to those at 0.1 mg liter^{-1}.

DISCUSSION

Various researchers have shown that coliforms can proliferate at extremely low carbon levels. Zobell and Grant (18), as early as 1943, reported that *E. coli* grew in solutions

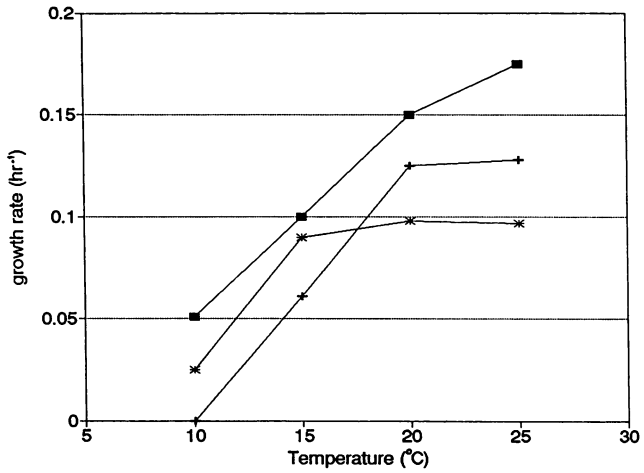

FIG. 2. Specific growth rates of environmental coliform isolates as a function of incubation temperature with 0.1 mg of yeast extract liter^{-1}. Symbols: ■, *K. pneumoniae*; *, *E. coli*; +, *Enterobacter aerogenes*.

containing 0.1 mg of glucose liter^{-1}. Van der Kooij et al. demonstrated that *Aeromonas hydrophila* was capable of growing with 0.1 mg of C liter^{-1} as glucose (13). Drinking water isolates of this organism were able to reach yields of up to 5×10^5 CFU ml^{-1} in the presence of 0.5 mg of peptone liter^{-1} and 7×10^4 CFU ml^{-1} in distilled water containing 4.2 mg of dissolved organic carbon liter^{-1}, with corresponding maximum growth rates of 0.3 and 0.08 h^{-1} (11). However, growth kinetics and cell yields are lacking for coliform isolates from drinking water, especially those of the fecal biotype. The specific growth rates for the coliform most frequently isolated from the New Haven distribution system, *K. pneumoniae*, ranged from a low of 0.10 h^{-1} in unsupplemented glass-distilled water to 0.29 h^{-1} at 1.0 mg of yeast extract liter^{-1} at 25°C. These specific growth rates, on average, exceeded those of the other coliforms tested, as did cell yields. The specific growth rate of the clinical isolate was approximately half that of the environmental strain. In addition, the specific growth rate of the drinking water *K. pneumoniae* isolate increased in a near-linear fashion with increasing temperature when the organism was grown on 0.1 mg of yeast extract liter^{-1}; the growth rates were also greater than those of the other two coliforms. These data indicate that this *K. pneumoniae* isolate is adapted to oligotrophic conditions and temperatures representative of distribution systems and may explain why it was the most frequently isolated coliform.

Of particular interest and concern are the results obtained with the three *E. coli* strains. The growth rate at the various carbon concentrations for the environmental strain exceeded that of the clinical isolate, but both were surpassed by that of the ETEC strain. The yields for the environmental strain and the ETEC strain were identical under the same conditions. Although these results are not definitive, because the ETEC strain had been passed for several generations in the laboratory, they indicate that the ETEC strain, a waterborne pathogen, may also be able to proliferate at low nutrient concentrations. The growth of the environmental isolate under the same conditions would yield similar results in a total coliform test, but the public health significance would be far lower.

A summary of the kinetic data and yields calculated for all the strains grown on yeast extract is presented in Table 4. The reported values for yield determined as milligrams of cell carbon per milligram of substrate carbon are an order of magnitude lower than those usually expected for copiotrophic growth (0.4 to 0.5 mg of cell C/mg of substrate C). Although it is possible that this difference is due to computational assumptions for cell volume, dry mass per cell, and percentage of dry mass as carbon, we speculate that these low yields are the result of significant energy expenditure by the cells in procuring the limited substrate available in solution. When examining yields obtained on a carbon basis and on a cell basis, we find that the yields for the clinical isolates and *Enterobacter cloacae* are significantly lower than those for the environmental strains. The ETEC yields are similar to those for the *K. pneumoniae* and *E. coli* water isolates. Values for K_s, as determined by nonlinear regression, are all well below 1 mg liter^{-1}, which is indicative of substrate uptake systems capable of operating under low nutrient (<1.0-mg liter^{-1}) conditions. K_s values for the environmental *K. pneumoniae* and *E. coli* strains and the ETEC strain are half or less than those for the clinical strains. The calculated maximum growth rates (μ_{max}) ranged from 0.16 to 0.37 h^{-1}, about half those observed for coli-

TABLE 4. Cell yield and kinetic parameters for environmental and clinical coliforms[a]

Isolate	Substrate coefficient, K_s (mg liter^{-1})	Maximum growth rate, μ_{max} (h^{-1})	SE for growth rate, σ_μ (h^{-1})	Cell yield on carbon basis, Y (mg mg^{-1})	SE for carbon yield, σ_Y (mg mg^{-1})	Correlation coefficient for yield, r^2	Cell yield on cell basis, Y (cells mg of C^{-1})	SE for cell yield, σ_Y (cells mg of C^{-1})
K. pneumoniae								
Water isolate	0.06	0.32	0.064	0.07	8.9×10^{-4}	0.998	1.7×10^9	2.2×10^7
Clinical isolate	0.12	0.16	0.038	0.01	3.8×10^{-4}	0.981	2.4×10^8	9.6×10^6
E. coli								
Water isolate	0.05	0.21	0.066	0.08	6.8×10^{-4}	0.999	1.9×10^9	1.7×10^7
Clinical isolate	0.15	0.25	0.005	0.03	2.5×10^{-3}	0.932	8.2×10^8	6.3×10^7
ETEC	0.05	0.26	0.055	0.08	6.5×10^{-4}	0.999	1.9×10^9	1.6×10^7
E. aerogenes	0.24	0.33	0.034	0.08	2.7×10^{-3}	0.985	1.9×10^9	6.8×10^7
E. cloacae	0.12	0.37	0.047	0.04	1.2×10^{-3}	0.987	9.1×10^8	3.0×10^7

[a] μ_{max} and K_s were determined by nonlinear regression. Cell number yields were determined by linear regression of the cell number with substrate carbon. Cell mass yields were based on 10^{-13} g (dry mass) per cell, 40% carbon within dry mass. Yeast extract was determined to contain 62% carbon.

forms under ideal growth conditions (ca. 0.67 h^{-1}). These data also support the hypothesis that the environmental coliforms, especially the *K. pneumoniae* and *E. coli* isolates, as well as the ETEC strain, are adapted to growth under oligotrophic conditions.

The source of carbon in distribution system water for growth of these organisms is also of concern. Several potential nutrient sources were tested in addition to the yeast extract. These included autoclaved biofilm, crushed tubercle material, and the assimilable organic carbon fraction of treated drinking water. *K. pneumoniae* was grown in the presence of the biofilm at a concentration of 0.5 mg of total organic carbon $liter^{-1}$. When compared with the yeast extract concentration most similar in carbon concentration to this value (1.0 mg $liter^{-1}$), similar increases of 1 log over the value for unsupplemented mineral salts in cell yields were seen. Biofilm itself appeared to be a suitable carbon source for the proliferation of this coliform. In our experiments, crushed tubercle material did not enhance the growth of the organism. These results disagree with those of Victoreen (16, 17), Martin et al. (8), and Allen et al. (1), all of whom showed that tubercle materials or their soluble fraction caused an increase in coliform numbers. The difference may be due to the coliforms chosen or the chemical composition of the tubercle material used.

There has recently been significant interest in the amount of assimilable organic carbon present in drinking water and its effect on coliform growth and distribution system operations (6). Van der Kooij et al. (14) developed the technique in which a specific *Pseudomonas* strain was grown in drinking water and the resulting increase in cell numbers was converted to levels of acetate utilization. A similar procedure was used in this study with the drinking water coliforms as the inoculum. Four unsupplemented drinking water samples were obtained during a period of normal system operation and were inoculated with *K. pneumoniae*, *E. coli*, and *Enterobacter aerogenes*. Organic carbon concentrations in these water samples varied from 1.44 to 5.44 mg $liter^{-1}$. All of the water samples supported the growth of at least one of the coliforms when incubated at 25°C. *K. pneumoniae* and *Enterobacter aerogenes* each grew in three of the water samples, and the *E. coli* isolate grew in one. The maximum cell yields were approximately 10^5 cells for each organism. The water sample with the greatest organic carbon concentration allowed for proliferation of all three coliforms. These results show that sufficient nutrients exist in the distribution water itself to support the growth of coliforms and that environmental isolates may be superior indicator organisms for determining the amount of assimilable organic carbon.

Clearly, the results support the hypothesis that growth of environmental coliform isolates is possible under environmental conditions typically found in distribution systems. Since these bacteria are the system nuisance organisms and appear to thrive on the conditions therein, it is only reasonable to use them to determine the ability of the water to support their growth. Considering that planktonic growth was demonstrated under low-nutrient and low-temperature conditions, biofilm growth is even more likely, especially since biofilm system conditions have been shown to support coliform growth under nutrient limitation (4, 7). Because the environmental coliforms tested in these studies were isolated from a distribution system and because they are capable of growth at low temperatures and low nutrient levels which occur in distribution systems, these coliforms can be a component of the biofilm consortia. The public health significance of these results is that fecal coliforms were capable of proliferation, even at low temperatures and under poor nutrient conditions. The tendency to consider these organisms of limited public health significance, even though they are fecal coliforms, must be cautioned against since an ETEC strain was also able to grow.

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