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Effects of Sudden Temperature Shifts on Pure Cultures of Four Strains of Freshwater Bacteria

Manfred G. Höfle

Limnological Institute, Konstanz-Egg, West Germany

Abstract. Three psychrotrophic and one mesophilic strains were isolated from winter water samples of different freshwater biotopes and identified as *Cytophaga johnsonae* (C-21), *Cytophaga* sp. (M-17), *Pseudomonas fluorescens* (KD), and *Enterobacter cloacae* (BS-2). Temperature shift-up experiments with emphasis on low temperatures were carried out with aerated pure batch cultures in glucose mineral medium. The effects of sudden temperature increases on growth rates and substrate conversion were investigated. All three psychrotrophic strains in the temperature increase experiments at low temperatures showed differing reactions within the linear zone of the Arrhenius plot. The *C. johnsonae* (C-21) shift-up cultures adjusted the growth rate immediately to the rate of the temperature adapted cultures, whereas *Cytophaga* sp. (M-17) shift-up cultures showed a lower and *P. fluorescens* (KD) a higher growth rate. The mesophilic *E. cloacae* (BS-2), like *C. johnsonae* (C-21), adjusted immediately to the new growth rate. Substrate conversion increased in all experiments immediately after the shift-up. The extracellular substrate conversion by *P. fluorescens* (KD) of glucose to gluconate and 2-ketogluconate was particularly affected by the sudden temperature increase.

Introduction

Sudden increases in the water temperature of freshwater biotopes are a widespread occurrence due to the input of household and industrial waste water and more particularly cooling water from power stations. Although damaging effects by high temperatures on bacteria are well considered in the literature (2, 9, 19), little is known about the consequences of a sudden temperature increase at low temperatures (14, 16, 18). As the main industrial areas are in cold temperate zones with freshwater temperatures below 10°C for half the year, such temperature increases, particularly from below 10°C, are frequent.

As the psychrotrophic bacteria with good growth at 0°C but growth optimum above 15°C (12) are of particular importance in these cold freshwater biotopes (5,

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13), three examples of this group and one mesophilic bacteria were chosen as test organisms. The aerated batch culture system allowed for the bacteria growth in the logarithmic phase without limitation due to oxygen and substrate, the bacteria being subject only to endogenic metabolic influences.

The Arrhenius plot, especially its linear region where the temperature characteristic is constant, reflects the effects of temperature on the population dynamic changes of the individual bacterial groups (7). With respect to the temperature, the growth rate of the bacteria follows in the linear region of the Arrhenius plot the Arrhenius equation

$$\ln \mu = -K_A/RT,$$

where μ is specific growth rate, K_A is the Arrhenius constant or temperature characteristic, R is the gas constant, and T is the absolute temperature.

The course of the substrate conversion provides information on the influence temperature has on the decomposition of organic substances within the ecosystem. The point of this work is therefore to investigate the effects of sudden temperature increases on growth rate and substrate conversion in pure batch cultures, particularly with respect to the psychrotrophic bacteria and low temperature range, where the temperature characteristic is constant.

Materials and Methods

Organisms

The four investigated strains were all isolated from water samples (water temperature 4° to 8°C) taken from different freshwater biotopes on casein-peptone-starch plates (6). After purification they were identified according to Bergey's Manual (1974) as strain C-21—*Cytophaga johnsonae*, strain M-17—*Cytophaga* sp., strain KD—*Pseudomonas fluorescens*, and strain BS—*Enterobacter cloacae*.

The first three strains were all psychrotrophic, the last one was mesophilic with no growth at 5°C. The stock cultures were grown on mineral medium agar slants (see below) supplemented with 0.2% glucose and held at 2°C. Agar-slant cultures were transferred monthly to new media.

Medium and Conditions of Cultivation

All growth experiments were carried out in the following glucose mineral medium (per liter): KH_2PO_4 1.4 g; $Na_2HPO_4 \cdot 2H_2O$ 2.1 g; $(NH_4)_2SO_4$ 1 g; $MgSO_4 \cdot 7H_2O$ 400mg; $CaCl_2$ 20 mg; $FeSO_4 \cdot 7\ H_2O$ 0.1 mg; trace element solution according to Allen (1) 1 ml; glucose 450 mg.

The chemicals were dissolved in deionized, distilled water. The final pH of 7.1 was adjusted with 1 N NaOH. The pH remained constant (± 0.1) during the entire growth experiments. The whole medium was sterilized by membrane filtration.

The tubes used for growing the bacteria were conical with a diameter of 45 mm and a length of 330 mm. They were aerated through glass tubing with 25 liter/hr air. The culture tubes with 250 ml of the medium were immersed in a water bath held at the desired temperature ± 0.05°C. All tubes used for growth experiments were triplicate and inoculated with 2% of an exponentially growing culture in the same medium and at the same growth conditions as used for subsequent experiments.

Five-milliliter samples for measuring optical density (at 578 nm with a Zeiss PM 4 spectrophotometer in 1-cm absorption cells) and carbohydrate content were removed with a sterile syringe by an inserted needle and immediately frozen at −20°C for later analysis.

Temperature Increase Experiments

To bring about an immediate temperature increase, we immersed the above described basic cultures at the end of the first third of the logarithmic phase in a 5°C warmer water bath. The temperature of the medium rose by 90% of the temperature difference within 7 minutes; after 12 minutes the total temperature increase was attained.

Carbohydrate Estimation

Glucose and gluconate concentrations were determined enzymatically by appropriate modifications of Bergmeyer (4). The concentration of 2-ketogluconate was determined by the method of Lanning and Cohen (10).

Yield and Dry Weight Estimation

A whole basic culture tube was harvested by centrifugation (15,000 *g*, 15 minutes at room temperature) and washed with distilled water. The pellet was freeze dried. This bacterial biomass was weighed and corrected for the optical density loss due to centrifugation. The optical density–dry weight relation so obtained was used for calculation of the yield.

Protein Estimation

The freeze-dried biomass from the dry weight estimation was weighed directly and dissolved in distilled water. The protein content of the solution was then determined by Herbert and associates' (8) modification of Lowry's method, using a bovine serum albumin standard.

Calculation of the Growth Rate

The growth rates were calculated according to the equation of Baig and Hopton (3) based on the optical density values. Only the values in the linear region of the semilogarithmic plot of the growth curve were used for these calculations. For *P. fluorescens* (KD) only the initial logarithmic growth rates were calculated; these are compared in Table 1.

Results

Psychrotrophic Strains

Cytophaga johnsonae (C-21). This strain showed a biphasic Arrhenius plot for the growth rate with an inflection at 15°C (Fig. 1). The temperature characteristic K_A for the first temperature range of 5° to 15°C was 14.6 kcal/mole, for the second range of 15° to 25°C 10.1 kcal/mole. The increase experiment for the first linear zone of the Arrhenius plot resulted in an immediate change in growth rate and in substrate utilization following the temperature shift (Fig. 2).

A similar result was obtained for the experiments with the higher basic temperature of 10° and 20°C (not illustrated). The new growth rate was equivalent to that of a basic culture at a temperature 5°C higher (Table 1). Protein

Table 1. Specific growth rates (hr^{-1}) and SDs of the triplicates of the 4 strains at different temperatures

Temperature (°C)	*C. johnsonae* (C-21)	*Cytophaga* sp. (M-17)	*P. fluorescens* (KD)	*E. cloacae* (BS-2)
5	0.059 ± 0.002	0.051 ± 0.003	0.067 ± 0.002	—
5–10 shift-up	0.099 ± 0.001	0.076 ± 0.001	0.187 ± 0.025	—
10	0.099 ± 0.001	0.086 ± 0.005	0.131 ± 0.007	0.091 ± 0.005
10–15 shift-up	0.147 ± 0.009	0.142 ± 0.002	—	0.193 ± 0.003
15	0.148 ± 0.010	0.143 ± 0.004	—	0.195 ± 0.003
20	0.197 ± 0.008	0.247 ± 0.006	—	0.378 ± 0.005
20–25 shift-up	0.262 ± 0.004	0.305 ± 0.003	—	0.559 ± 0.008
25	0.278 ± 0.007	0.308 ± 0.009	—	0.549 ± 0.021
30	0.126 ± 0.004	0.166 ± 0.005	—	—

content and yield remained relatively constant over the whole temperature range (Table 2), only the yield being markedly reduced at the above-optimum temperature.

Cytophaga sp. (M-17). This strain had a constant temperature characteristic from 5° to 20°C with a K_A value of 16.9 kcal/mole (Fig. 1). After the temperature shift (Fig. 3) the same result as with *C. johnsonae* (C-21) was obtained for temperatures from 5° to 10°C, i.e., an immediate change in growth rate and substrate utilization. The growth rate here was, however, markedly lower (13%) than that of the 10°C basic culture (Table 1). In contrast, in the temperature increase experiments with higher initial temperatures, the growth rate reached the same level as in the basic cultures. For this strain a maximum yield at 5°C was evident together with a minimum protein content (Table 2). The yield in the 10°C shift-up culture was exactly the same as in the 10°C basic culture.

Pseudomonas fluorescens (KD). This strain was chosen because the temperature dependence of the extracellular conversion of glucose to gluconate and 2-

Table 2. Yield and protein content of the 4 strains at different temperatures expressed as % dry weight

			5°C	10°C	15°C	20°C	25°C	30°C
C. johnsonae	Protein	%	56.4	54.2	55.0	56.2	—	54.3
(C-21)	Yield	%	50.6	50.8	47.9	46.0	47.5	6.4
Cytophaga sp.	Protein	%	43.6	53.2	—	52.4	52.6	51.2
(M-17)	Yield	%	61.6	47.6	—	50.2	46.9	36.6
P. fluorescens	Protein	%	41.9	55.4	—	—	—	—
(KD)	Yield	%	46.3	45.6	—	—	—	—
E. cloacae	Protein	%	—	63.3	64.1	58.5	58.5	—
(BS-2)	Yield	%	—	38.8	43.5	46.5	46.5	—

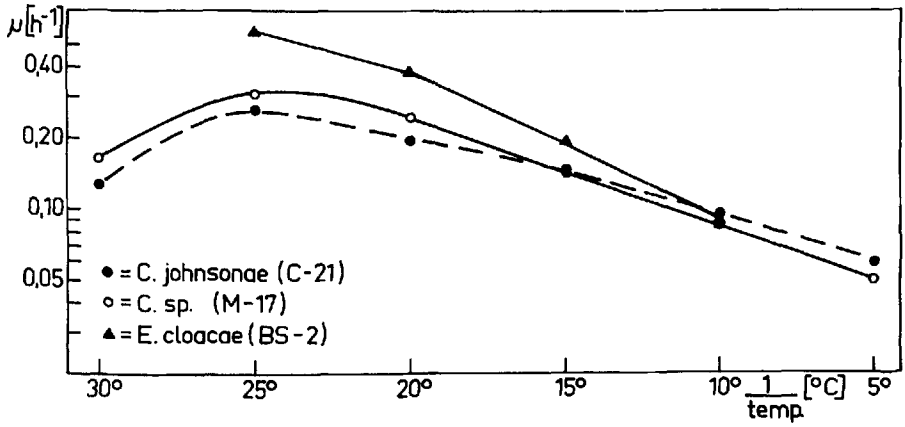

Fig. 1. Arrhenius plots of *C. johnsonae* (C-21), *Cytophaga* sp. (M-17) and *E. cloacae* (BS-2).

ketogluconate was known from work by Lynch et al. (11) and also because the temperature characteristic in glucose mineral medium between 5° and 20°C was constant (11, 15). As is to be expected, the amount of these oxidation products formed decreased with increasing temperature (Figs. 4–6). The 5°C basic culture had a maximum of 1.67 mM/liter gluconate and 0.87 mM/liter 2-ketogluconate, whereas the 10°C basic culture had a maximum of 1.44 mM/liter gluconate and 0.56 mM/liter 2-ketogluconate. The 10°C shift-up culture showed with 1.68 mM/liter gluconate and 0.87 mM/liter 2-ketogluconate the same maxima as the 5°C basic culture. The metabolite conversion rate, however, increased immediately

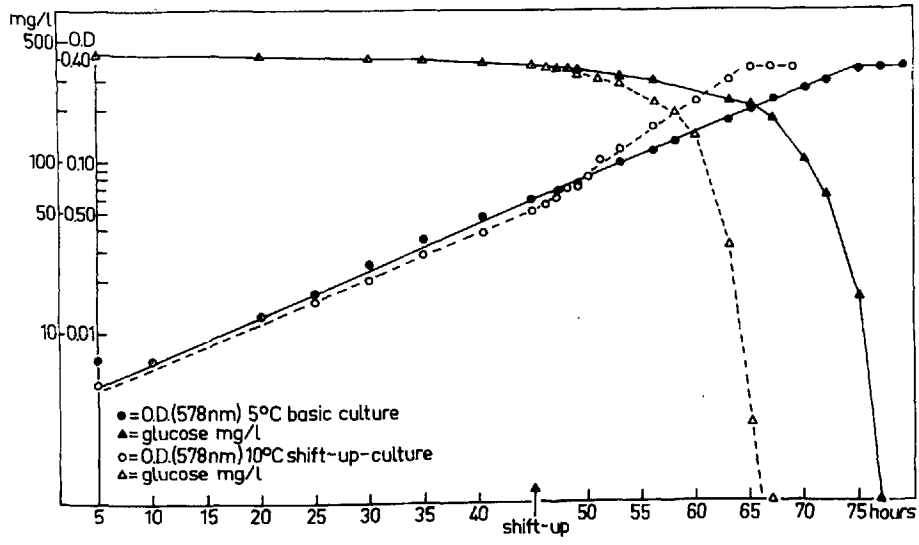

Fig. 2. Effect of sudden temperature shift-up from 5°C to 10°C on growth and substrate breakdown of *C. johnsonae* (C-21).

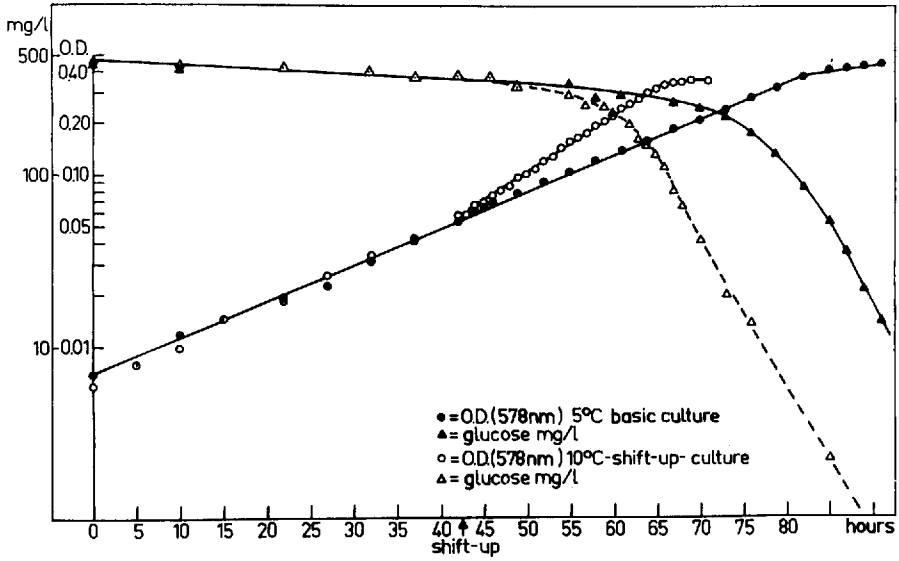

Fig. 3. Effect of sudden temperature shift-up from 5°C to 10°C on growth and substrate breakdown of *Cytophaga* sp. (M-17).

as a result of the temperature shift-up, as seen in Fig. 5, and the growth rate rose immediately to 43% above that of the 10°C basic culture (Table 1). The yield of each of these cultures was the same, whereas the protein content increased markedly from the 5°C to the 10°C basic culture (Table 2).

Mesophilic Strain

Enterobacter cloacae (BS-2). The first linear region of the Arrhenius growth rate plot occurred between 10° and 20°C for this strain with a temperature characteristic K_A of 23.4 kcal/mole. No growth occurred at 5°C in glucose mineral medium. The shift-up experiment in this first range with constant temperature characteristic from 10° to 15°C resulted in the same growth rate as in the basic culture (Table 1). The change in growth rate occurred immediately together with a relevant substrate breakdown (Fig. 7). Yield was reduced at lower temperatures.

Strains *Cytophaga* sp. (M-17) and *P. fluorescens* (KD) showed in the 10°C shift-up experiment a transient growth rate, whereas the strains *C. johnsonae* (C-21) and *E. cloacae* (BS-2) adapted immediately with the high temperature growth rate. The duration of the transient growth rate of *Cytophaga* sp. (M-17) was not much greater than the time required to reach the stationary phase (Fig. 3), since there was no significant difference between the growth rate of the 10°C basic culture and those cells which were transferred from the late logarithmic phase of the 10°C shift-up culture into new culture tubes.

Because of the irregular growth curve, an estimation of the duration of the transient growth rate of *P. fluorescens* (KD) was not possible.

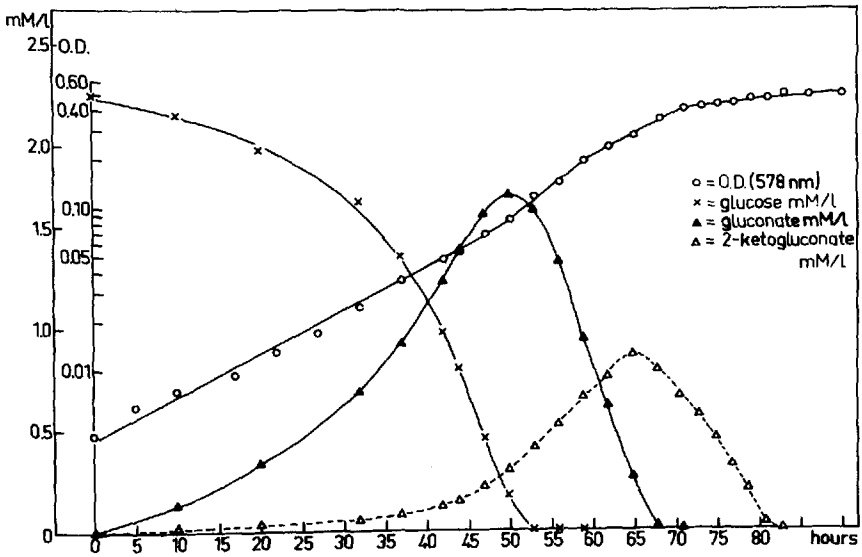

Fig. 4. Conversion of glucose to gluconate and 2-ketogluconate during growth of *P. fluorescens* (KD) at 5°C.

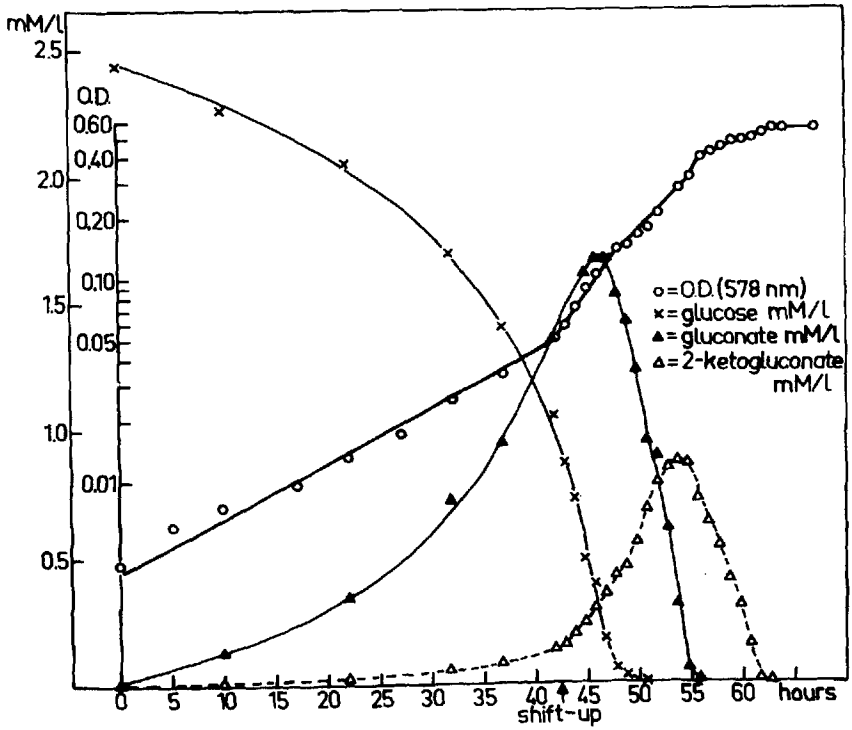

Fig. 5. Effect of sudden temperature shift-up from 5°C to 10°C on the conversion of glucose to gluconate and 2-ketogluconate during growth of *P. fluorescens* (KD).

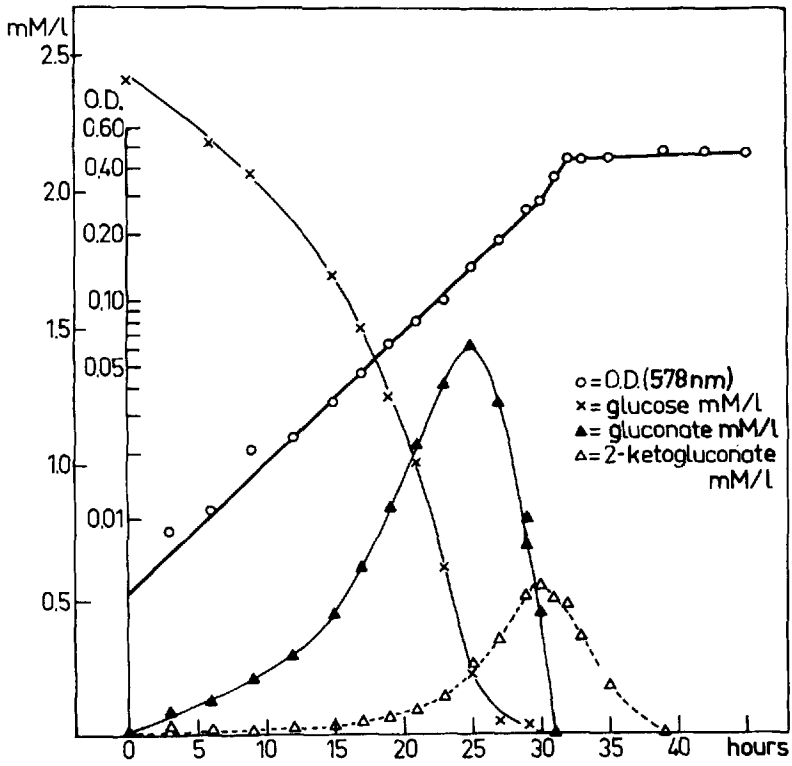

Fig. 6. Conversion of glucose to gluconate and 2-ketogluconate during growth of *P. fluorescens* (KD) at 10°C.

Discussion

Whereas the mesophilic *E. cloacae* (BS-2) reacts similarly to *E. coli* and yeasts as described by Ng et al. (14) and Shaw (17), respectively, and within the temperature range where the temperature characteristic is constant adjusts growth rate immediately after the temperature shift with respect to the new temperature, the three psychrotrophic strains all react differently.

The growth rate in the shift-up cultures from 5° to 10°C decreases for *Cytophaga* sp. (M-17), remains the same for *C. johnsonae* (C-21), and increases for *P. fluorescens* (KD) in comparison to the 10°C basic culture. Psychrotrophic and mesophilic strains have contrasting reactions probably because lower temperatures require greater metabolic adaptation with respect to substrate degradation pathways and protein synthesis (11, 15). There is evidence that the two strains *Cytophaga* sp. (M-17) and *P. fluorescens* (KD), which deviate from the normal reaction have a markedly reduced protein content at 5°C which could be caused by an increased RNA content to compensate for reduced ribosome activity at these low temperatures (9). Furthermore, the drastically increased yield at 5°C in *Cytophaga* sp. (M-17) suggests that this strain has a special

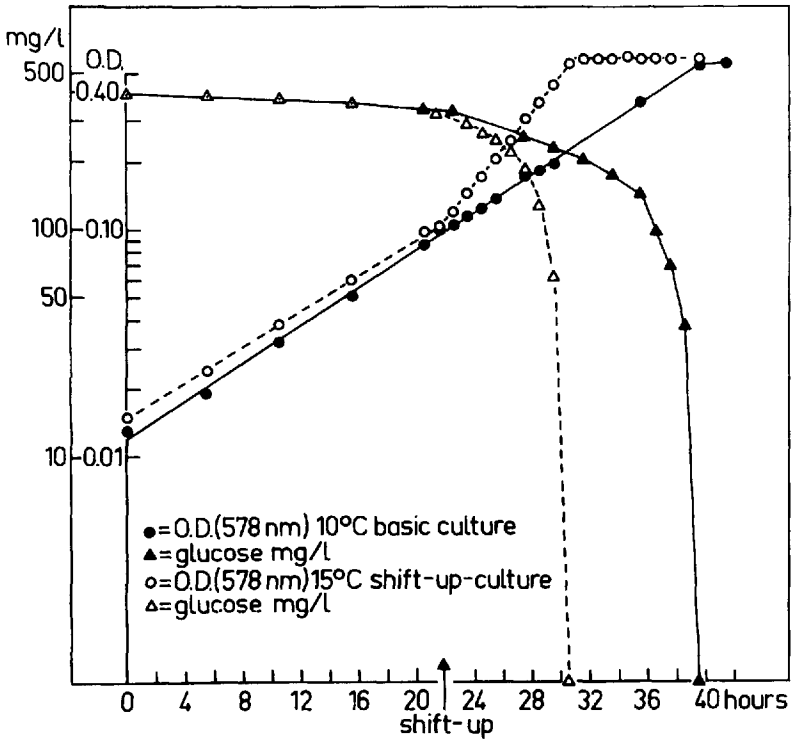

Fig. 7. Effect of sudden temperature shift-up from 10°C to 15°C on growth and substrate breakdown of *E. cloacae* (BS-2).

adaptive mechanism for lower temperatures, although the growth rate shows a constant temperature characteristic.

Due to the three substrates, interpretation of *P. fluorescens* (KD) is obviously more difficult. The results in the 10°C shift-up culture can be interpreted in that the cells still possess the 5°C enzyme quantities and can consequently convert as much substrate as in the 5°C basic culture, but only at the 10°C turnover rate; thus the peaks for gluconate and 2-ketogluconate are just as high as in the 5°C basic culture but narrower (Fig. 5). The lower production of glucose-converting enzymes by the cells at 10°C and consequent reduction in growth rate are probably due to the relevant enzyme production either being influenced by substrate concentration (20) or being dependent on growth rate.

Despite the difficulties of applying laboratory results to *in situ* conditions, it can be expected that unlike the mesophilic bacteria, the psychrotrophic bacteria in freshwater biotopes do not always exhibit a simple reaction to temperature shift-ups at low temperatures. For psychrotrophic bacteria the new exponential growth rate obtained after the temperature shift-up does not always agree with that predicted by the temperature characteristic of the basic culture.

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