THE EFFECT OF TEMPERATURE ON THE MOLAR GROWTH YIELD AND MAINTENANCE REQUIREMENT OF *ESCHERICHIA COLI* W DURING AEROBIC GROWTH IN CONTINUOUS CULTURE

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1. Introduction

A number of reports are present in the literature which describe the effect of temperature on the efficiency of bacterial growth under aerobic conditions [1–8]. Unfortunately, most of these studies have been carried out using batch cultures and therefore either predate or ignore the concept of maintenance energy [9,10]. Rather surprisingly, very few studies have been carried out using continous cultures where it is relatively easy to measure maintenance requirements and to make the corrections which are necessary for the determination of true molar growth yields. Furthermore, the few available results are by no means consistent (cf. refs. [6,8]) and their interpretation is somewhat complicated by the use of a fermentable carbon source, viz. glucose.

We have therefore investigated the effect of temperature on the energetics of *Escherichia coli* W growing aerobically in glycerol-limited continous culture. The results indicate that an increment of 20°C in the growth temperature of this mesophile increases the energy requirements for maintenance purposes by twelve-fold, whilst leaving the energy requirements for the production of cell material only about one-third higher; the efficiency of oxidative phosphorylation, as measured by respiration-linked proton translocation, appears to be temperature-independent.

2. Materials and methods

E. coli W was grown in carbon-limited continous culture using a 500 ml capacity chemostat fitted with a simple oxygen analyser [11–13]. The culture medium consisted of a minimal salts solution [14] supplemented with nitrilo-triacetic acid (0.1 g · litre^{-1}) plus 7 mM glycerol. The selected temperature (± 0.1°C) was maintained by immersing the growth chamber in a controlled-temperature water tank. In situ respiratory activities of growing cells were determined from the difference in the oxygen concentration of the input and effluent air, the air flow rate and the steady-state culture density as described in previous communications from this laboratory [11–13]. True molar growth yields with respect to glycerol utilisation ($Y^{max}_{glycerol}$) were determined by essentially standard procedures in which observed growth yields obtained at a series of dilution rates were extrapolated to an infinite dilution rate [10,15].

Whole cell suspensions were prepared and assayed for $\rightarrow H^+/O$ ratios [16] as described previously [13,17]; an assay temperature of 30°C was routinely used since control experiments indicated that the efficiency of respiration-linked proton translocation was independent of temperature within the range 20–40°C. Protein contents were assayed colourimetrically using Folin's reagent [18]. The concentration of acetate in the effluent growth medium was determined by standard colourimetric procedures [19] and the concentration of lactate was assayed enzymatically [20].

3. Results

The growth of *E. coli* W in glycerol-limited continuous culture (dilution rate, 0.12 h^{-1}) at increasing

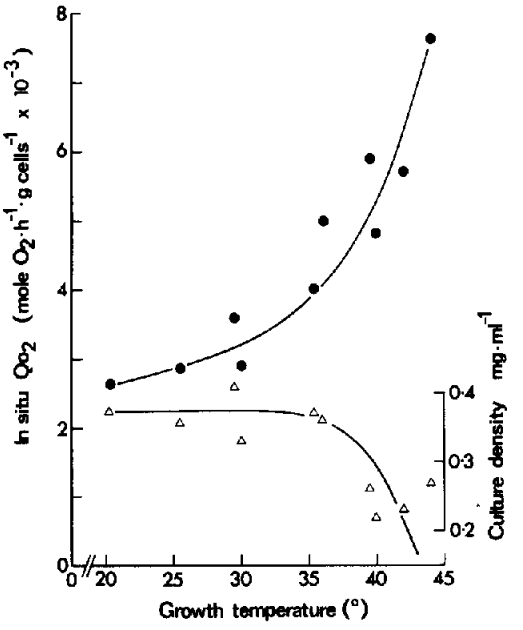

Fig.1. The effect of temperature on selected parameters during the aerobic growth of *E. coli* W in glycerol-limited continuous culture at a constant dilution rate. In situ Q_{O_2} (•) and cell density (△) were determined as described in Materials and methods. Dilution rate 0.12 ± 0.01; pH 6.92 ± 0.05.

temperatures in the range 20–44°C was characterised by a progressive increase in the in situ respiratory activity and a concomitant decrease in the cell density (fig.1). These changes were relatively slight below 30°C, but were particularly marked at the higher growth temperatures.

The energetics of an energy-limited continuous culture can be described by the equation

$$Q_{O_2} = \frac{\mu}{Y_{O_2}^{max}} + M_{O_2}$$

where Q_{O_2} is the in situ respiratory activity (mole $O_2 \cdot h^{-1} \cdot$ g cells^{-1}), μ is the specific growth rate (equivalent to the dilution rate, D, of a continuous culture; h^{-1}), $Y_{O_2}^{max}$ is the true molar growth yield with respect to oxygen consumption (g cells · mole O_2^{-1}) and M_{O_2} is the maintenance respiration rate (mole $O_2 \cdot h^{-1} \cdot$ g cells^{-1}) [6]. Thus, the temperature-dependent increase in the Q_{O_2} which was observed at constant dilution rate could have reflected a decrease in $Y_{O_2}^{max}$ and/or an increase in M_{O_2}. These possibilities were therefore investigated experimentally by measuring in situ Q_{O_2} as a function of dilution rate during the growth of *E. coli* W at 20.3, 30.0 and 40.0°C, on the expectation that any changes in $Y_{O_2}^{max}$ and M_{O_2} would be reflected in the slope and intercept respectively of the resultant straight line graphs (fig.2). The results indicated that the values of both $Y_{O_2}^{max}$ and M_{O_2} altered with the increase in temperature, albeit disproportionately; $Y_{O_2}^{max}$ decreased from 46.8 to 33.4 g cells.mole O_2^{-1} (a change which was limited entirely to the upper half of the temperature range), whereas M_{O_2} increased from 0.1 to 1.2 mmol $O_2 \cdot h^{-1} \cdot$ g cells^{-1} (a change which occurred progressivcly over the entire temperature range; table 1). The changes in $Y_{O_2}^{max}$ were accompanied by very similar changes in the values of $Y_{glycerol}^{max}$ (the true molar growth yield with respect to glycerol

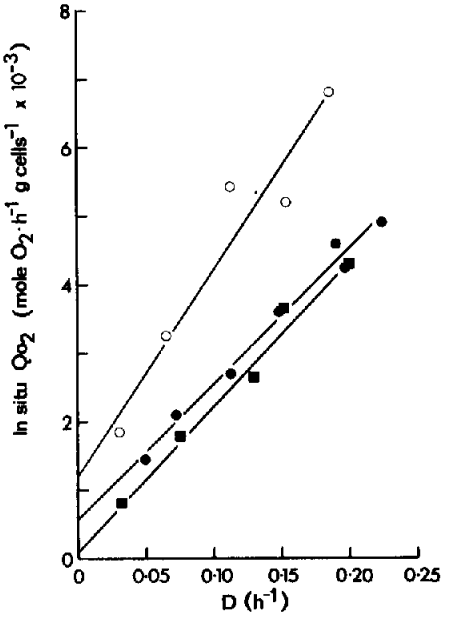

Fig.2. The effect of temperature on the in situ respiratory activity of *E. coli* W during growth in glycerol-limited continuous culture at varying dilution rates. In situ Q_{O_2} was determined as described in Materials and methods. Temperature 20.3°C (▪), 30.0°C (•) and 40.0°C (○); pH 7.02 ± 0.05. The best line was fitted to each set of data by linear regression analysis using a Hewlett-Packard 1600B bench-top computer.

Table 1
The effect of temperature on the energetics of *E. coli* W during aerobic growth in glycerol-limited continuous culture

Temperature	$Y_{O_2}^{max}$ (g cells·mole O_2^{-1})	M_{O_2} (mole $O_2 \cdot h^{-1}$ ·g cells^{-1})	$\rightarrow H^+/O$ (endogenous) (g ion $H^+ \cdot g$ atom O^{-1})	N (mole ATP·mole O_2^{-1})	Y_{ATP}^{max} (g cells·mole ATP equiv.$^{-1}$)	M (mole ATP equiv·h^{-1} ·g cells^{-1})	$Y_{glycerol}^{max}$ (g cells·mole glycerol^{-1})	Protein content (%)
20.3°C	46.8	0.00010	4.07 (2)	4.00	11.7	0.00040	52.3	62.2 ± 2.0 (3)
30.0°C	50.9	0.00058	3.96 ± 0.07 (5)	4.00	12.7	0.00232	50.9	65.0 ± 2.4 (5)
40.0°C	33.4	0.00120	3.79 ± 0.24 (6)	4.00	8.4	0.00480	35.3	60.9 ± 4.4 (3)

$Y_{O_2}^{max}$ and M_{O_2} were determined from the plots of in situ Q_{O_2} versus dilution rate shown in fig.2. $\rightarrow H^+/O$ ratios, protein contents and $Y_{glycerol}^{max}$ values were determined as described in Materials and methods; where appropriate, these are expressed as the average ± the standard error of the mean, with the number of determinations in brackets. N was calculated from a knowlegde of the pathway of glycerol catabolism and the efficiency of oxidative phosphorylation in *E. coli* W as described previously [12,13].

utilisation; g cells · mole glycerol^{-1}). No significant concentrations of acetate or lactate were detected in any of the effluent growth media, thus indicating that catabolism was completely oxidative at all of the growth temperatures investigated; only slight changes were observed in the protein content of the cells.

$Y_{O_2}^{max}$ and M_{O_2} can be defined in more detail by the simple equations,

$$Y_{O_2}^{max} = Y_{ATP}^{max} \cdot N$$

and,

$$M_{O_2} = M/N$$

where Y_{ATP}^{max} is the true molar growth yield with respect to the utilisation of ATP equivalents (g cells · mole ATP equivalents^{-1}), M is the energy requirement for maintenance purposes (mole ATP equivalents · h^{-1} · g cells^{-1}) and N is the overall efficiency of aerobic energy conservation, i.e. oxidative phosphorylation *plus* a small contribution from substrate-level phosphorylation (mole ATP equivalents · mole O_2^{-1}) [6]. Theoretically, therefore, the observed changes in $Y_{O_2}^{max}$ and M_{O_2} could have been brought about by alterations in the values of Y_{ATP}^{max}, M and/or N. However, since $Y_{O_2}^{max}$ and M_{O_2} changed by approximately one-third and twelve-fold respectively over the temperature range investigated, it was considered unlikely that changes in the value of the common component, N, were responsible. This conclusion was supported by the observed constancy of the endogenous → H^+/O ratios which continued to reflect the presence of two proton-translocating respiratory chain segments at all growth temperatures [13,21–23]; no evidence was obtained to support the concept of a temperature-dependent doubling of the efficiency of oxidative phosphorylation as proposed recently for *E. coli* B growing under glucose-limited conditions [8]. Thus, with N remaining constant at 4.00 mol ATP equivalents · mole O_2^{-1}, it must be concluded that the observed temperature-dependent changes in $Y_{O_2}^{max}$ and M_{O_2} reflect changes in the values of Y_{ATP}^{max} and M respectively, the former decreasing from 11.7 to 8.4 g cells · mole ATP equivalents^{-1} and the latter increasing from 0.4 to 4.8 mmole ATP equivalents · h^{-1} · g cells^{-1} (with an activation energy of 24.2 kcal · mole^{-1}).

4. Discussion

The effect of temperature on the Y_{ATP}^{max} of glycerol-limited, aerobic cultures of *E. coli* W is almost identical to that recently reported by Mainzer and Hempfling for glucose-limited, anaerobic cultures of *E. coli* B where Y_{ATP}^{max} could of course be determined without reference to oxidative phosphorylation [8]. It would appear therefore that the biosynthetic pathways of aerobic and anaerobic cultures respond similarly to increases in the growth temperature, i.e. the efficiency with which energy initially conserved via oxidative or substrate-level phosphorylation is subsequently utilised for the synthesis and organisation of cell materials is essentially unchanged at the lower growth temperatures, but is significantly diminished at the higher end of the temperature range. Thus in *E. coli*, as in several other mesophilic bacteria [1,2,5,8] but not in the thermophile *Bacillus stearothermophilus* [7], decreases in growth efficiency occur principally at temperatures which are approximately equal to, or higher than, those which support the fastest growth rates.

In contrast, the maintenance requirements (M_{O_2} or M) of glycerol-limited cultures of *E. coli* W are less than one-third of those exhibited by glucose-limited cultures of *E. coli* B at corresponding growth temperatures [8]. Furthermore, the calculated activation energy for maintenance (24.2 kcal · mole^{-1}) is considerably lower than the value of 68 kcal · mole^{-1} reported elsewhere for the aerobic growth of *E. coli* ML 308 under slightly different conditions [24]. This lower activation energy is similar to that reported for protein turnover in the latter organism and is within the range normally encountered for metabolic reactions [25]. It is likely therefore that the temperature-dependent increases in M_{O_2} and M in *E. coli* W reflect increased energy requirements occasioned by the more rapid turnover of cellular macromolecules at elevated temperatures.

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