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Effect of Decreasing Growth Temperature on Cell Yield of *Escherichia coli*

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Studies of the relationship between yield coefficient and growth rate, as affected by temperature of growth, in *Escherichia coli* have shown that, over a wide range of temperature, yield is relatively constant until the specific growth rate falls below about 0.2 hr^{-1}, at which point the yield begins to fall off precipitously. No intermediates of glucose metabolism in a form utilizable at higher temperatures could be found in the medium, and no toxic product was produced which limited growth. At 10 C, 37% of the carbon from glucose-*UL*-^{14}C was assimilated into cellular material, whereas, at 30 C, 53% was assimilated. Cells grown at 10 C contained more carbohydrate than did cells grown at 37 C, and the glycogen-to-protein ratio of cells grown at 10 C was approximately three times higher than that of cells grown at 37 C. Adenosine triphosphatase activities of cells grown at 10 and 35 C were similar. Growth rates on glucose, glycerol, and succinate were quite similar at 10 C, but at 35 C growth was most rapid on glucose and slowest on succinate. The data suggest that the decrease in yield with decrease in temperature is a result of uncoupling of energy production from energy utilization.

For many years after the report of Graham-Smith (4), it was generally accepted that yields of cells were greater at temperatures below those at which most rapid growth occurred. More recently, however, Sinclair and Stokes (20) demonstrated that, when oxygen is not a limiting factor, maximal cell numbers produced by selected mesophiles and psychrophiles are as high at 30 C as they are at 10 C. Besides oxygen limitation, other factors which undoubtedly have led to confusion in interpreting the effect of temperature on growth have been (i) the use of cell numbers instead of cell mass as a measure of yield (20), (ii) the use of chemically undefined media (13, 20), and (iii) the different methods used for calculating and reporting yield (1, 5, 11, 13, 14, 18).

Monod (11) found that, in aerated cultures of *Escherichia coli* incubated between 20 and 37 C, the yield was between 0.26 and 0.25 g of cells (dry weight) per g of glucose. At 39 and 41 C, however, the yields dropped to 0.21 and 0.20, respectively. Senez (18) observed a similar decrease in yield with *Aerobacter aerogenes* above 37 C. The rate of growth of *A. aerogenes* is maximum at 37 C, but the rate of respiration of glucose continues to increase with increases in temperature and does not reach a maximum until 42 C. He interpreted these data to mean that, at the higher temperature, biosynthesis is proceeding at a slower rate than is catabolism, with resultant energy uncoupling, a condition in which energy is being made available faster than it can be utilized.

This paper provides evidence to show that the yield of *E. coli* decreases if growth temperature is decreased below the temperatures at which the growth rates deviate from the Arrhenius equation (15). It is suggested that the decrease in yield may be the result of an energy uncoupling.

MATERIALS AND METHODS

Organism. The organism used throughout this study was *Escherichia coli* ML 30, obtained from Jacques Monod.

Media. The basal medium used was medium 56 of Monod, Cohen-Bazire, and Cohn (12). Glucose, glycerol, or sodium succinate was sterilized by filtration through a membrane filter (Millipore Corp., Bedford, Mass.).

Chemicals. The uniformly labeled ^{14}C-glucose was purchased from New England Nuclear Corp., Boston, Mass. The scintillation mixture was "Liquefluor," obtained from Nuclear-Chicago Corp., Des Plaines, Ill., and the hydroxide of hyamine was purchased from Packard Instrument Co., Inc., La Grange, Ill.

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Other chemicals were of reagent grade from the usual commercial sources.

Cultural conditions and measurement of growth. Cultures were grown, their growth was measured, and the specific growth rate, k, was calculated as described previously (15) with the following exceptions: cells for preparation of standard curves were obtained from cultures growing exponentially at 35 C, and a Spectronic-20 (Bausch & Lomb, Inc., Rochester, N.Y.) and model DB spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) were used for measurements of optical density. Generation time, g, was computed by the formula $g = 0.69/k$.

Determination of yield. Two independent methods were used to determine yield. Method A consisted of inoculating 5 ml of a starter culture, grown at the same temperature, into 145 ml of minimal medium lacking a carbon source, and then allowing the culture to exhaust the carried-over substrate before adding glucose (Fig. 1). At zero-time, sterile glucose was added aseptically to give a final concentration of 250 μg of glucose/ml. Turbidity measurements were made on the culture until growth ceased. Glucose determinations were made at the beginning and end of the growth cycle. The yield coefficient, K, was computed from the equation $K = (G - G_0)/C$, where G and G_0 are cell mass [estimated as μg (dry weight)ml] at the end of the experiment and at zero-time, respectively, and C is the glucose concentration in μgrams/milliliter at zero-time.

Method B was as follows. Minimal medium containing 250 μg of glucose per ml was placed in a side-arm flask adapted for a Klett colorimeter and was inoculated with a washed suspension of cells from an exponentially growing culture (10% inoculum). The flask was incubated on a water-bath shaker at the desired temperature. The turbidity of the culture was followed on a Klett colorimeter until a constant reading was obtained. Turbidity readings were made at the beginning and at the end of the growth phase on the Beckman Model DB spectrophotometer to permit estimation of the cell mass. Glucose determinations also were made on the filtered medium. The yield coefficient was then computed as described for Method A. The results obtained with either method were in good agreement.

Utilization of ^{14}C-glucose. The procedure was essentially the same as that described for determining yield by method A except that uniformly labeled glucose was used as the substrate. After removing a sample for the determination of turbidity, glucose, and radioactivity, the vessel was stoppered and connected to two test tubes in series containing 0.1 N NaOH to trap the respiratory CO_2, which was swept through by sparging with air.

After growth had ceased, the radioactivity incorporated into cellular material was determined by liquid scintillation counting of cells collected and dried on membrane filters; the radioactivity remaining in the culture fluid also was determined. The bound CO_2 dissolved in the culture fluid was released into a closed container by 10 N H_2SO_4 and, then, was absorbed by hyamine after overnight incubation. The value for CO_2, thus determined, was subtracted from the total radioactivity in the culture fluid. The CO_2 removed by the alkali traps was determined in a similar manner, and this value was added to that obtained as bound CO_2 in the supernatant fluid to give total CO_2. All aqueous samples (0.1 ml) were made miscible with 10 ml of "Liquefluor" by the addition of 3 ml of absolute ethyl alcohol. Radioactivity was determined in a Nuclear-Chicago scintillation spectrometer at the ambient temperature of an air-conditioned room maintained at about 20 C. Counting efficiency, as determined by the channels ratio method, was approximately 50%.

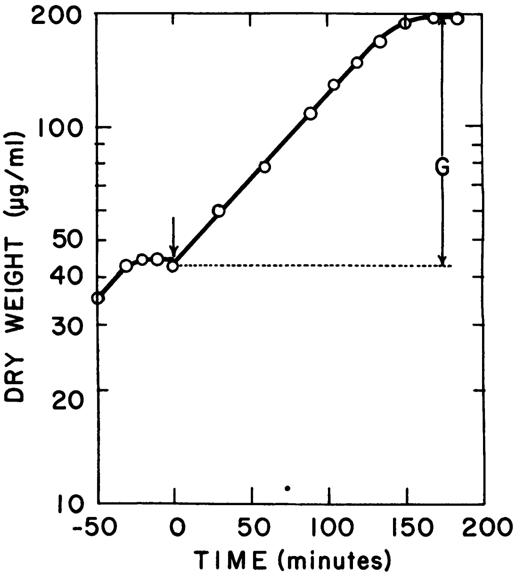

FIG. 1. *Typical experiment for determining yield by means of method A. Arrow denotes the time at which sterile glucose was added aseptically to give a final concentration of 250 μg/ml. The temperature was 30 C. G represents the increment of growth resulting from the glucose added.*

Respiratory rate. Uptake of oxygen was determined by conventional Warburg techniques (22) on samples from exponentially growing cultures at the temperature of growth of the cultures. Cells were resuspended in 0.1 M phosphate buffer (pH 7.4) to a density of 600 to 800 μg (dry weight)/ml. Each Warburg vessel contained 2.5 ml of washed cell suspension, 0.3 ml of buffer in the main compartment, and 0.2 ml of 1% (w/v) glucose in the side arm, which was tipped in after temperature equilibration. The center well contained 0.2 ml of 20% KOH to absorb respiratory CO_2. Two sets of data were obtained; one set was corrected for endogenous respiration, whereas the other was not. QO_2 is defined as μliters of O_2 taken up per milligram of protein per hour

Protein and carbohydrate determinations. Protein was determined by the method of Lowry et al. (7). The total carbohydrate was determined by the anthrone method as modified by Fales (2). Glucose was determined by the enzymatic Glucostat reagent (Worthington Biochemical Corp., Freehold, N.J.).

Preparation of cell extract. Cells grown in a glucose minimal medium at 35 C or at 10 C were resuspended in 0.1 M tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 8.0) to a density equivalent to about 160 µg of protein/ml. The resuspended cells were treated for 5 min at the full power of a 10-kc MSE sonicator (Measuring and Scientific Equipment, Ltd., London, England) by use of the large probe. The sample was kept cool in an ice bath during sonic treatment.

Adenosine triphosphatase assay. The method of assay used was that of Marsh and Militzer (8). The incubation mixture consisted of 0.5 ml of 0.2 M Tris buffer (*p*H 8.0), 0.3 ml of adenosine triphosphate (ATP)-Mg mixture (0.032 M ATP and 0.015 M $MgCl_2$), and 0.5 ml of cell extract. The mixture was incubated for 30 min at 35 C. After the reaction was stopped with 0.2 ml of 50% trichloroacetic acid, the precipitate was removed by centrifugation. The inorganic phosphate liberated was determined on a 0.5-ml sample of the supernatant liquid by use of the Fiske-SubbaRow procedure (22). Phosphat liberatede by a boiled enzyme extract was subtracted to correct for instability of ATP. One unit of enzyme is defined as that amount which liberates 1 µg of phosphorus per hr under these conditions.

RESULTS

Yield of cells at various temperatures. Figure 2 shows the relationship between yield coefficient and specific growth rates as affected by temperature of growth. The yield is quite constant over a wide range of growth rates until rates fall below about 0.2 hr^{-1}, a point corresponding to a temperature of about 18 C, at which the yield decreases precipitously. For example, at 30 C, the average growth rate in this medium is about 0.55 hr^{-1}, and the average yield coefficient is about 0.5; i.e., 0.5 g of cells (dry weight) is produced from 1 g of glucose. At 10 C, however, the growth rate decreases to about 0.04 hr^{-1}, and the yield coefficient drops to 0.39.

Growth at 10 C on culture supernatant fluid. To explore the possibility that intermediates of glucose metabolism may have accumulated which could not be metabolized at 10 C, the supernatant liquid from a culture at 10 C that had ceased growing was examined for its ability to support growth at 30 C. This supernatant liquid failed to support growth of either cells grown at 10 or 30 C and subsequently incubated at 30 C (Table 1). Thus, no intermediates are excreted into the medium in a form that can be utilized at a higher temperature. The fact that even cells grown at 30 C cannot grow eliminates the possibility that cells may not have been adapted at 10 C to use the intermediate. The growth obtained when 0.1% glucose was added indicates that cessation of growth at 10 C was due not to a toxic condition but rather to a lack of a carbon and energy source. The growth in the uninoculated glucose-supplemented medium after 20 hr results from the incomplete removal of cells by the centrifugation.

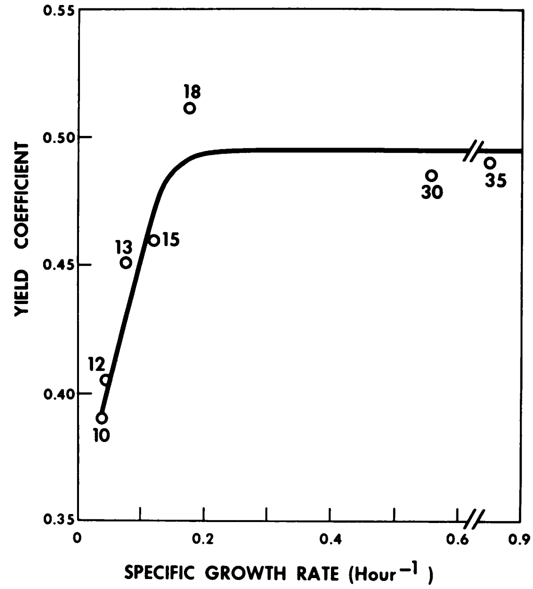

FIG. 2. *Yield of Escherichia coli ML 30 as a function of specific growth rate. Numbers above the experimental points indicate the temperature of growth that gives the growth rates shown on the abscissa. The 30, 13, and 10 C points are averages of six different experiments in which both methods A and B were used; the other points represent single determinations.*

TABLE 1. *Ability of the supernatant fluid from a culture grown at 10 C to support additional growth at 30 C*[a]

Inoculum	Cell density (µg/ml)					
	No glucose at hour			Glucose added (0.1%) at hour		
	0	4	20	0	4	20
Cells grown at 10 C........	53	50	35	53	106	213
Cells grown at 30 C........	115	101	85	117	287	250
None..........	2	2	3	1	2	211

[a] Incubated at 30 C on shaking water bath.

Carbon balance of E. coli growing on glucose. The proportion of carbon from glucose-*UL*-^{14}C going into cellular material, into CO_2, and remaining in the culture fluid is presented in Table

TABLE 2. *Percent of* ^{14}C *present in cells,* CO_2*, and culture supernatant fluid at 10 and 30 C.*

Expt	Temp	Cells	CO_2	Supernatant fluid	Total
	C				
1	10	36.6	42.8	22.7	102.1
	30	52.7	29.2	21.6	103.5
2	10	38.0	38.3	20.9	97.2
	30	53.5	33.2	18.0	104.7
Avg	10	37.3	40.6	21.8	99.7
	30	53.1	31.2	19.8	104.1

2. At 10 C, only about 37% of the glucose carbon is assimilated into cellular material, whereas at 30 C, 53% is recovered as cells. These values are in good agreement with the yield based on dry weight. The lower rate of conversion of glucose carbon into cellular material at 10 C is concomitant with an increased recovery of carbon in the form of CO_2 at 10 C, 41% as compared to only 31% at 30 C. The percentage of carbon remaining in the culture supernatant fluid at both temperatures is about the same.

Respiratory rate and growth rate at various temperatures. The data in Fig. 3 are in the form of an Arrhenius plot, in which the logarithm of the specific growth rate and the logarithm of the respiratory rate are plotted as functions of the reciprocal of the absolute temperature. This figure shows that this function for the respiratory rate is linear throughout the range of temperatures studied, whereas the corresponding function for the growth rate deviates from linearity at temperatures below about 20 C.

Respiration of glucose at 35 C by cells grown at 10 C. Cells grown at 10 C respired glucose at 35 C at a faster rate than do cells grown at 35 C; the former had a QO_2 (corrected for endogenous) of 180 as compared to only 159 for the latter. Furthermore, the rate of endogenous respiration was higher for the cells grown at 10 C than for those grown at 35 C, and a longer period of endogenous metabolism was required to deplete the endogenous reserve of cells grown at 10 C.

Carbohydrate-to-protein ratio of cells grown at 10 and 37 C. The data of Table 3 confirm the finding that cells grown at 10 C are richer in carbohydrate (22% of dry weight) than are cells grown at 37 C (8.4% of dry weight). The protein content of cells grown at 10 C is slightly lower than that of cells grown at 37 C; the carbohydrate-to-protein ratio was 0.37 at 10 C as compared to 0.13 at 37 C. There was no significant difference in optical densities at 420 nm of suspensions each containing an equivalent dry weight of cells grown at the two temperatures.

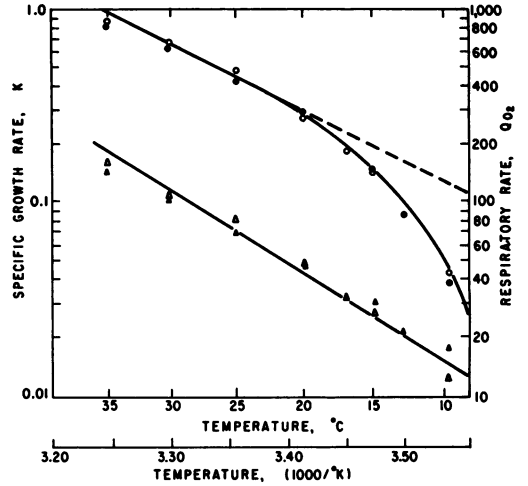

FIG. 3. *Arrhenius plot of growth rates and respiratory rates of E. coli ML 30. The circles are specific growth rates (k), and the triangles are the rates of oxygen uptake (*QO_2*). Open and closed symbols indicate two different sets of data.*

Adenosine triphosphatase activity of cells grown at 10 and at 35 C. The lower yield of cells at 10 C could be the result of higher adenosine triphosphatase activity in the cells. However, the data in Table 4 show that the specific activity of adenosine triphosphatase in cells grown at 10 C may be slightly lower than that in those grown at 35 C.

Growth rate on various carbon sources at high and low temperatures. In Table 5, growth rates of *E. coli* on glucose, glycerol, and succinate media are shown. These data show that, at the higher temperatures, growth is most rapid on glucose, slowest on succinate, and intermediate on glycerol. When temperature is low (13 C), however, the rate of growth is approximately equal on all three sources.

DISCUSSION

The results of this study demonstrate that, over a wide range of temperature, cell yield of *E. coli* is independent of growth rate until the rates fall below the temperature which approximately coincides with that at which growth rate begins to deviate from the Arrhenius equation (Fig. 3). Senez (18) has found that growing *A. aerogenes* at temperatures above the optimal growth temperature, 37 C, also results in lower cell yields. He has postulated that biosynthetic reactions at the high temperatures do not keep pace with catabolic reactions, which results in a

TABLE 3. *Carbohydrate and protein content in E. coli grown at 10 and at 37 C*

Growth temp	Dry weight	Optical density[a] at 420 nm	Protein[b]	Carbohydrate[b]	Carbohydrate/ protein (w/w)
C	*µg/ml*				
10	1,068	0.303	635(59.5)	234(21.9)	0.37
37	1,094	0.323	718(65.5)	92(8.4)	0.13

[a] Determined in a 1:10 dilution of cell suspension.

[b] Expressed as micrograms per milliliter. Values in parentheses represent per cent (dry weight).

TABLE 4. *Adenosine triphosphatase activity in cells of E. coli grown at 10 and at 35 C*

Growth temp	Phosphorus liberated in 30 min[a]			Adenosine triphosphatase activity[b]	Protein in extract	Specific activity
	Unheated extract	Heated extract[a]	Net			
C	*µg*	*µg*	*µg*	*units/ml*	*µg/ml*	*units/µg*
10	48.0	15.2	32.8	65.6	164.6	0.40
35	55.8	13.6	42.2	84.4	171.6	0.49

[a] Heated extract was boiled for 5 min.

[b] Units of activity are defined as micrograms of phosphorus liberated per hour per milliliter.

TABLE 5. *Effect of temperature on specific growth rates of E. coli ML 30 on three carbon sources*

Growth temp	Glucose	Glycerol	Succinate
C			
37	0.993		0.621
35		0.722	
13	0.099	0.088	0.105

condition he terms energy uncoupling. On the basis of evidence provided in this paper, this hypothesis may apply equally well at low temperatures.

It was demonstrated that, whereas the rate of growth at low temperature is slower than predicted by the Arrhenius equation, respiratory rate seems to obey the equation at least down to 10 C. The inability of biosynthesis (anabolism) to keep pace with catabolism should result in the diversion of energy from catabolism into forms of storage, e.g., glycogen, and this is reflected by the higher carbohydrate-to-protein ratio in cells grown at 10 C as compared to those grown at 37 C. That catabolic reactions predominate over biosynthetic reactions is also suggested by the fact that cells grown at 10 C respire glucose at 35 C at a rate in excess of that of cells grown at 35 C; furthermore, a longer period of endogenous respiration is required to reduce the rate of endogenous respiration of cells grown at 10 C. The observation that growth rates on glucose and succinate differ considerably at 37 C but are similar at 13 C suggests that biosynthesis limits growth at low temperatures, whereas catabolism of the carbon source is the limiting factor at higher temperatures. Glucose, being more readily utilized than succinate or glycerol, will allow a faster growth rate. Marr, Ingraham, and Squires (9) also concluded that growth at low temperatures results in a higher level of catabolic repression of β-galactosidase in *E. coli.*

O'Donovan, Kearney, and Ingraham (17) have isolated a variety of conditional lethal mutants which can grow at low temperatures only when supplemented with one or more amino acids, whereas the parent strain is capable of growing without supplementation. One of these cold-sensitive mutants, *E. coli* strain K-II-27, requiring histidine for growth at 20 C, has been analyzed by O'Donovan and Ingraham (16), and the lesion has been traced to an enzyme in the histidine-synthesizing pathway which is extremely sensitive to allosteric (feedback) inhibition at low temperatures. Thus, a preferential inhibition, at low temperatures, of allosteric enzymes involved in biosynthetic reactions could bring about an uncoupling of energy utilization from energy production. A related observation is that uncoupled growth caused decreased yield and increased glycogen content for a leaky threonine auxotrophic *E. coli* growing under threonine limitation (19). Similar findings have been reported for *A. aerogenes* growing linearly in the presence of an amino acid analogue, *p*-fluorophenylalanine (19), and for *E. coli* growing in the presence of selenate in the place of sulfate (6). All these conditions lead to restriction of protein synthesis.

Finally, the decreased permeability of bacterial cells to sugars at low temperature has often been suggested as a possible basis for the existence of a minimal temperature for growth of bacteria (3). The fact that my results show that the respiratory rate at low temperature is well in excess of that required for growth renders this explanation unlikely. Stokes and Larkin (21) have recently shown that the extract of a psychrophilic bacillus is capable of oxidizing glucose at low temperature (5 C) faster than is the extract of a mesophilic bacillus. Therefore, the failure of some bacteria to grow at low temperature is probably not due to a limitation in permeability of cell membrane to substrates.

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LITERATURE CITED

1. Bauchop, T., and S. R. Elsden. 1960. The growth of microorganisms in relation to their energy supply. J. Gen. Microbiol. 23:457–469.
2. Fales, F. W. 1951. The assimilation and degradation of carbohydrates by yeast cells. J. Biol. Chem. 193:113–124.
3. Farrell, J., and A. H. Rose. 1967. Temperature effects on microorganisms, p. 147–218. *In* A. H. Rose (ed.), Thermobiology. Academic Press Inc., New York.
4. Graham-Smith, G. S. 1920. The behavior of bacteria in fluid cultures as indicated by daily estimates of the numbers of living organisms. J. Hyg. 19:133–204.
5. Herbert, D., R. Elsworth, and R. C. Telling. 1956. The continuous culture of bacteria; a theoretical and experimental study. J. Gen. Microbiol. 14:601–622.
6. Huber, R. E., I. H. Segel, and R. S. Criddle. 1967. Growth of *Escherichia coli* on selenate. Biochim. Biophys. Acta 141:573–586.
7. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
8. Marsh, C., and W. Militzer. 1956. Thermal enzymes. VII. Further data on an adenosine triphosphatase. Arch. Biochem. Biophys. 60:433–438.
9. Marr, A. G., J. L. Ingraham, and C. L. Squires. 1964. Effect of the temperature of growth of *Escherichia coli* on the formation of β-galactosidase. J. Bacteriol. 87:356–362.
10. Mayberry, W. R., G. J. Prochazka, and W. J. Payne. 1967. Growth yields of bacteria on selected organic compounds. Appl. Microbiol. 15:1332–1338.
11. Monod, J. 1942. Recherches sur la croissance des cultures bactériennes. Hermann et Cie., Paris.
12. Monod, J., G. Cohen-Bazire, and M. Cohn. 1951. Sur la biosynthese de la β-galactosidase (lactase) chez *Escherichia coli*. La specificite de l'induction. Biochim. Biophys. Acta 7:585–599.
13. Morita, R. Y., and L. J. Albright. 1965. Cell yields of *Vibrio marinus*, an obligate psychrophile, at low temperature. Can. J. Microbiol. 11:221–227.
14. Morris, J. G. 1960. Studies on the metabolism of *Arthrobacter globiformis*. J. Gen. Microbiol. 22:564–582.
15. Ng, H., J. L. Ingraham, and A. G. Marr. 1962. Damage and derepression in *Escherichia coli* resulting from growth at low temperatures. J. Bacteriol. 84:331–339.
16. O'Donovan, G. A., and J. L. Ingraham. 1965. Cold-sensitive mutants of *Escherichia coli* resulting from increased feedback inhibition. Proc. Natl. Acad. Sci. U.S. 54:451–457.
17. O'Donovan, G. A., C. L. Kearney, and J. L. Ingraham. 1965. Mutants of *Escherichia coli* with high minimal temperatures of growth. J. Bacteriol. 90:611–616.
18. Senez, J. C. 1962. Some considerations on the energetics of bacterial growth. Bacteriol. Rev. 26:95–107.
19. Sigal, N., J. Cattaneo, and I. H. Segel. 1964. Glycogen accumulation by wild-type and uridine diphosphate glucose pyrophosphorylase-negative strains of *Escherichia coli*. Arch. Biochem. Biophys. 108:440–451.
20. Sinclair, N. A., and J. L. Stokes. 1963. Role of oxygen in the high cell yields of psychrophiles and mesophiles at low temperatures. J. Bacteriol. 85:164–167.
21. Stokes, J. L., and J. M. Larkin. 1968. Comparative effect of temperature on the oxidative metabolism of whole and disrupted cells of a psychrophilic and a mesophilic species of *Bacillus*. J. Bacteriol. 95:95–98.
22. Umbreit, W. W., R. H. Burris, and J. F. Stauffer. 1957 Manometric techniques, 3rd ed. Burgess Publishing Co., Minneapolis, Minn.