COMPARATIVE STUDY OF EFFECT OF TEMPERATURE ON METABOLISM OF PSYCHROPHILIC AND MESOPHILIC BACTERIA

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Quantitative growth rate studies have shown that psychrophilic bacteria have a markedly lower temperature characteristic for growth and a lower minimum growth temperature than mesophiles (Ingraham, 1958). The terms temperature characteristic and temperature coefficient are used as by Porter (1946). Temperature characteristic is synonymous with μ of the Arrhenius equation, while temperature coefficient is synonymous with Q_{10}. Temperature coefficients were calculated when rates were determined at only two temperatures. Both terms are a measure of the rate of decrease of rate of a given process with temperature. The present investigations were initiated to determine the metabolic basis for the low temperature characteristic of growth of psychrophilic bacteria.

During the course of this study, Brown (1957) published a comparison of a psychrophilic and a mesophilic pseudomonad and showed the end products of glucose oxidation to be the same for both strains in spite of a marked difference between the two strains with respect to the temperature coefficient of glucose and gluconate oxidation. He also found that the temperature at which the cells of the mesophilic strain were grown affected the temperature coefficient of glucose oxidation by resting cells. Mesophilic cells grown at a lower temperature had a lower coefficient of glucose oxidation. Since mesophilic cells grown at lower temperatures behaved more like psychrophiles, Brown doubted that the ability of psychrophiles to grow at lower temperatures was related to differences in the nature of glucose oxidation. He found, however, that cells of the psychrophilic strain grown at the same temperature as the mesophilic strain continued to exhibit a lower temperature coefficient of glucose oxidation.

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MATERIALS AND METHODS

Organisms and media used. *Escherichia coli* strain K-12 and *Pseudomonas aeruginosa* strain NRRL B-23 were selected as representative mesophiles. *Pseudomonas perolens* strain NRRL B-1124, and *Pseudomonas* sp. strain 21-3c, isolated from chicken allowed to spoil at 3 C (Ingraham, 1958) were studied as representative psychrophiles. All cultures were grown on either nutrient agar (Difco) or trypticase-soy agar (BBL).

Manometric experiments. Cells were grown on solid media at the indicated temperatures, harvested, and washed twice in 0.06 M phosphate buffer at pH 7.0. Suspensions were adjusted to desired densities in a Spectronic 20 spectrophotometer at 600 mμ. Usual manometric techniques were employed. Temperature coefficients were calculated from oxygen uptake rates determined simultaneously in baths at 30 and 10 C.

Preparation of cell-free extracts. Washed cells were suspended in 0.06 M phosphate buffer at pH 7.0 at indicated cell densities and subjected to 10 kc sonic oscillations in a Raytheon 250-watt sonic oscillator (model DF101) at full power for 12 min. Liquid from an ice bath to which 20 per cent ethanol had been added was circulated through the sonic head so that the temperature of the contents did not rise above 5 C. The disrupted cells were then centrifuged 10 min at 5000 rpm to remove intact cells. With some preparations of *P. aeruginosa* it was necessary to dialyze against phosphate buffer to eliminate endogenous respiration.

Dehydrogenase assays. The dehydrogenase activities were measured by following the increase in absorption of coenzyme at 340 mμ in a Cary model 14 recording spectrophotometer. Temperature control was achieved by placing the cuvette in a copper water jacket through which water from a thermostatically controlled bath was circulated. A thermocouple was inserted into the cell and continuous temperature records were

TABLE 1

Temperature coefficients of glucose oxidation by psychrophilic and mesophilic bacteria

Organism	Growth Conditions		Q_{O_2}*		Q_{10}†
	Medium‡	Temp	30 C	10 C	
		C			
Psychrophiles:					
Pseudomonas sp. (21-3c)	NA	10	56.6	12.2	2.15
	NA	10	18.9	4.66	2.02
	NA	22	10.3	2.5	2.03
	NA	22	6.6	2.2	1.73
	NA	30	25.3	5.6	2.12
	NA	30	22.2	4.6	2.20
Pseudomonas perolens	NA	15	95.0	31.7	1.73
	NA	30	144.0	40.0	1.90
	TSA	15	191.0	80.3	1.54
	TSA	15	163.0	69.7	1.53
	TSA	30	270.0	123.0	1.48
	TSA	30	270.0	123.0	1.48
Mesophiles:					
Escherichia coli	NA	35	186.0	18.0	3.26
	NA	35	96.0	8.2	3.43
	NA	25	70.9	7.1	3.17
	TSA	37	65.0	7.2	3.01
	TSA	18	113.0	11.8	3.10
Pseudomonas aeruginosa	TSA	18	126.0	16.6	2.76
	TSA	18	114.0	20.5	2.37
	TSA	37	84.6	7.8	3.30
	TSA	37	86.2	6.7	3.59

* Q_{O_2} = μL O_2 uptake per mg dry weight of cells per hr.

† $Q_{10} = \sqrt{\frac{(Q_{O_2} \text{ at } 30 \text{ C})}{(Q_{O_2} \text{ at } 10 \text{ C})}}$

‡ NA = nutrient agar; TSA = trypticase soy agar.

made as a check on the temperature control system during photometry. All reactants, with the exception of the enzyme preparation, were mixed in a small test tube which was immersed in the water bath. After a temperature equilibration period of 15 min, the enzyme preparation was pipetted into the bowl of a small glass spoon which had previously been brought to the temperature of the water bath. The enzyme was added to the reaction mixture, rapidly mixed, and poured into the cuvette. The rate of change of absorbance at 340 mμ was recorded.

At temperatures below the dew point, solid CO_2 was placed in the bottom of the cuvette compartment to prevent condensation of moisture on the optical surfaces.

Reagents. Diphosphopyridine nucleotide (DPN), 95 per cent, and triphosphopyridine nucleotide (TPN), 95 per cent, were obtained from the Sigma Chemical Company. Glucose-6-phosphate and isocitric acid (trisodium salt) were obtained from the Nutritional Biochemicals Corporation. DL-Malic acid was an Eastman product.

RESULTS

Glucose oxidation by intact cells. The growth process of psychrophiles has been shown to be characterized by a lower temperature characteristic than that of mesophiles. It was considered important, therefore, to determine whether a lower temperature characteristic also applies to the catabolic reactions of psychrophiles. Glucose oxidation was studied as a representative catabolic reaction. The rate of glucose oxidation was simultaneously determined at 30 and 10 C on aliquots of a resting cell suspension and temperature coefficients were calculated. Table 1 summarizes the results of these experiments with two psychrophiles and two mesophiles. The temperature coefficient of glucose oxidation is clearly much less for psychrophiles than for mesophiles, and in this respect parallels the differences in the temperature coefficient of growth. The temperature coefficients for different batches of cells are reasonably consistent, although there is considerable variation in Q_{O_2}, the specific rate of oxygen consumption. Variation in media has little or no effect on the temperature coefficient of glucose oxidation. *Pseudomonas* sp. strain 21-3c produced large amounts of capsular material on trypticase-soy medium, and the resulting high endogenous respiration precluded temperature coefficient measurements with cells grown on this medium. *P. aeruginosa* grew sparsely on nutrient agar.

The temperature coefficient of glucose oxidation was also unaffected by the temperature at which the cells were grown in the case of the two psychrophiles and *E. coli*. On the other hand, when *P. aeruginosa* was grown at lower temperatures, the temperature coefficient of glucose oxidation decreased. However, the value remained significantly greater than that for psychrophiles grown at the same temperature. These results agree well with those of Brown (1957).

TABLE 2

Temperature coefficients of acetate and formate oxidation

Organism	Growth Conditions		Substrate	Q_{10}
	Medium*	Temp		
		C		
Psychrophiles:				
Pseudomonas sp. (21-3c)	NA	25	Acetate	1.94
Pseudomonas perolens	TSA	15	Formate	2.03
Mesophiles:				
Escherichia coli	NA	25	Acetate	2.80
Pseudomonas aeruginosa	TSA	37	Formate	3.17

* NA = nutrient agar; TSA = trypticase soy agar.

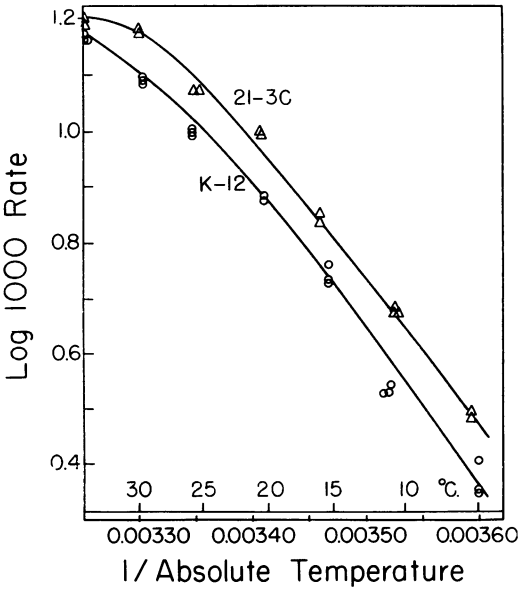

Figure 1. A comparison of the effect of temperature on the activity of malic dehydrogenase from a psychrophile and a mesophile. 21-3c, a sonic extract from a psychrophilic *Pseudomonas* sp. K-12, a sonic extract from *Escherichia coli*. Reaction mixture contained: 1.0 ml 0.36 M glycine buffer at pH 10.0, 1.0 ml 0.12 M K malate, 1.0 ml containing 3.5 mg DPN, 3.2 ml distilled water, and 0.03 ml of enzyme preparation. The rates of the reaction were determined from the linear portions of records of absorbance at 340 mμ as a function of time. Rate is expressed in units of change of absorbance per minute.

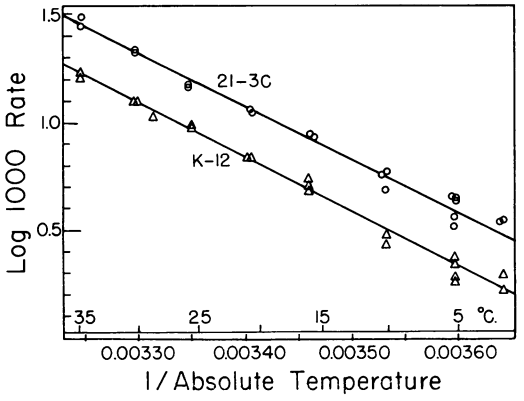

Figure 2. A comparison of the effect of temperature on the activity of isocitric acid dehydrogenase from a psychrophile and a mesophile. 21-3c, a sonic extract from a psychrophilic *Pseudomonas* sp. K-12, a sonic extract from *Escherichia coli*. Reaction mixture contained: 1.0 ml 0.0012 M K isocitrate, 1.0 ml 0.0036 M $MnCl_2$, 1.0 ml 0.15 M tris(hydroxymethyl)aminomethane at pH 7.4, 1.0 ml 0.00135 M TPN, 2.2 ml distilled water, and 0.03 ml of enzyme preparation. The rates of reaction were determined from the linear portions of records of absorbance at 340 mμ as a function of time. Rate is expressed in units of change of absorbance per minute.

Oxidation of acetate and formate. In order to determine whether the lower temperature coefficients for psychrophiles is limited to glucose oxidation, acetate and formate oxidations were investigated (table 2). For these substrates, lower temperature coefficients were found to be associated with the psychrophiles, but the difference was not as great for acetate as for glucose and formate oxidation.

The temperature coefficient of formate oxidation by *E. coli* was as low as that of the psychrophiles. Since these rate determinations may be complicated by the presence of formic hydrogenlyase, not much importance was attached to these results.

Dehydrogenase activity of cell-free extracts. The foregoing respirometric experiments established that the effect of temperature on the catabolic process, glucose oxidation, parallels the effect of temperature on growth of psychrophiles and mesophiles.

Experiments were then performed to determine whether corresponding cell-free enzyme preparations from psychrophiles and mesophiles also differed in their response to temperature.

Dehydrogenases were chosen for study because reaction rates could be readily estimated by photometric measurement of absorption of reduced DPN and TPN at 340 mμ. The results with malic, isocitric, and glucose-6-phosphate dehydrogenases are shown in figures 1, 2, and 3. It is clear from these plots that no significant differences exist in the effect of temperature on dehydrogenase preparations obtained from the mesophile and from the psychrophile.

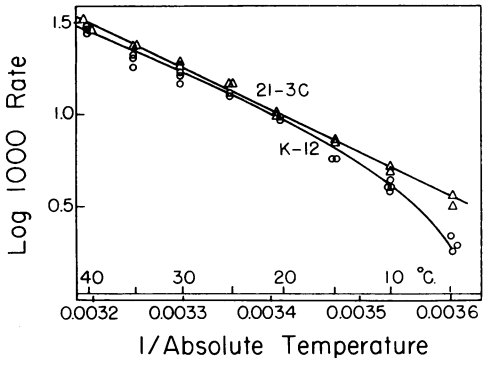

Figure 3. A comparison of the effect of temperature on the activity of glucose-6-phosphate dehydrogenase from a psychrophile and a mesophile. 21-3c, a sonic extract from a psychrophilic *Pseudomonas* sp. K-12, a sonic extract from *Escherichia coli*. Reaction mixture contained: 0.5 ml containing 0.5 mg TPN, 3.0 ml 0.1 M tris(hydroxymethyl)-aminomethane buffer at pH 7.8, 0.2 ml 0.1 M $MgCl_2$, 0.4 ml 0.02 M glucose-6-phosphate, 2.0 ml distilled water, and 0.03 ml enzyme preparation. The rates of reactions were determined from the linear portions of records of absorbance at 340 mμ as a function of time. Rate is expressed in units of change of absorbance per minute.

Glucose oxidation by cell-free extracts. The parallel behavior of mesophiles and psychrophiles with respect to temperature dependence of activity for 3 different dehydrogenases suggests that the growth differences depend upon structural integrity of the cell rather than on enzymatic differences. For this reason it was considered important to measure the same system in intact and in ruptured cells. The temperature coefficient of glucose oxidation by intact cells was measured and then a heavy suspension of the cells was treated by sonic oscillation and the determinations were repeated. In certain cases, the determinations with the cells and extracts were made simultaneously. Viable cell counts were made to insure that oxidation was not caused by unbroken cells. Extracts which oxidized glucose at the same rates as the corresponding resting cell preparations had viable cell counts 100 times smaller. It was found that the sizable differences in temperature coefficient between psychrophiles and mesophiles largely disappeared when the cells were broken. Two representative experiments of this type are shown in table 3. Paper chromatography indicated the end product of glucose oxidation by cell-free extracts to be gluconic acid. Preparations of *P. perolens* took up 0.5 mole of oxygen per mole of glucose oxidized, and the rate of oxygen uptake by *P. aeruginosa* markedly decreased after 0.5 mole of oxygen was utilized. Intact cells, on the other hand, presumably oxidize glucose to CO_2 and H_2O.

TABLE 3

Comparison of the Q_{10} of glucose oxidation by intact and ruptured cells of a mesophile and a psychrophile

Organism	Growth Temp	Q_{O_2}				Q_{10}	
		Cells		Extract* Sonic		Cells	Extract Sonic
		30 C	10 C	30 C	10 C		
	C						
Psychrophile:							
Pseudomonas perolens	15	176.0	51.6	11.1	4.71	1.85	1.53
	15	98.0	33.0	12.3	4.63	1.72	1.63
Mesophile:							
Pseudomonas aeruginosa	37	52.6	4.86	3.35	0.82	3.29	2.02
	37	45.3	4.91	1.39	0.43	3.04	1.80

* Calculated on the basis of dry weight of cells used to prepare the sonic extract.

In the case of formate oxidation, one would expect the same products from intact and ruptured cells, namely, CO_2 and H_2O. For this reason an attempt was made to study the temperature coefficient of formate oxidation by cell-free preparations. However, cell-free preparations proved to be very rapidly inactivated during the course of oxidation, and the period of linear oxygen uptake was not sufficient to determine the rate with any accuracy.

DISCUSSION

It has been shown that the growth rate of psychrophiles decreases more slowly than that of mesophiles, with decreasing temperature. A similar situation is found with certain catabolic reactions, namely, glucose, acetate, and formate oxidations. The results presented here on glucose oxidation agree well with those of Brown (1957). In the case of glucose oxidation the differences between psychrophiles and mesophiles largely disappear when the cells are broken. The temperature coefficient of mesophiles decreases while that of psychrophiles remains almost constant. No significant differences were found in the temperature coefficient of dehydrogenase activity of preparations from psychrophiles and mesophiles and only slight differences were found in glucose oxidation by cell-free preparations. The parallelism between temperature coefficients of the catabolic reactions of the cell and of growth may not have a cause-and-effect relationship but the catabolic reactions do provide a system where mechanism is more easily studied. And there seems to be a reasonable likelihood that information gained from the study of catabolic systems would also be applicable to the over-all growth process. The results presented here, although preliminary in nature, indicate that the difference in temperature response of psychrophiles and mesophiles is dependent upon the structural integrity of the cells.

Why cell rupture eliminates temperature coefficient differences cannot be answered on the basis of data now available. But two explanations seem reasonable: The ability of the psychrophile and the mesophile to concentrate substrates within the cell differ; or the structural arrangement of the enzymes in the cells differs. The fact that cell breakage lowers the temperature coefficient of the mesophile rather than raising the temperature coefficient of the psychrophile does not appear to preferentially support either of the above explanations.

SUMMARY

Temperature coefficient differences between mesophiles and psychrophiles have been found for the catabolic processes: glucose oxidation, acetate oxidation, and formate oxidation by resting cells. These differences parallel those previously reported for the temperature characteristics of growth.

The effect of temperature on rate of reactions catalyzed by malic, isocitric, and glucose-6-phosphate dehydrogenases was found to be the same for preparations from psychrophiles and mesophiles. Very little difference in the effect of temperature on glucose oxidation by cell-free preparations was found between psychrophiles and mesophiles.

Our data indicate that the temperature response differences between psychrophiles and mesophiles for growth and catabolism is probably a result of some aspect of cellular organization rather than of enzymatic differences.

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