JOURNAL OF BACTERIOLOGY, Jan. 1968, p. 95-98

Vol. 95, No. 1
Printed in U.S.A.

Comparative Effect of Temperature on the Oxidative Metabolism of Whole and Disrupted Cells of a Psychrophilic and a Mesophilic Species of *Bacillus*

J. L. STOKES AND J. M. LARKIN

Department of Bacteriology and Public Health, Washington State University, Pullman, Washington 99163

Received for publication 25 October 1967

We investigated the influence of temperature, in the range of 45 to 5 C, on the rate of oxidation of glucose and citrate by intact cells and cell-free extracts of psychrophilic *Bacillus psychrophilus* and mesophilic *B. thuringiensis*. Both glucose and endogenous oxidation by whole and disrupted cells of the psychrophile decreased more slowly with decrease in temperature than did glucose and endogenous oxidation by whole and disrupted cells of the mesophile. Similar results were obtained for the oxidation of citrate by cell-free extracts. Since substrate permeability is not involved in the oxidative metabolism of the cell-free extracts, we concluded that the internal enzymes of the psychrophile differ from those of the mesophile.

The mechanism which permits psychrophilic microorganisms to grow at temperatures as low as −10 C is not yet known. Comparative metabolic investigations with whole cells have shown that, in general, enzymatic activity of psychrophilic bacteria and yeasts decreases more slowly with decrease in temperature than does enzymatic activity of mesophilic bacteria and yeasts. This is true for the oxidation of glucose and fatty acids by psychrophilic and mesophilic species of *Pseudomonas* and other bacteria (2, 4, 8, 11) and for the oxidation and fermentation of glucose by psychrophilic and mesophilic yeasts (1, 8, 9). Investigations with nonmetabolizable glucosamine and with glucose and sorbose have indicated that, at low temperatures, substrate transport is better in psychrophilic yeasts than in mesophilic yeasts (1, 3).

It appears, therefore, that either permeability, or the nature of the enzymes, or both may be involved in establishing the lower temperature limits of growth. To obtain further information on the role of the enzymes, comparative oxidative studies were done on whole cells and cell-free extracts of a psychrophilic and a mesophilic species of *Bacillus*. It was thought that investigations with cell-free extracts would provide information on the internal enzymes free from the complicating effects of substrate permeability.

MATERIALS AND METHODS

The experiments were done on the psychrophile *B. psychrophilus* strain W16A (5, 6) and on the mesophile *B. thuringiensis* ATCC 10792. The former has a maximal growth temperature of 30 C and a minimal growth temperature below 0 C. The corresponding cardinal temperatures for the mesophile are 40 and 10 C. These strains were chosen because of their ability to oxidize glucose readily and to resist autolysis in cell suspensions. We found extensive lysis in psychrophilic and mesophilic species of *Bacillus* when the cells were washed and suspended in phosphate buffer.

Inocula were prepared by growing the cells in 25 ml of Trypticase Soy Broth in 250-ml Erlenmeyer flasks (at 20 and 30 C for the psychrophile and mesophile, respectively) on a New Brunswick rotary shaker (New Brunswick Scientific Co., New Brunswick, N.J.) at 300 oscillations per min. Cell suspensions for manometric experiments were prepared in the following manner. Sterile Trypticase Soy Broth, 350 ml of medium contained in a 2-liter Erlenmeyer flask, was inoculated with 1.0 ml of a 26-hr broth culture of *B. psychrophilus* or with 0.1 ml of a 13-hr broth culture of *B. thuringiensis*. The psychrophile was incubated at 20 C for 21 hr and the mesophile at 30 C for 7.5 hr, with vigorous shaking in both cases. As a result of these conditions, we obtained cultures which were approximately in the middle of the exponential phase of growth at the time of harvest. The psychrophile grew more slowly and less extensively than did the mesophile. At harvest, the former had reached a turbidity of 200 units, as measured in the Klett-Summerson photoelectric colorimeter (no. 66 filter), and the latter a turbidity of 400 units. The cells were harvested by centrifugation at 4 C, washed twice with 0.1 M K_2HPO_4-KH_2PO_4 buffer, *p*H 7.0, and finally suspended in sufficient buffer to give a turbidity of 450 Klett units for the psychrophile and 200 Klett units for the mesophile. These suspensions contained 2.0 mg of the psychrophile and 1.4 mg of the mesophile per ml on a dry weight basis, and the suspensions

consumed O_2 at a moderate rate during glucose oxidation, manometrically, so that the O_2 supply was not rate limiting.

To prepare cell-free extracts, the cells of both organisms were grown, harvested, and washed, as described above, and then resuspended in sufficient buffer so that a 1:10 dilution gave a Klett reading of 450 units. These suspensions were passed through a French pressure cell at 4 C and at about 20,000 psi. The disrupted cell suspensions were used without further treatment. Microscopic examination revealed only one or two intact cells per field or over 99.9% breakage. The disruption of the cells caused a marked decrease in their ability to oxidize glucose, and this made it necessary to use concentrated cell suspensions for breakage.

Manometric methods were used for the oxidation experiments. Each Warburg vessel received 2.0 ml of cell suspension or extract in the main compartment, 0.2 ml of substrate (20 μmoles) dissolved in water in the side arm, and 0.2 ml of 20% KOH in the center well to absorb CO_2. The gas phase was air and the bath temperature ranged from 5 to 50 C at 5 C intervals.

RESULTS

Preliminary experiments with *B. psychrophilus* indicated that suspensions of intact cells oxidized glucose, glycerol, glycine, L-alanine, and L-glutamic acid, and that cells in the middle of the exponential phase of growth were most active. Acetate was oxidized slowly, and xylose, citrate, succinate, and pyruvate were not oxidized at all. Cell-free extracts, however, oxidized not only glucose, glycerol, and glycine but also citrate and pyruvate. Presumably, the latter two compounds cannot penetrate intact cells. Comparable, but not as extensive, experimentation with *B. thuringiensis* showed that intact cells readily oxidized glucose but not citrate, and that cell-free extracts oxidized both compounds. Cells in the middle of the exponential phase of growth were satisfactory for oxidative experiments.

Effect of temperature on glucose oxidation by intact cells. A comparison between the rates of oxygen consumption by whole cells of *B. psychrophilus* and *B. thuringiensis* with glucose as substrate, at temperatures ranging from 45 to 5 C, showed that both organisms oxidized glucose most rapidly at 35 C (Table 1). As the incubation temperature was lowered, the oxidation rate for the psychrophile decreased less rapidly than did the oxidation rate for the mesophile. The oxidation rate was considerably higher at 10 and 5 C for the former, even when the overall higher activity of the psychrophile was taken into consideration. This difference is made clearer if O_2 consumption at different temperatures is plotted as a percentage of maximal activity (Fig. 1). At

TABLE 1. *Effect of temperature on rate of oxidation of glucose by intact cells of Bacillus psychrophilus and B. thuringiensis*

Temp (C)	O_2 consumed per vessel per hr[a] (μliters)	
	B. psychrophilus	*B. thuringiensis*
45		9
40	105	100
35	258	172
30	234	131
25		104
20	131	61
15		35
10	52	9
5	28	4

[a] Endogenous substracted.

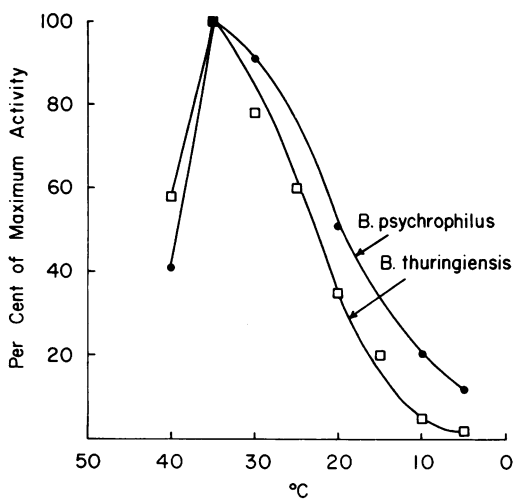

FIG. 1. *Influence of temperature on rate of glucose oxidation by intact cells of Bacillus psychrophilus and B. thuringiensis.*

20, 10, and 5 C, the psychrophile retained 51, 20, and 11%, respectively, of its maximal activity, whereas the mesophile retained only 35, 5, and 2% of its maximal activity. In contrast, at temperatures above 35 C, glucose oxidation by the psychrophile declined more sharply than did glucose oxidation of the mesophile. Additional experiments have shown that oxidation by the psychrophile, but not by the mesophile, was abolished completely at 45 C.

Thus, *B. psychrophilus* oxidized glucose more readily than did *B. thuringiensis* at the lower temperatures and less readily at the higher temperatures. These results are in accord with those of similar experiments described above, and also with the frequent finding that enzymatic reactions

of psychrophiles are more heat sensitive than corresponding reactions of mesophiles (7, 10).

Effect of temperature on glucose oxidation by cell-free extracts. Although disruption reduced the ability of the cells to oxidize glucose approximately 90%, sufficient activity remained to permit comparison of the effect of temperature on the oxidation rates of the two organisms. The data in Table 2 indicate that the activity of the cell-free extracts responded to temperature in the same manner as did the activity of the intact cells. With a decrease in incubation temperature, glucose oxidation by *B. psychrophilus* extract decreased to a lesser extent than did glucose oxidation by *B. thuringiensis* extract. For example, at 5 C, oxidation still occurred with the psychrophile but not with the mesophile, despite the greater overall activity of the latter. Again, the difference is seen more easily when the O_2 consumption data are plotted as a percentage of maximal activity (Fig. 2). At 15, 10, and 5 C, the cell-free extract of the psychrophile retained, respectively, 41, 29, and 22% of its maximal activity, whereas the corresponding values for that of the mesophile were 17, 7, and 0%.

The experiments with whole cells and cell-free extracts were repeated several times with consistent results. The average oxidation rates, as a percentage of maximal activity, are shown in Table 3. With both intact cells and cell-free extracts, a greater percentage of glucose oxidative activity was retained at lower temperatures by the psychrophile than by the mesophile.

In addition, the endogenous oxidation rates of the cell-free extracts of the two organisms were similarly affected by temperature (Table 4). For example, at 10 and 5 C the psychrophile extract exhibited 37 and 20%, respectively, of its maximal activity, whereas the mesophile extract exhibited only 13 and 7%.

TABLE 2. *Effect of temperature on rate of oxidation of glucose by cell-free extracts of Bacillus psychrophilus and B. thuringiensis*

Temp (C)	O_2 consumed per vessel per hr[a] (μliters)	
	B. psychrophilus	*B. thuringiensis*
40	25	111
35	37	174
30	34	162
25	28	122
20	23	74
15	15	29
10		12
5	8	0

[a] Endogenous subtracted.

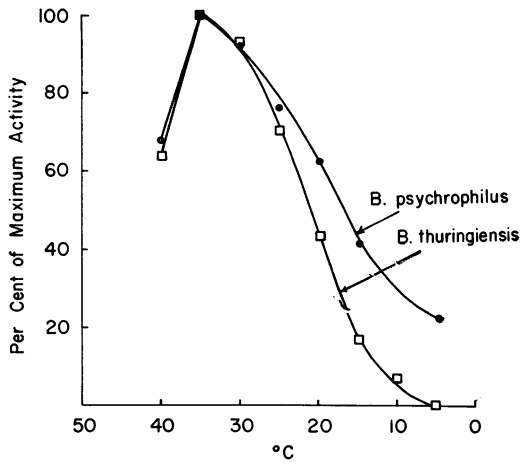

FIG. 2. *Influence of temperature on rate of glucose oxidation by cell-free extracts of Bacillus psychrophilus and B. thuringiensis.*

TABLE 3. *Influence of temperature on glucose oxidation by intact cells and cell-free extracts of Bacillus psychrophilus and B. thuringiensis (averages of several experiments)*

Temp (C)	Percentage of maximal activity[a]			
	Intact cells		Cell-free extracts	
	B. psychrophilus	*B. thuringiensis*	*B. psychrophilus*	*B. thuringiensis*
45	0	5	0	27
40	30	60	50	66
35	100	100	100	100
30	92	87	92	90
25	66	61	80	75
20	50	40	64	34
15	32	23	46	16
10	20	11	26	4
5	12	3	18	0

[a] Endogenous subtracted.

Citrate oxidation by cell-free extracts. Although the intact cells of both organisms do not oxidize citrate, their extracts oxidized this compound readily and more rapidly than glucose. The influence of temperature on citrate oxidation is shown in Table 5. With *B. psychrophilus* extract, oxidation of citrate, like that of glucose, is slowed to a lesser extent by decreases in temperature than with *B. thuringiensis* extract.

DISCUSSION

All of the experiments with intact and disrupted cells of *B. psychrophilus* and *B. thuringiensis*

TABLE 4. *Influence of temperature on endogenous oxidation of cell-free extracts of Bacillus psychrophilus and B. thuringiensis*

Temp (C)	O_2 consumed per vessel per hr (μliters)		Percentage of maximal activity	
	B. psychrophilus	*B. thuringiensis*	*B. psychrophilus*	*B. thuringiensis*
45		126		46
40	68	240	56	87
35	84	276	69	100
30	122	225	100	82
25	108		89	
20	76	75	62	27
15	50		41	
10	38	37	31	13
5	23	20	19	7

TABLE 5. *Influence of temperature on oxidation of citrate by cell-free extracts of Bacillus psychrophilus and B. thuringiensis*

Temp (C)	O_2 consumed per vessel per hr[a] (μliters)		Percentage of maximal activity	
	B. psychrophilus	*B. thuringiensis*	*B. psychrophilus*	*B. thuringiensis*
45		0		0
40	48		32	
35	71	228	48	74
30	107	307	72	100
25	148		100	
20	140	206	95	67
15	115	156	78	51
10	73	80	49	26
5	53	53	26	17

[a] Endogenous subtracted.

showed that the psychrophile functions better oxidatively at low temperatures than the mesophile. Since substrate permeability was not involved in the experiments with cell-free extracts, it can be concluded that the enzymes of the psychrophile differ in some manner from those of the mesophile. They function better at low temperatures and less effectively at high temperatures. This does not eliminate permeability as one determining factor of low temperature growth and metabolism of psychrophilic microorganisms, but it does indicate that the nature of the internal enzymes may be of great importance.

Our results differ, in part, from those of Ingraham and Bailey (4). In their experiments, the temperature difference for the oxidation of glucose between psychrophilic and mesophilic strains of *Pseudomonas* disappeared when cell-free extracts were used. Perhaps the difference in the results of the two investigations is due to the difference in cultures used. We were unable to extend our results to other pairs of psychrophilic and mesophilic species of *Bacillus* because of difficulty with autolysis. Experiments which we are currently carrying out with psychrophilic and mesophilic strains of yeast indicate that the temperature difference for the fermentation of glucose can be obtained with both whole cells and cell-free extracts.

ACKNOWLEDGMENT

This investigation was supported by a grant from the National Science Foundation.

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