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Protein synthesis in psychrotrophic and psychrophilic bacteria at various temperatures

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Effect of temperature on amino acid uptake and protein synthesis by intact resting cells of two mesophilic, five psychrotrophic, and one psychrophilic bacteria was investigated. All these bacteria could incorporate L-leucine into hot TCA-insoluble fraction at 0°C, and the incorporation activities of the psychrotrophs and psychrophile were always higher than those of the mesophiles. The temperature coefficients (Q_{10}^{0-10}) of protein synthesis were in the range of 1.2 to 6.1 for the psychrotrophs and psychrophile contrasting with 28.0 and 54.7 for the mesophiles. These results indicated that the activity of protein synthesis of these bacteria at low temperature may reflect on the capacity of growth at low temperature circumstances, but it was strange that the activities of *Flavobacterium* sp. strain G-3 and *Vibrio* sp. strain ABE-1 were unexpectedly low in consideration of their high growth rates at 0°C.

We have examined whether the difference between mesophiles and psychrotrophs or psychrophiles in the protein-synthesizing activities at low temperature correlates directly with the difference of their capacities of growth at that temperature. In a previous paper (SARUYAMA *et al.*, 1979), it was described that there seemed to be a correlation between cold-sensitivity of the protein-synthesizing system of *Pseudomonas aeruginosa* and diminution of its viability under low temperature, and that protein synthesis at 0°C was always less in the mesophiles than in the psychrotrophs as far as we examined. However, results of psychrotrophs were limited only by *Pseudomonas* sp. strain 351 (*P.* 351) in view of the purpose of that report. Thus, we considered it to be better to publish the other results which were the basis of the above description.

In this paper, incorporation activity of ^{3}H-leucine as well as growth rate at various temperatures between 0° and 50°C in five strains of psychrotrophic, one psychrophilic, and two mesophilic bacteria are described.

Materials and Methods

Bacteria and growth conditions: Psychrotrophic bacteria, strain I-9*, strain G-4*, *Bacillus* sp. strain I-12 (*B.* I-12), and *Flavobacterium* sp. strain G-3 (*F.* G-3) (SARUYAMA *et al.*, 1978), and psychrophilic bacterium, *Vibrio* sp. strain ABE-1 (*V.* ABE-1) (TAKADA *et al.*, 1979) were used in this study. Growth conditions for psychrotrophs were the same as those reported previously (SARUYAMA *et al.*, 1979), and *V.* ABE-1 was cultured under the same conditions as above except that 0.5 M NaCl was added to the nutrient medium and the growth temperature was at 10°C instead of 20°C (for the psychrotrophs). Cultures in the late log phase were used for examinations.

Assay of amino acid incorporation: Uptake of ^{3}H-leucine and the activity of protein synthesis of psychrotrophs were measured as described previously (SARUYAMA *et al.*, 1979), and those of *V.* ABE-1 were measured by the same methods as above except that 0.5 M NaCl was added to all buffers. ^{3}H-Leucine was added to the reaction mixture in the following concentration, for strain I-9, 0.56 μM; strain G-4, 0.94 μM; *B.* I-12, 0.94 μM; *F.* G-3, 0.75 μM; and *V.* ABE-1, 0.86 μM.

Chemicals: L-[4, 5-^{3}H] Leucine (50~58 Ci/mmol) was purchased from the Radiochemical Centre Ltd., England.

Results and Discussion

Total uptake of ^{3}H-leucine and incorporation of leucine to hot TCA-insoluble fraction by five psychrotrophic, one psychrophilic, and two mesophilic bacteria were determined at different temperatures between 0° and 50°C. These results are represented in Table. 1. A part of the results by mesophilic bacteria, *Pseudomonas aeruginosa* and *Bacillus subtilis*, and a psychrotrophic bacterium, *P.* 351, were already reported in the previous paper (SARUYAMA *et al.*, 1979). As the maximum temperatures for the protein-synthesizing activity were higher than the optimum temperatures for growth in *B.* I-12, *F.* G-3, strain G-4, and *V.* ABE-1, the protein-synthesizing system in these bacteria did not limit the maximum temperature for growth. Other limiting factors such as thermolabile enzyme, cellular organization, membrane and so on which were reviewed by MORITA (1975) may exist.

Although both total uptake and protein-synthesizing activity of psychrotrophs and psychrophile at 10°C were similar as or rather low than those of two mesophiles, the activities of psychrotrophs and psychrophile

* Strains I-9 and G-4 seem to belong in genera *Pseudomonas* and *Bacillus*, respectively, but have not been identified.

were always higher than those of mesophiles at 0°C.

Further, Q_{10} values showed more definite cold-stability of the protein-synthesizing systems of psychrotrophs and psychrophile. Q_{10} values were calculated from the results in Table 1, and summarised in Table 2. Low

TABLE 1. Growth rate, total uptake, and incorporation to hot TCA-insoluble fraction of ^{3}H-leucine at various temperatures

Bacteria		0°C	10°C	15°C	20°C	25°C	30°C	40°C	50°C
Strain I-9	Growth rate	1.4	10.3	17.3	37.1	43.3	31.0	0.0	0.0
	Total uptake	13.5	49.0	—	97.0	119.3	79.0	3.7	0.0
	Hot TCA	3.6	22.0	—	39.0	33.8	32.7	2.3	0.0
Bacillus I-12	Growth rate	1.4	8.0	15.4	39.6	17.9	0.0	0.0	0.0
	Total uptake	4.9	9.8	—	20.7	—	24.9	9.1	0.0
	Hot TCA	0.5	2.0	—	7.5	—	12.0	5.3	0.0
Flavobacterium G-3	Growth rate	4.3	17.2	26.5	34.8	30.1	7.7	0.0	0.0
	Total uptake	0.9	1.5	—	2.2	—	3.9	1.6	0.0
	Hot TCA	0.6	0.7	—	1.0	—	2.4	1.3	0.0
Strain G-4	Growth rate	2.7	10.7	19.0	27.7	31.7	10.3	0.0	0.0
	Total uptake	4.8	17.0	—	38.0	—	60.0	12.0	0.0
	Hot TCA	1.3	3.9	—	14.0	—	23.0	1.3	0.0
**Pseudomonas* sp. 351	Growth rate	1.5	11.7	—	19.1	—	27·7	7.4	0.0
	Total uptake	11.1	43.1	—	66.9	—	108.6	17.0	0.2
	Hot TCA	2.9	15.8	—	29.9	—	60.5	4.8	0.0
Vibrio ABE-1	Growth rate	6.5	18.5	25.9	19.6	0.0	0.0	0.0	0.0
	Total uptake	0.7	0.9	1.3	1.8	2.0	1.1	0.5	0.0
	Hot TCA	0.6	0.7	0.8	1.1	1.3	1.1	0.5	0.0
**P. aeruginosa*	Growth rate	0.0	3.7	—	25.2	—	62.5	71.2	—
	Total uptake	1.1	42.2	—	157.0	—	203.7	178.2	2.2
	Hot TCA	0.3	16.4	—	98.3	—	164.0	79.6	1.8
**B. subtilis*	Growth rate	0.0	4.1	—	16.4	—	61.3	85.5	—
	Total uptake	1.2	9.6	—	36.1	—	77.1	54.3	0.0
	Hot TCA	0.1	2.8	—	16.4	—	54.7	19.3	0.0

Growth rates are expressed as $hr^{-1} \times 100$, and total uptake and incorporation to hot TCA-insoluble fraction are as pmol/min/10^9 cells.

Growth rates of *V.* ABE-1 and the other bacteria were calculated from the results by TAKADA *et al.* (1979), and SARUYAMA *et al.* (1978).

* A part of these data were reported in a previous paper (SARUYAMA *et al.*, 1979).

TABLE 2. Temperature coefficient (Q_{10}) for the activities of the total uptake of ^{3}H-leucine and the incorporation of ^{3}H-leucine into hot TCA-insoluble fraction

Bacteria	Q_{10}^{0-10}		Q_{10}^{10-20}	
	Total uptake	Hot TCA	Total uptake	Hot TCA
Strain I-9	3.6	6.1	2.0	1.8
Bacillus I-12	2.0	4.0	2.1	3.8
Flavobacterium G-3	1.7	1.2	1.5	1.4
Strain G-4	3.5	3.0	2.2	3.6
**Pseudomonas* sp. 351	3.9	5.4	1.6	1.9
Vibrio ABE-1	1.3	1.2	2.0	1.6
**P. aeruginosa*	38.4	54.7	3.7	6.0
**B. subtilis*	8.0	28.0	3.8	5.9

* These data were reported in a previous paper (SARUYAMA *et al.*, 1979).

Q_{10}^{0-10} values were shown not only in *P.* 351 as reported previously but also in all other psychrotrophs and psychrophile. According to HOCHACHKA (1973), low Q_{10} values mean an advantage for the strategies of low temperature adaptation, and our results obtained by psychrotrophs and psychrophile may powerfully support his theory.

As seen in Table 2, Q_{10}^{0-10} value was lowest in *F.* G-3 and *V.* ABE-1, both of which could grow more rapidly at 0°C and gave a larger cell mass than the other psychrotrophs, but the specific activities of protein synthesis at the temperature range tested were unexpectedly low. It was a strange phenomenon, but has not yet been clarified.

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