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Heat and Cold Shock Protein Synthesis in Arctic and Temperate Strains of Rhizobia†

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We compared heat shock proteins (HSPs) and cold shock proteins (CSPs) produced by different species of *Rhizobium* having different growth temperature ranges. Several HSPs and CSPs were induced when cells of three arctic (psychrotrophic) and three temperate (mesophilic) strains of rhizobia were shifted from their optimal growth temperatures (arctic, 25°C; temperate, 30°C) to shock temperatures outside their growth temperature ranges. At heat shock temperatures, three major HSPs of high molecular weight (106,900, 83,100, and 59,500) were present in all strains for all shock treatments (29, 32, 36.4, 38.4, 40.7, 41.4, and 46.4°C), with the exception of temperate strains exposed to 46.4°C, in which no protein synthesis was detected. Cell survival of arctic and temperate strains decreased markedly with the increase of shock temperature and was only 1% at 46.4°C. Under cold shock conditions, five proteins (52.0, 38.0, 23.4, 22.7, and 11.1 kDa) were always present for all treatments (−2, −5, and −10°C) in arctic strains. Among temperate strains, five CSPs (56.1, 37.1, 34.4, 17.3, and 11.1 kDa) were present at temperatures down to 0°C. The 34.4- and the 11.1-kDa components were present in all temperate strains at −5°C and in one strain at −10°C. Survival of all strains decreased with cold shock temperatures but was always higher than 50%. These results show that rhizobia can synthesize proteins at temperatures not permissive for growth. In all shock treatments, no correspondence between the number of HSPs or CSPs produced and rhizobial survival was found. In conclusion, cold-adapted arctic rhizobia synthesized HSPs at temperatures higher (46.4 versus 41.4°C) than temperate rhizobia and produced more CSPs under freezing conditions (−10°C). However, their cold adaptation does not seem to provide them with better survival at freezing temperatures.

Temperature is one of the most important factors affecting any organism (9). When cells are exposed to high temperatures or any other environmental perturbations such as UV irradiation, nalidixic acid (17), or ethanol (23), a group of proteins called heat shock proteins (HSPs) are rapidly synthesized (25, 36). These HSPs have been encountered in a wide variety of species from bacteria to humans (extensive reviews are in references 19, 24, and 33), but their physiological significance remains to be elucidated. However, they may have a role in cellular homeostasis, since their synthesis has been correlated with the acquisition of thermotolerance and ethanol tolerance in bacteria (23).

In response to shocks at low temperatures, bacteria show a specific cold response. For instance, 13 polypeptides were synthesized in *Escherichia coli* after a shift from 37 to 10°C (11). More recently, a major cold shock protein (CSP), CS7.4, was reported to be rapidly induced upon a shift from 37 to 10 or 15°C. This peptide may act as an antifreeze protein (6). The CSPs also seemed involved in transcription and translation, and it has been suggested that the cold shock response may be adaptive for the growth of cells (11). In fact, the minimum growth temperatures of microorganisms are determined by many factors, among which protein synthesis is very important (10). It has been reported that the protein-synthesizing system of psychrophilic bacteria is more active at low temperatures than that of mesophilic bacteria, whose decrease in viability coincides with a decrease in the level of protein synthesis (30, 31).

There is no report on the differences in heat and cold shock responses that may exist between psychrotrophic and mesophilic bacteria. In previous papers (27–29), we reported that rhizobia isolated from arctic legumes (*Astragalus* and *Oxytropis* spp.) are of considerable interest because of their potential to improve symbiotic nitrogen fixation of legumes cultivated in temperate climates, where low temperature limits the efficiency of the symbiosis (5, 8). In fact, arctic rhizobia showed an adaptation to low temperatures for their growth in culture media, their competitiveness in forming nodules, and their nitrogenase activity in symbiosis with the temperate forage legume sainfoin (*Onobrychis viciifolia*).

In order to understand the factors related to temperature adaptation, we undertook a study of protein synthesis in rhizobial strains having different thermoadaptation levels. We report here on a comparison of the HSP and CSP responses of three arctic (psychrotrophic) and three temperate (mesophilic) strains of rhizobia exposed to temperatures outside their growth temperature ranges.

MATERIALS AND METHODS

Bacteria and culture conditions. The three arctic rhizobia used in this study were isolated from the arctic legumes *Astragalus alpinus* (N31 and N44) and *Oxytropis maydelliana* (N40): each strain can nodulate both arctic legumes and the temperate legume *O. viciifolia* (28). For purposes of comparison, three temperate strains were selected: *Rhizobium* sp. (*O. viciifolia*) strain SM2 (27), capable of nodulating the arctic legume *A. alpinus*; *Rhizobium* sp. (*Oxytropis*

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TABLE 1. Limits of growth temperature of arctic and temperate strains of rhizobia used in this study

Growth temp category	Temp (°C) for:					
	Arctic strain			Temperate strain		
	N31	N40	N44	A2	SM2	118H1
Minimal	0.0	0.0	0.0	7.0	5.5	4.0
Optimal	25.0	25.0	25.0	30.0	30.0	30.0
Maximal	30.0	30.0	27.0	38.7	36.4	36.4

riparia) strain 118H1 (Liphatech Inc., Milwaukee, Wis.), capable of nodulating the two arctic legumes *A. alpinus* and *O. maydelliana*; and *Rhizobium meliloti* A2 (Agriculture Canada), which is commonly used as an inoculant for alfalfa grown in temperate regions. The taxonomic status of arctic rhizobia is uncertain, and they may constitute a specific group. They have physiological characteristics in common with both *Rhizobium* spp. and *Bradyrhizobium* spp. and, on the basis of DNA homology, they show little relationship with these two genera but are closely related to each other (3, 28).

Cells were grown in a minimal medium containing (per liter) 5 g of glucose, 0.6 g of KNO_3, 0.05 g of $CaCl_2 \cdot 2H_2O$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of NaCl, 0.5 g of K_2HPO_4, 0.5 g of KH_2PO_4, 0.5 mg of ferric citrate, 100 μg of biotin, 250 μg of thiamine, 100 μg of pantothenic acid, and 1 ml of trace mineral element (2.86 g of H_3BO_3, 1.81 g of $MnCl_2 \cdot 4H_2O$, 0.22 g of $ZnSO_4 \cdot 7H_2O$, 0.08 g of $CuSO_4 \cdot 5H_2O$, 0.09 g of H_2MoO_4, and 0.04 g of $CoCl_2 \cdot 6H_2O$ per liter). Phosphates were autoclaved separately, vitamins were sterilized by filtration (with a 0.22-μm-pore-size filter), and the pH of the medium was adjusted to 6.9.

Selection of shock temperatures. The normal growth temperature range of each strain was determined in preliminary experiments by using the total protein content of the cells (20) for growth evaluation (Table 1).

Shock temperatures were selected in order to compare protein synthesis in all strains at specific temperatures and at specific ranges (shock temperature minus optimal temperature) outside the normal growth temperature range. A shift of 2°C outside the growth temperature limits was applied to each strain. For heat shocks, arctic strains were shifted from their optimal growth temperature (25°C) to the maximal growth temperature of temperate strains 118H1 and SM2 (36.4°C). Both types of rhizobia were also shifted from their optimal growth temperatures to 41.4 and 46.4°C. For cold shocks, temperate strains were shifted from their optimal growth temperature (30°C) to the minimal growth temperature of arctic strains (0°C). Both types of rhizobia were also shifted from their optimal growth temperatures to −5 and −10°C.

Labelling of cells. One hundred milliliters of mid-log-phase cells (optical density at 600 nm = 0.6) grown at the optimal temperature were labelled with 0.30 mCi of [^{35}S]methionine (specific activity, 1,000 to 1,500 Ci/mmol; Amersham). For shock treatment, mid-log-phase cells were incubated in a water bath shaker (for HSPs) or in a computer-controlled freezer (for CSPs) for 10 to 20 min until the desired shock temperature was reached and were immediately labelled with [^{35}S]methionine (0.75 mCi for heat shocks and 0.30 mCi for cold shocks). After a 3-h shock period, three 15-ml subcultures and one 5-ml subculture were sampled and transferred to sterile centrifuge tubes, and unlabelled L-methionine (final concentration, 1 mM) was added to stop radioactive incorporation. Cells were centrifuged at 10,000 × *g* for 5 min. After an initial wash with saline solution (0.85% NaCl), pellets were resuspended in sterile water and stored at −80°C until used. Pellets from the 5-ml samples were used for determination of radioactivity incorporation. After suspension in 5 ml of sterile distilled water, cells were disrupted with a Fisher Sonic Dismembrator (model 300) at 195 W for 1 min, and proteins were precipitated with 15% trichloroacetic acid. Aliquots of 1 ml were centrifuged at 13,000 × *g* for 5 min. The precipitates were then dissolved in 0.5 ml of distilled water and neutralized with 50 μl of 1 N NaOH. Radioactivity was measured with a scintillation counter (LKB Rack beta 1217) by using a Beckman Ready Protein scintillation cocktail.

PAGE of proteins. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) system of Laemmli (18) was used. Separations were performed on a 12-cm gradient separation gel (12 to 18%), 0.8 mm thick, with a 2-cm-high stacking gel (4.5%). Pellets from the 15-ml samples were suspended in cold 20% trichloroacetic acid and sonicated. The precipitates were washed twice by centrifugation (at 13,000 × *g* for 5 min) in 100% acetone, dissolved in sample buffer (62.5 mM Tris [pH 6.8], 2.3% SDS, 10% glycerol, 5% β-mercaptoethanol) and heated at 100°C for 2 min. Equal volumes of samples equivalent in protein content were loaded into each well. Electrophoretic migration was performed for 16 h at 85 V (constant voltage). Gels were stained with Coomassie brilliant blue R-250. ^{35}S-labelled proteins were detected by fluorography by using X-Omat film (1). Molecular weights of proteins were determined by using several proteins as standards: myosin, 205,000; β-galactosidase, 116,000; phosphorylase *b*, 97,400; bovine albumin, 66,000; egg albumin, 45,000; carbonic anhydrase, 29,000; soybean trypsin inhibitor, 20,100; and α-lactalbumin, 14,200 (Sigma). The intensity of each protein band was measured from densitometer scannings (Bio-Rad densitometer, model 620) of fluorograms. Area and relative abundance of the proteins were determined by computer analysis.

Survival estimation. Survival of each rhizobial strain was evaluated for each shock temperature. Percent survival was estimated as the number of viable cells after the 3-h shock period relative to the number of cells before the treatment. The number of cells was evaluated by colony counts obtained from serial dilutions plated on YMA (yeast-mannitol agar) containing Congo red (34). Plates of arctic and temperate rhizobia were incubated at their optimal growth temperatures.

RESULTS

Protein synthesis pattern at optimal temperature. The basic protein pattern obtained by the incorporation of [^{35}S]methionine was determined for each strain at its optimal growth temperature (Fig. 1). A total of 46 different bands was observed for arctic strains compared with 55 bands for temperate strains. Moreover, 27 bands of similar molecular weight were common to both groups of rhizobia.

Protein patterns among arctic strains were similar. Although most of the bands were present in each strain, three bands were not observed in strain N31, six bands were not observed in strain N44, and two bands were not observed in strain N40. Only one strain-specific band was observed in strain N40.

Protein patterns of temperate strains showed a greater diversity than those observed with arctic strains. In fact, *R.*

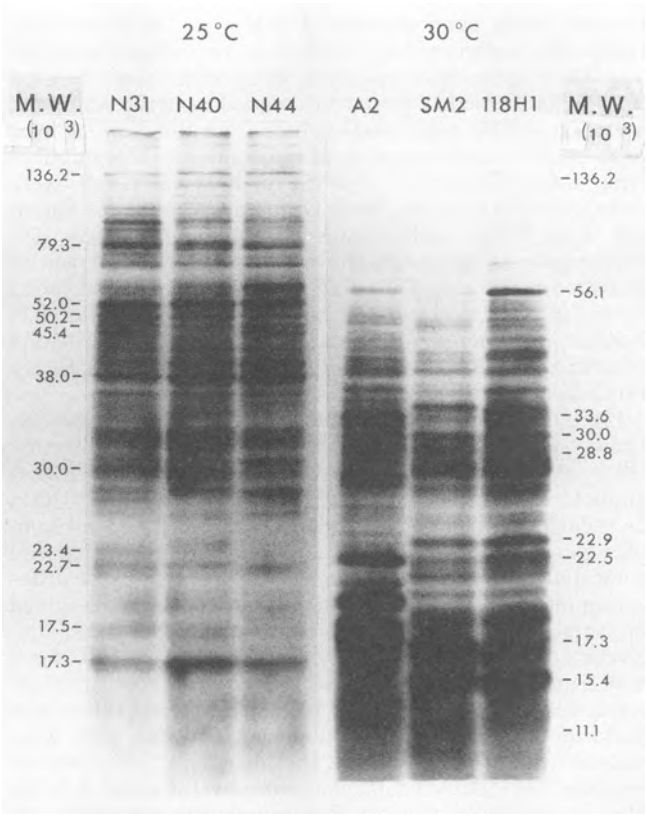

FIG. 1. Fluorograms of protein synthesized at optimal growth temperatures by arctic (N31, N40, and N44) and temperate (A2, SM2, and 118H1) strains of rhizobia.

meliloti A2 showed seven specific bands, strain 118H1 from *O. riparia* showed six specific bands, and strain SM2 from *O. viciifolia* only one specific band.

In addition, no differences in total stained protein patterns between optimal and shock temperatures were observed for any strain (results not shown).

HSPs. All arctic strains of rhizobia showed a similar response to all temperature shifts over their maximal growth temperature by synthesizing in common a basic set of three HSPs (106.9, 83.1, and 59.5 kDa) in closely similar proportions (Table 2; Fig. 2A). At 29 and 32°C (2°C over the maximal growth temperature), only this set of three proteins was present. Additional proteins (one to four, depending on the strain) were observed at higher temperatures. Two of these proteins were strain specific, as was shown by the absence of the 18.3-kDa component in strain N40 and the absence of the 17.6-kDa component in strain N31. In all strains, the relative abundance of the major component (59.5 kDa) decreased (from ≈80% to 14%) with increased shock temperatures (Table 2).

Although arctic strains produced HSPs at all shock temperatures studied, temperate strains had a more restricted response, because no protein synthesis was detected at 46.4°C and there was no increase in the number of protein bands with shock temperatures (Table 3; Fig. 2A). However, temperate strains also produced, in similar proportions, the basic set of HSPs (106.9, 83.1, and 59.5 kDa), which was present in arctic strains. Furthermore, a specific HSP (51.9 kDa) was synthesized by strain A2 in proportions similar to those of the 59.5-kDa component. The patterns of protein synthesis were similar between strains SM2 and 118H1 because, in addition to the basic set, three proteins (32.3, 20.0, and 18.0 kDa) were present at 38.4°C. The 18.0-kDa protein represented nearly 30% of all proteins synthesized by both strains at 41.4°C. As was found with arctic strains, the major HSP in all temperate strains was the 59.5-kDa component, and its abundance decreased with increased shock temperatures in strains SM2 and 118H1.

Cell survival decreased with shock temperature for both types of rhizobia (Tables 2 and 3). For all arctic strains, survival was approximately 75% when cells were submitted to a shift of 2°C over their maximal growth temperature (minimal number of HSPs obtained) and was less than 1% after a shift to 46.4°C, at which many HSPs were present. There were no differences in survival among temperate strains at various shock temperatures (Table 3). As for arctic strains, survival was over 70% for a shift of 2°C over their maximal growth temperature and less than 1% after a shift to 46.4°C. However, in contrast to arctic strains, temperate strains did not synthesize HSPs at this temperature. At 41.4°C, survival of temperate strains was considerably higher than that of arctic strains.

CSPs. The arctic strains N31, N40, and N44 responded to the cold shock treatments by synthesizing proteins at temperatures as low as −10°C (Table 4; Fig. 2B). In general, few proteins were strain specific (e.g., four minor bands in N31), thus revealing a high level of similarity. The maximal num-

TABLE 2. Distribution of HSPs and survival of arctic strains of rhizobia at various shock temperatures

HSP (mol wt, 10^3)	Relative abundance (% of total area)[a] in strain at temp											
	N31				N40				N44			
	32°C	36.4°C	41.4°C	46.4°C	32°C	36.4°C	41.4°C	46.4°C	29°C	36.4°C	41.4°C	46.4°C
106.9	9.7	5.4	16.8	8.8	13.0	3.9	4.3	3.5	6.3	4.4	3.0	5.1
83.1	7.9	7.0	18.9	12.0	11.0	8.8	17.3	6.6	7.4	5.5	10.8	8.5
59.5	82.4	43.7	45.3	36.6	76.0	37.5	55.3	13.9	86.3	35.0	45.6	26.9
31.7	—[b]	10.7	—	2.9	—	10.8	—	—	—	14.3	9.8	3.4
30.3	—	4.5	—	9.0	—	6.5	—	—	—	12.5	3.7	12.5
18.3	—	28.7	19.0	30.7	—	—	—	—	—	21.6	18.2	28.6
17.6	—	—	—	—	—	32.5	23.1	76.0	—	6.7	8.9	15.0
Survival (%)[c]	78.6	68.1	0.9	<0.001	70.6	48.5	39.6	<0.001	72.2	0.3	0.07	<0.001

[a] Each value represents the proportion of a given protein band over the total protein bands in the treatment.
[b] —, not detected.
[c] Percentage of viable cells relative to the population number before the treatment. Each value is the mean of three replicates (standard error, <3.7).

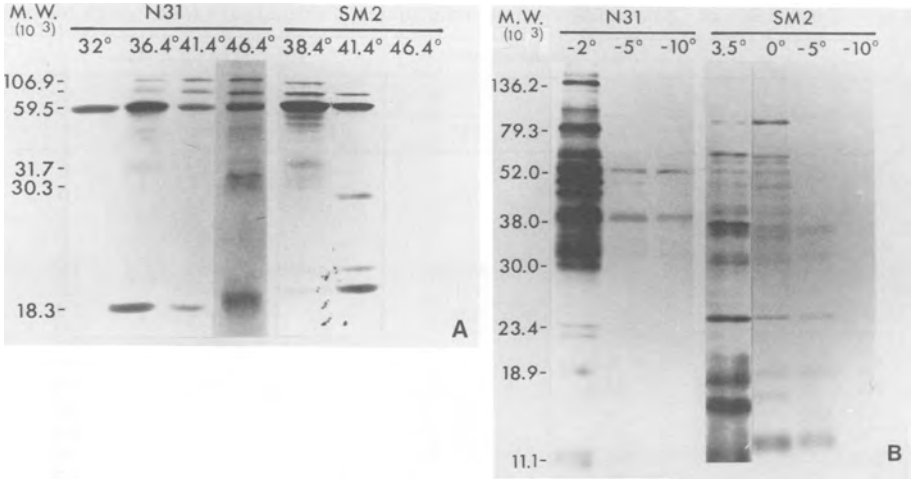

FIG. 2. Fluorograms of the HSPs (A) and CSPs (B) synthesized by the arctic strain N31 and the temperate strain SM2.

ber of bands was observed at −2°C in all strains (24, 20, and 17 bands for strains N31, N40, and N44, respectively) and, at −10°C, the numbers of bands were reduced to 10, 6, and 14, respectively. In all arctic strains, only proteins with molecular weights below 79,300 were present at the lowest shock temperatures (−5 and −10°C). At all temperatures, 5 CSPs were always present (52.0, 38.0, 23.4, 22.7, and 11.1 kDa). The 52.0-, 38.0-, and 11.1-kDa CSPs were the major components, but their relative abundance varied considerably according to strain and shock temperature. The proportion of the 11.1-kDa component increased constantly as the temperature decreased from −2 to −10°C.

As was observed with arctic strains, the three temperate strains responded to all cold shock treatments (Table 5; Fig. 2B). Strains SM2 and 118H1 responded similarly, showing 9 corresponding bands, which were absent in strain A2, while strain A2 showed a more distinctive pattern with 10 specific bands. For all strains, the number of bands decreased sharply with low temperature shocks. In fact, at −10°C, the number of components was reduced to 1 to 4 bands of very low intensity compared with 18 to 22 bands observed at the highest temperatures of cold shock. At −5 and −10°C, no proteins with molecular weights higher than 39,700 were observed, with the exception of a 56.1-kDa component in 118H1. The 56.1-, 37.1-, 34.4-, and 11.1-kDa components were common to all strains at temperatures down to 0°C and the relative abundance of each was close to 5%. Interestingly, as for arctic strains, the very abundant 11.1-kDa constituent (17 to 57%) was always present at temperatures down to −5°C.

After a 3-h shock period, survival of cells decreased slightly with lowering of the temperature (Tables 4 and 5). In fact, even at −10°C, survival of rhizobial cells was always greater than 55% for all strains. Even though the survival of temperate strains was slightly superior to that of arctic strains, the number of CSPs was similar for both types from 2°C below their minimal growth temperatures to −5°C. However, at −10°C, the number of bands and their intensity were largely superior in arctic strains (6 to 10 bands) than in temperate strains (1 to 4 bands), even though temperate strains showed 10% higher survival.

We estimated relative levels of protein synthesis by com-

TABLE 3. Distribution of HSPs and survival of temperate strains of rhizobia at various shock temperatures

HSP (mol wt, 10^3)	Relative abundance (% of total area)[a] in strain at temp								
	A2			SM2			118H1		
	40.7°C	41.4°C	46.4°C	38.4°C	41.4°C	46.4°C	38.4°C	41.4°C	46.4°C
173.2	2.4	—[b]	—	—	—	—	—	—	—
106.9	9.4	7.8	—	7.3	3.4	—	9.5	4.3	—
83.1	8.6	10.0	—	11.3	8.7	—	11.9	9.2	—
59.5	31.2	36.0	—	54.3	36.1	—	59.0	38.2	—
51.9	39.2	38.0	—	—	—	—	—	—	—
32.3	3.1	—	—	12.5	—	—	10.2	—	—
30.0	—	—	—	—	11.4	—	—	9.0	—
20.0	—	—	—	5.5	12.8	—	3.6	5.8	—
19.0	3.6	8.2	—	—	—	—	—	—	—
18.0	—	—	—	9.1	27.6	—	5.8	33.5	—
16.5	2.5	—	—	—	—	—	—	—	—
Survival (%)[c]	75.5	68.6	0.02	84.3	81.8	0.004	77.2	73.3	<0.001

[a] Each value represents the proportion of a given protein band over the total protein bands in the treatment.
[b] —, not detected.
[c] Percentage of viable cells relative to the population number before the treatment. Each value is the mean of three replicates (standard error, <9.9).

TABLE 4. Distribution of CSPs and survival of arctic strains of rhizobia at various shock temperatures

CSP (mol wt, 10^3)	Relative abundance (% of total area)[a] in strain at temp								
	N31			N40			N44		
	−2°C	−5°C	−10°C	−2°C	−5°C	−10°C	−2°C	−5°C	−10°C
151.3	1.2	—[b]	—	—	—	—	—	—	—
136.2	2.6	—	—	0.3	—	—	1.1	—	—
123.2	0.7	—	—	—	—	—	—	—	—
116.9	0.7	—	—	—	—	—	—	—	—
103.2	3.7	—	—	0.4	—	—	1.0	—	—
79.3	8.7	+[c]	—	1.3	—	—	4.5	—	+
64.0	—	—	—	0.6	—	—	2.2	—	—
63.2	1.8	—	—	0.9	—	—	2.8	—	—
58.2	5.4	3.1	6.5	—	—	—	—	—	2.7
52.0	9.1	17.4	14.9	10.2	49.8	23.9	14.0	+	4.7
50.2	7.5	6.6	—	5.6	4.6	+	10.8	6.4	14.7
45.4	7.6	—	—	1.7	—	—	7.6	1.7	5.8
43.5	2.5	—	—	—	—	—	—	—	—
39.1	7.3	9.1	18.6	—	5.6	—	4.7	8.2	6.1
38.0	10.1	35.0	4.4	10.6	27.5	24.7	21.5	46.9	8.4
34.4	3.9	2.0	2.5	1.7	—	—	4.2	—	2.8
32.8	3.9	—	—	8.9	—	—	3.1	—	4.0
31.3	6.0	2.7	4.9	2.6	—	—	7.3	—	7.3
30.0	4.4	8.4	5.7	2.3	—	—	3.8	—	+
23.4	1.3	+	5.6	2.5	+	7.5	1.5	3.8	5.4
22.7	1.3	+	6.8	1.7	+	+	1.9	5.3	4.2
20.1	1.1	—	—	1.8	—	—	—	—	—
18.9	2.7	—	—	7.9	—	—	—	—	—
17.5	1.6	—	—	10.1	—	—	—	—	
17.3	—	—	—	12.3	—	—	—	—	—
11.1	4.8	15.8	30.0	16.4	12.5	43.9	7.9	27.7	33.8
Survival (%)[d]	74.8	69.5	58.6	67.6	62.6	57.1	76.1	59.7	55.1

[a] Each value represents the porportion of a given protein band over the total protein bands in the treatment.
[b] —, not detected.
[c] +, presence of a protein band which had a very low intensity on the fluorogram.
[d] Percentage of viable cells relative to the population number before the treatment. Each value is the mean of three replicates (standard error, <7.9).

paring the area values from scannings of the fluorograms at all temperatures. In the arctic strains, N31 showed protein synthesis five times higher at −2°C than that of strains N40 and N44, while at −5 and −10°C, all strains had a protein synthesis level which was 3% of the level of N31 at −2°C. In the temperate strains, A2 showed a protein synthesis level three to six times higher than those of the other two strains (at 3.5 and 0°C), while at −10°C, residual synthesis of all strains was less than 1% of that observed for A2 at 3.5°C.

DISCUSSION

From these experiments, we observed that different species of *Rhizobium* showing different thermoadaptation characteristics do produce HSPs and CSPs at temperatures outside their normal growth ranges. Heat and cold shock responses of microorganisms have been reported for a wide range of growth-permissive temperatures (6, 11, 22, 33) and, in a few cases, as with *E. coli* (23), at higher temperatures.

HSPs and CSPs synthesized by arctic and temperate strains were less numerous than protein bands obtained at optimal temperatures. Also, as observed with the protein patterns at optimal growth temperatures, HSP and CSP patterns among arctic strains were more uniform than those of temperate strains, in which specific HSPs and CSPs were more numerous, particularly in *R. meliloti* A2. These differences between the two groups of rhizobia may be due to the fact that arctic rhizobia, isolated from *Astragalus* and *Oxytropis* species, constitute a specific group since they are closely related to each other on the basis of DNA homology and show little relatedness to known species of *Rhizobium* or *Bradyrhizobium* (3, 28). The temperate strains used in our study belong to different species of the genus *Rhizobium*, and the differences observed between *R. meliloti* and the two other rhizobial species (those isolated from *Onobrychis* and *Oxytropis* spp.) may be due to their distinct taxonomic position (12).

In spite of their different characteristics (taxonomy and growth temperature range), arctic and temperate strains of rhizobia have shown some similarities in their HSPs and CSPs. Indeed, when HSPs were synthesized, three major proteins with high molecular weights (106,900, 83,100, and 59,500) were always present in both types of rhizobia, the 59.5-kDa protein being the most abundant. These results are consistent with the observations of McCallum et al. (22), for which the synthesis of HSPs consisted of a core group included in all shock treatments. Furthermore, they observed additional shock proteins whose synthesis was dependent on the severity of the thermal shock. In our experiments, however, the appearance of additional protein bands was not related to the severity of the treatment (increasing shock temperature). Except at 46.4°C, the numbers of HSPs were similar between arctic (psychrotrophic) and temperate (mesophilic) strains. However, differences between strains with different thermoadaptation levels were reported at temperature shocks from 0°C to 32°C, at which 19 HSPs were induced in the arctic psychrophile Res-10, whereas 25

TABLE 5. Distribution of CSPs and survival of temperate strains of rhizobia at various shock temperatures

CSP (mol wt, 10^3)	Relative abundance (% of total area)[a] in strain at temp											
	A2				SM2				118H1			
	5°C	0°C	−5°C	−10°C	3.5°C	0°C	−5°C	−10°C	2°C	0°C	−5°C	−10°C
136.2	+[b]	1.6	—[c]	—	+	+	—	—	—	+	—	—
103.2	+	0.5	—	—	—	—	—	—	—	—	—	—
95.9	+	1.6	—	—	—	—	—	—	+	—	—	—
88.8	—	—	—	—	—	—	—	—	0.9	—	—	—
79.3	1.0	4.4	—	—	+	9.6	—	—	1.6	—	—	—
69.0	0.6	1.4	—	—	—	—	—	—	—	—	—	—
60.7	—	1.5	—	—	—	—	—	—	1.9	—	—	—
56.1	4.0	3.6	—	—	0.9	4.0	—	—	7.3	+	+	+
54.1	—	—	—	—	+	2.9	—	—	2.1	—	—	—
52.0	5.8	7.2	—	—	—	—	—	—	—	—	—	—
50.2	—	2.7	—	—	+	3.2	—	—	4.6	—	—	—
46.9	—	1.7	—	—	—	—	—	—	—	—	—	—
45.4	1.2	4.1	—	—	+	3.4	—	—	3.7	—	—	—
42.7	1.1	2.3	—	—	+	1.1	—	—	2.3	—	—	—
41.3	—	2.2	—	—	—	—	—	—	—	—	—	—
39.7	3.4	3.2	10.2	—	—	1.9	—	—	—	—	—	—
38.2	—	—	—	—	+	—	—	—	+	—	—	—
37.1	5.3	10.6	6.6	+	1.4	4.5	—	—	8.3	+	+	—
34.8	—	—	—	—	5.2	7.5	1.0	—	6.6	1.0	2.2	—
34.4	5.2	7.4	20.3	+	1.5	6.5	7.4	—	+	0.5	2.2	—
33.6	—	—	—	—	2.1	2.1	—	—	9.8	+	0.9	—
32.8	2.6	3.4	7.6	—	—	—	—	—	—	—	—	—
31.3	1.8	2.6	—	—	—	—	—	—	—	—	—	—
30.5	—	—	9.1	—	3.0	3.5	2.6	—	2.7	+	1.9	—
30.0	2.5	3.1	—	—	—	—	—	—	—	—	—	—
29.2	—	—	—	—	3.1	2.8	+	—	3.7	+	3.3	—
28.8	3.0	1.0	—	—	—	—	—	—	0.9	—	—	—
28.7	+	0.7	—	—	—	—	—	—	—	—	—	—
22.9	—	—	—	—	4.2	2.9	3.8	+	2.6	3.1	6.5	+
22.5	2.8	0.7	6.7	+	—	—	—	—	—	—	—	—
20.2	5.0	2.6	—	—	—	—	—	—	3.8	—	—	—
18.9	6.0	3.3	22.7	—	5.8	—	—	—	—	—	—	—
17.3	11.7	4.3	—	—	13.5	5.3	7.0	—	7.7	9.8	15.9	—
15.4	—	—	—	—	23.9	4.5	3.5	—	6.8	9.0	9.0	—
13.3	7.7	1.4	—	—	—	—	—	—	—	—	—	—
12.6	—	—	—	—	—	—	18.3	—	—	7.9	16.1	—
11.1	29.2	20.0	16.7	+	34.5	34.1	56.6	—	22.4	67.8	42.0	—
Survival (%)[d]	90.9	87.0	78.1	74.1	85.8	81.2	71.6	66.9	85.7	76.2	72.4	64.8

[a] Each value represents the proportion of a given protein band over the total protein bands in the treatment.
[b] +, presence of a protein band which had a very low intensity on the fluorogram.
[c] —, not detected.
[d] Percentage of viable cells relative to the population number before the treatment. Each value is the mean of three replicates (standard error, <4.8).

HSPs were synthesized in the psychrotroph *Bacillus psychrophilus* (22).

The number of HSPs found in all rhizobial strains under the different shock temperatures was not related to their survival, even though there is evidence that heat shock response confers thermal resistance in *E. coli* cells (38). Although the survival was less than 1% at 46.4°C, only arctic rhizobia maintained protein synthesis under this treatment, which for arctic strains represents 21.4°C over the maximum growth temperature in comparison with 16.4°C for temperate strains. However, we did not determine whether protein synthesis was performed by all cells at the beginning of the treatment or by surviving cells throughout the shock. Usually, the thermostability of proteins in bacteria increases with the optimum growth temperature of the species (16). On the contrary, in our experiments, protein synthesis is more tolerant to high temperatures in arctic strains which have an optimal growth temperature (25°C) lower than that (30°C) for temperate strains. Many hypotheses could explain the lack of induction of HSPs in the temperate strains of rhizobia at 46.4°C. A defect in a temperature-sensitive membrane carrier for the transport of the methionine or denaturation in the transcriptional or translational enzymes could have a drastic effect on the synthesis of HSPs (16, 22, 37).

Just as was observed for the heat shock response, arctic and temperate strains of rhizobia responded similarly to cold shocks by synthesizing proteins under their minimal growth temperatures at freezing temperatures as low as −10°C. These proteins may play a protective role against freezing damage to membranes or in maintaining basic cell functions. As was observed with HSPs, cold shock temperatures reduced protein synthesis in comparison with that obtained under optimal temperatures. Such a reduction was observed in *E. coli* cells and was probably due to the inhibition of the initiation of translation (4) and the accumulation of 70S ribosomal particles (2).

Interestingly, 13 common CSPs were found in all strains, and the 11.1-kDa component was the major constituent at all shock temperatures. It is unlikely that this component originates from proteolytic activity, since this phenomenon is reduced at low temperatures, as has been reported for the psychrotrophic bacteria *Arthrobacter* spp. (26). The major CSP produced by *E. coli* was also reported to have a low molecular weight (7,400) (6, 11).

The different thermoadaptation levels of arctic and temperate strains were not strongly reflected in their cold responses because they produced similar numbers of CSPs at temperatures representing the same range from the optimal temperature to the shock temperature. For instance, at 35°C below the optimal growth temperature (−5°C for temperate strains and −10°C for arctic strains), the number of CSPs was 8 to 11 for temperate strains and 6 to 14 for arctic strains. It has been shown with the psychrotrophic yeast *Trichosporon pullulans* that a reduced range of cold shock temperatures (15 to 5°C, compared with 24 to 5°C) induced a minimal number of proteins (13). Conversely, in our experiments, arctic and temperate strains gave a maximal response with the minimal cold shock temperature range (27°C). At the very low shock temperature of −10°C, arctic strains responded better than temperate strains, by synthesizing more proteins. The incorporation activities of amino acids have been reported to be higher in psychrophiles and in psychrotrophs than in mesophiles (32). More detailed studies on the rate of protein synthesis would be necessary to evaluate differences between arctic and temperate strains.

For all cold shock treatments, arctic rhizobia had a survival rate slightly lower than that of temperate strains, indicating that their cold adaptation for growth does not confer on them a better survival rate under freezing conditions. Freezing temperatures affect the viability of bacterial cells by causing injuries to the cell membrane (7). Cold adaptation for cell growth may be related to an increase in the degree of insaturation of membrane lipids (14, 21), as has been shown with the enhancement of the chilling tolerance of a cyanobacterium by the transfer of a gene controlling fatty acid desaturation (35) and by the role of a cold resistance gene as found with *E. coli* (15).

Differences between the two groups of rhizobia, reflected by the production of HSPs at 46.4°C and by more CSPs at −10°C in arctic strains, may be related more to their ecological origin than to their taxonomic position. In spite of the fact that the temperate strain 118H1 and the arctic strain N40 were isolated from the same legume genus (*Oxytropis*), strain 118H1 has a minimal growth temperature and a shock temperature response comparable to those observed with the two other temperate strains, which belong to different species of *Rhizobium*. Thus, arctic strains, isolated from harsh environmental conditions (the Canadian high arctic) and showing a lower minimal growth temperature than temperate strains (0°C compared with 4 to 7°C), may have developed a protein-synthesizing system adapted to different stresses.

In our experiments, proteins similar in size to HSPs and CSPs were observed in cells grown at optimal temperatures. Since it has been reported that the number of HSPs may differ according to the method used for electrophoresis (23, 37), extensive use of two-dimensional gel electrophoresis could bring a better perspective to the real number of HSPs and CSPs synthesized under various conditions and help to determine which of these proteins are induced only under shock conditions. Further studies will be necessary to identify the contribution of these proteins to the establishment of thermoadaptation.

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