

Characteristics of rhizobia isolated from three legumes indigenous to the Canadian high arctic: *Astragalus alpinus*, *Oxytropis maydelliana*, and *Oxytropis arctobia**

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Summary Forty-eight strains of rhizobia were isolated from the root nodules of *Astragalus alpinus* (21), *Oxytropis maydelliana* (19) and *Oxytropis arctobia* (8), three species of arctic legumes found in the Melville Peninsula, Northwest Territories, Canada. On the basis of 74 characteristics (cultural, physiological, biochemical and host nodulation range) the 48 arctic rhizobia could be divided into 11 distinct groups by numerical analysis techniques. All 48 arctic rhizobia were able to nodulate the three arctic legume species and also sainfoin (*Onobrychis viciifolia*), however, milkvetch (*Astragalus cicer*) was only nodulated by 33 strains. In general, the arctic rhizobia showed properties found in both Rhizobium and Bradyrhizobium. The adaptation of the arctic strains to low temperature is indicated by their ability to grow in liquid culture at 5°C.

Introduction

Nitrogen fixation in leguminous plants involves a symbiotic relationship between nitrogen fixing bacteria and legume roots, and occurs within specialized root nodules. Low temperature stress is known to have an adverse effect on leguminous root nodule development and activity²⁴. However, in several arctic legumes, the ability of the symbiotic nitrogen fixation process to function in a psychrophilic environment suggests a unique evolutionary adaptation¹ and, also the strain of Rhizobium involved in a symbiotic association plays an important role in determining the efficiency of nitrogen fixation at low temperatures^{11,17}. Nodulation and some characteristics of rhizobia isolated from legumes in Arctic Russia and Alaska were previously reported^{2,10}.

Field measurements suggest that nitrogen fixation by legumes

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provides a major input to the nitrogen budget of the Canadian high arctic⁹. In the present study we report on the cultural, physiological and nodulation characteristics of arctic rhizobia isolated from *Astragalus alpinus* L., *Oxytropis maydelliana* Trautv. and *O. arctobia* Bunge, three legumes indigenous to the Sarcpa Lake region of the Melville Peninsula, Northwest Territories, Canada.

Materials and methods

Origin and maintenance of rhizobia

Arctic rhizobia used in this study were obtained from nodules or *via* host-plant infection from soil samples²⁷, collected in the Melville Peninsula near Sarcpa Lake (68°33'N, 83°19'W) Northwest Territories, Canada. Isolates were obtained from *Astragalus alpinus* (21 strains), *Oxytropis maydelliana* (19 strains) and *Oxytropis arctobia* (8 strains). Temperate reference strains also included in this work were: *Bradyrhizobium japonicum* ATCC 10324; *Rhizobium leguminosarum* biovar *viceae* ATCC 10004; *R. leguminosarum* biovar *phaseoli* ATCC 14482; *R. leguminosarum* biovar *trifolii* ATCC 14480; *Bradyrhizobium* sp. (*Lupinus*) Nit 96E3 (Nitragin Co, Milwaukee, WI); *R. meliloti* A₂ and *Rhizobium* sp. (*Onobrychis*) SM-2 (Agriculture Canada, Sainte-Foy, Québec). All cultures were maintained on yeast extract mannitol agar (YEM)²⁷ slants at 4°C and subcultured every 2 months.

Nodulation tests

Each strain was tested for ability to nodulate arctic legumes: *A. alpinus*, *O. maydelliana* and *O. arctobia*, and the following temperate legumes: *Astragalus cicer*, *Coronilla varia*, *Lotus corniculatus*, *Medicago sativa*, *Onobrychis viciifolia* and *Trifolium pratense*. Surface sterilized seeds²⁷ were sown in 200 × 25 mm sterile cotton-plugged tubes containing approximately 20 ml of vermiculite and 15 ml of a nitrogen free nutrient solution⁴ and placed in growth cabinets. Arctic legumes were grown at 20°C, 16 h light period at 300 μEm⁻²s⁻¹ alternating with 15°C, 8 h light period at 200 μEm⁻²s⁻¹. For temperate legumes, a 16 h light period alternating with 8 h of darkness was used, except for clover with 12 h darkness. Duplicate tubes of 5-day old seedlings were inoculated with 10⁶–10⁷ cells of the test strain. Nodulation tests were performed twice. Three weeks after inoculation, a positive score was recorded only when rhizobia could be reisolated from nodules and cultured on YEM plates containing 0.0025% congo red.

Cultural and morphological characteristics

Gram stain and cell dimensions. Fresh cultures were Gram stained⁵ and cell shape and dimensions determined by light microscopy.

Colonial characteristics. Colony size, growth rate and morphological features were determined for cultures grown on YEM at 25°C for up to 10 days.

Growth at 30°C and 5°C. Strains were cultured in 50 ml Erlenmeyer flasks containing 10 ml of liquid YEM medium, for 4 days at 30°C or 40 days at 5°C on a rotary shaker (125 rpm). Cells from 1 ml of culture were washed twice in saline, resuspended in 1 ml 1 N NaOH, then heated at 90°C for 10 min prior to protein determination by the Folin reaction¹³.

Acid production. Strains were tested for acid production as described for *Rhizobium* species¹⁵.

Tolerance of NaCl. Growth in the presence of 2% NaCl on YEM agar was determined after 7 days incubation at 25°C.

Tolerance of high temperatures. Plates of YEM agar were inoculated, incubated for 7 days at 37°C and then examined for growth.

Motility. Motility was assessed by stabbing a young culture into a semi-solid YEM medium (0.3% agar). With motile strains, a migration away from the spot of inoculation was observed after 7 days incubation at 25°C²¹.

Antibiotic resistance. Plates of YEM containing antibiotics were inoculated and incubated at 25°C for 7 days. The antibiotics used were: ampicillin (10 µg/ml), chloramphenicol (10 µg/ml), erythromycin (10 µg/ml), kanamycin (10 µg/ml), nalidixic acid (20 µg/ml), rifampicin (60 µg/ml), streptomycin (2 µg/ml), tetracycline (2 µg/ml). Strains were recorded resistant when growth occurred, and sensitive when no growth or very poor growth occurred.

Utilization of carbohydrates and organic acids

Strains were tested for their ability to grow with different carbohydrates and organic acids as sole source of carbon. A method previously described³ was used, but the culture medium was modified by adding per liter of medium: 100 µg thiamine and 100 µg panthotenic acid. Organic acids were used at concentrations of 2, 10 and 20 mM and carbohydrates at 1%. Growth was recorded after 10 days incubation at 25°C.

Enzyme assays

Constitutive enzymes (API-ZYM). Nineteen constitutive enzymes were tested using the API-ZYM System (Analytab Products, Plainsview, NY). The microcupules were inoculated according to the directions of the manufacturer. After 24 h incubation at 25°C, reactions were developed and any degree of color intensity was recorded as positive.

Catalase. Catalase activity was detected by the addition of a drop of 1.5% hydrogen peroxide to 2 drops of a fresh bacterial suspension. The mixture was observed for gas evolution.²¹

Acetylene reduction ex-planta. Nitrogenase was induced in free-living rhizobia by growing the cultures on CS-7 medium¹⁶ and was assayed by the acetylene reduction technique²⁶. Reference strain was *Bradyrhizobium* sp. (Vigna) 32H1.

Numerical analysis

Tests in which all cultures gave the same result were disregarded for computer analysis. For all other features, a positive test was scored 1 and a negative test was scored 0. Cluster analysis was carried out by using a clustran computer program (Wishart D., Edinburgh University, Scotland, 1978). The simple matching coefficient and Jaccard's coefficient were calculated and the results are shown as a dendrogram prepared by the unweighted pair group method using arithmetic averages.²².

Results

Relationship between the strains

The 48 strains of arctic rhizobia formed 11 distinct groups and one unclustered strain N₂₇ (Fig. 1) by both simple matching coefficient and Jaccard's coefficient. The highest similarity level was 85% between members of group 8 and the lowest was 65% with strains of group 11. The unclustered strain N₂₇ was related to group 7 and group 8 at 76% similarity level, but was not included in either of these groups which clustered at 83% and 85% respectively. Group 1 to 5 included 27 strains. Group 1 was clustered at 82% similarity, group 2 at 84%, group 3 at 85%, group 4 at 80% and group 5 and 85%. Groups 6 to 11 were formed

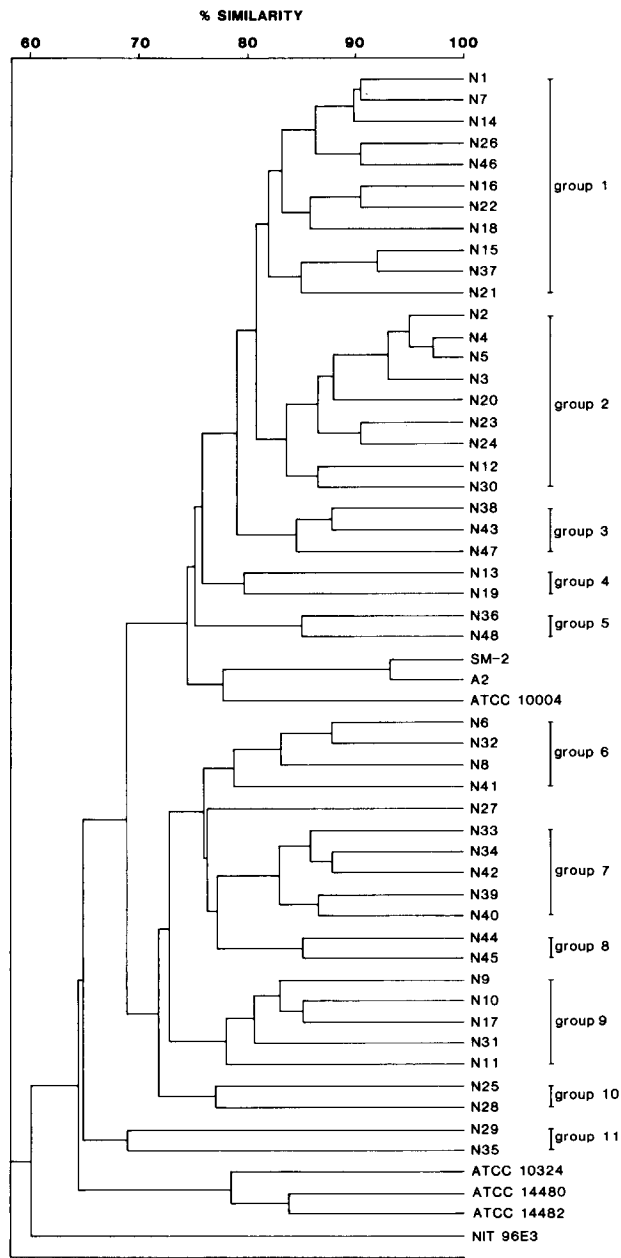


Fig. 1. Dendrogram showing the relationships among rhizobia studied as obtained by the simple matching coefficient; arctic rhizobia: from *A. alpinus* N₁ to N₅, N₁₈, N₁₉, N₂₁, N₂₄, N₂₇, N₂₉ to N₃₁, N₃₄ to N₃₆, N₃₉, N₄₂ and N₄₄ to N₄₆; from *O. maydelliana* N₆ to N₁₃, N₁₅, N₁₇, N₂₀, N₂₂, N₂₆, N₂₈, N₃₂, N₃₇, N₄₀, N₄₁ and N₄₃; from *O. arctobia* N₁₄, N₁₆, N₂₃, N₂₅, N₃₃, N₃₈, N₄₇ and N₄₈; reference strains: SM-2, *Rhizobium* sp. (*Onobrychis*); A₂, *R. meliloti*; ATCC 10004, *R. leguminosarum* biovar *viceae*; ATCC 10324, *Bradyrhizobium japonicum*; ATCC 14480, *R. leguminosarum* biovar *trifolii*; ATCC 14482, *R. leguminosarum* biovar *phaseoli*; Nit 96E3, *Bradyrhizobium* sp. (*Lupinus*).

with 20 strains at the similarity levels of 79%, 83%, 85%, 78%, 77% and 68% respectively. All strains of arctic rhizobia were similar at the 65% level.

None of the reference strains clustered with the arctic rhizobia and they formed 2 distinct groups. *R. meliloti* A₂, *Rhizobium* sp. (*Onobrychis*) SM-2, and *R. leguminosarum* biovar *viceae* ATCC 10004 were clustered at 78% similarity. This group was located between group 5 and group 6 of arctic rhizobia and was related to the 27 arctic strains included in group 1 to group 5 at the 75% level. *B. japonicum* ATCC 10324, *R. leguminosarum* biovar *trifolii* ATCC 14480 and *R. leguminosarum* biovar *phaseoli* ATCC 14482 formed a group at 78% similarity. They were related to the arctic population at 64% similarity. *Bradyrhizobium* sp. (Lupinus) Nit 96E3 was not clustered and was related to the other rhizobia at 58% similarity.

Nodulation patterns

The host range patterns of the 11 groups of arctic rhizobia are shown in Table 1. The 48 strains tested were able to form nodules with the three arctic legumes *A. alpinus*, *O. arctobia* and *O. maydelliana* and with the temperate legume *Onobrychis viciifolia*. However, nodulation was not observed on the remaining temperate legumes, *C. varia*, *L. corniculatus*, *M. sativa* and *T. pratense*, except *A. cicer* which was nodulated by 33 strains (including the unclustered strain N₂₇).

Morphology and growth characteristics

The arctic rhizobia were all gram negative rods varying in length between 1.8 and 2.5 μm and in width between 0.3 and 1.2 μm . Detectable colonies appeared after 2–7 days incubation and full colony development occurred after 7–10 days (Table 1). Colonies grown on YEM at 25°C were almost colourless, circular and convex, and variable amounts of extracellular gum were produced. Most of the strains included in groups 1 to 5 had colonies appearing after 2 to 4 days, with a diameter of 1 mm or more at 7 days and greater than 2 mm after 10 days. In contrast, the majority of the strains from groups 6 to 11 showed slower growth, with colonies appearing 5 to 7 days after inoculation, and a diameter not exceeding 2 mm after 10 days.

In liquid medium, only 4 strains all from group 7 showed very good growth at 30°C while the majority of the isolates grew poorly. On the other hand, at 5°C, 30 strains grew very well. These strains were distributed among all groups except group 10 in which one isolate showed very poor growth at this temperature (Table 1). None of the arctic strains grew at 37°C or in the presence of 2% NaCl. All these strains were acid producers, lowering the pH of Norris's medium from 6.82 to an average

Table 1. Nodulation patterns, cultural and physiological characteristics of arctic rhizobia

	Group according to numerical analysis										
	1	2	3	4	5	6	7	8	9	10	11
Number of strains in each group	11	9	3	2	2	4	5	2	5	2	2
Nodulation of:											
<i>Astragalus alpinus</i>	+ ^a	+	+	+	+	+	+	+	+	+	+
<i>Astragalus cicer</i>	5 ^b	8	1	+	- ^c	+	+	+	4	1	-
<i>Coronilla varia</i>	-	-	-	-	-	-	-	-	-	-	-
<i>Lotus corniculatus</i>	-	-	-	-	-	-	-	-	-	-	-
<i>Medicago sativa</i>	-	-	-	-	-	-	-	-	-	-	-
<i>Onobrychis viciifolia</i>	+	+	+	+	+	+	+	+	+	+	+
<i>Oxytropis arctobia</i>	+	+	+	+	+	+	+	+	+	+	+
<i>Oxytropis maydelliana</i>	+	+	+	+	+	+	+	+	+	+	+
<i>Trifolium pratense</i>	-	-	-	-	-	-	-	-	-	-	-
Growth at 25°C											
First colony appearance after:											
2-4 days	+	+	+	1	+	2	3	-	-	-	1
5-7 days	-	-	-	1	-	2	2	+	+	+	1
Colony diameter after 7 days:											
≥ 1 mm	9	8	+	+	+	-	-	-	-	-	-
< 1 mm	2	1	-	-	-	+	+	+	+	+	+
Colony diameter after 10 days:											
> 2 mm	+	+	+	+	+	-	2	-	-	-	1
≤ 2 mm	-	-	-	-	-	+	3	+	+	+	1
Growth at 30°C (µg protein/ml):											
Poor (0-100)	+	+	2	+	1	+	1	+	3	1	1
Good (100-200)	-	-	1	-	1	-	-	-	2	1	1
Very good (> 200)	-	-	-	-	-	-	4	-	-	-	-
Growth at 5°C (µg protein/ml):											
Poor (0-100)	-	-	-	-	-	-	-	-	-	1	-
Good (100-300)	5	7	-	1	1	1	-	-	-	1	1
Very good (> 300)	6	2	+	1	1	3	+	+	+	-	1
Growth at 37°C											
Motility	+	+	+	+	+	1	3	1	1	-	-
Tolerance of 2% NaCl	-	-	-	-	-	-	-	-	-	-	-
Acid production	+	+	+	+	+	+	+	+	+	+	+
Resistance to antibiotics:											
Ampicillin	1	-	-	-	1	1	-	-	-	-	+
Chloramphenicol	6	2	-	-	+	1	-	-	-	-	-
Erythromycin	+	+	+	+	+	+	+	+	4	+	+
Kanamycin	8	2	2	-	-	1	3	+	4	1	+
Nalidixic acid	9	+	-	-	-	2	-	-	+	+	-
Rifampicin	-	-	-	-	-	-	-	-	-	-	-
Streptomycin	+	1	-	-	+	-	4	-	1	1	-
Tetracycline	-	-	-	-	-	-	-	-	-	-	-

^a all strains positive.^b numbers indicate number of positive strains.^c all strains negative.

of 5.68. All members of groups 1 to 5 were motile and those of groups 10 and 11 were non-motile; only 6 out of the 16 isolates included in groups 6 to 9 were motile.

All arctic isolates were sensitive to tetracycline and rifampicin while all except one were resistant to erythromycin. Most of the bacteria were sensitive to ampicillin with only five resistant strains, included in groups 1, 5, 6 and 11. Resistance of the population was variable against chloramphenicol, streptomycin, nalidixic acid and kanamycin. None of the strains of groups 3 and 4, and groups 7 to 11 were resistant to chloramphenicol (Table 1).

Carbohydrate and organic acid utilization

All groups of arctic rhizobia were able to utilize a wide variety of both carbohydrates and organic acids (Table 2). Oxaloacetate was poorly utilized as a sole carbon source and concentration dependant inhibition was observed for nine strains when the concentration was increased from 10 to 20 mM. A high concentration (20 mM) of α -ketoglutarate, glyoxylate and pyruvate appeared to be required for growth when used as sole carbon sources. For example, 2 mM glyoxylate was used only by 6 strains of groups 1 and 4, and 20 mM glyoxylate was utilized by 35 strains distributed among all groups except group 5. Lactate at 10 mM and 20 mM was used by all strains except one from group 8, and acetate and citrate were also used by a large number of strains. Acetate was more efficiently utilized at 10 mM (46 positive strains), and citrate at 2 mM (36 positive strains), whereas fumarate, malate and succinate were used by most of the strains at 2, 10 or 20 mM. Strains unable to grow on these organic acids were included mainly in groups 8 and 9. Dulcitol, lactose and raffinose were readily used by 43, 46, and 43 strains respectively, including the unclustered strain N₂₇ (Table 2).

Enzymes profile

Table 3 shows the constitutive enzymes, catalase and *explanta* nitrogenase expression in arctic rhizobia. Acid phosphatase, α -glucosidase, trypsin and phosphoamidase were present in the majority of the strains (45 and 42 strains respectively). Esterase lipase was active in 22 strains belonging to each group except group 8, leucine aminopeptidase was detected in 18 strains belonging to the groups 1, 2, 3, 5, 9, 10 and 11. Alkaline phosphatase and N-acetyl- β -glucosaminidase were present in only four strains. Catalase was detected in 21 isolates which were distributed throughout each group except group 4. Nitrogenase activity in free-living cells was expressed in 39 isolates, with rates similar to that found for the reference strain *Bradyrhizobium* sp. (Vigna) 32H1.

Table 2. Utilization of carbohydrates and organic acids by arctic rhizobia

	Groups according to numerical analysis										
	1	2	3	4	5	6	7	8	9	10	11
Number of strains in each group	11	9	3	2	2	4	5	2	5	2	2
Carbohydrates (1%)											
L-arabinose	+ ^a	+	+	+	+	+	+	+	+	+	+
Dulcitol	10 ^b	8	+	+	+	3	+	+	+	+	- ^c
Fructose	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+	+
α -Lactose	+	+	+	+	+	+	+	+	+	+	1
D-Maltose	+	+	+	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+	+	+
Raffinose	9	+	+	+	+	+	4	+	4	+	1
L-Rhamnose	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+
D-Trehalose	+	+	+	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+	+	+	+
Organic acids (mM)											
Acetate	2	+	+	2	1	+	+	+	+	-	+
	10	+	+	+	1	+	+	+	+	+	1
	20	+	8	2	1	+	1	4	+	+	-
Citrate	2	9	4	+	-	+	3	+	4	1	+
	10	9	3	2	-	-	1	3	1	2	-
	20	7	2	-	-	-	-	3	-	1	-
Fumarate	2	+	+	+	+	+	+	+	1	+	+
	10	+	+	+	+	+	+	+	4	+	+
	20	+	+	+	+	1	+	+	-	3	+
Glyoxylate	2	5	-	-	1	-	-	-	-	-	-
	10	9	3	1	1	+	2	1	-	-	1
	20	10	8	+	1	-	2	3	+	3	1
α -Ketoglutarate	2	8	6	-	1	+	2	3	+	-	1
	10	+	+	+	+	+	+	4	+	+	+
	20	+	+	+	+	+	+	+	+	+	1
D-Lactate	2	+	+	+	+	+	+	+	+	+	+
	10	+	+	+	+	+	+	+	1	+	+
	20	+	+	+	+	+	+	+	1	+	+
L-Malate	2	+	+	+	+	+	+	+	1	1	+
	10	+	+	+	+	+	+	+	1	4	+
	20	+	+	+	+	+	+	+	1	4	1
Oxaloacetate	2	-	-	-	-	-	-	-	-	-	-
	10	4	1	-	+	1	1	-	-	1	-
	20	1	-	-	1	-	-	-	-	-	-
Pyruvate	2	10	+	2	1	+	3	4	+	-	+
	10	+	+	+	+	+	+	+	4	+	1
	20	+	+	+	+	+	+	+	4	+	1
Succinate	2	10	+	+	+	+	+	+	-	2	+
	10	+	+	+	+	+	+	+	1	3	+
	20	+	+	+	+	+	+	+	-	2	+

^a all strains positive.^b numbers indicate number of positive strains.^c all strains negative.

Table 3. Enzymes profile of arctic rhizobia

	Group according to numerical analysis										
	1	2	3	4	5	6	7	8	9	10	11
Number of strains in each group	11	9	3	2	2	4	5	2	5	2	2
Constitutive enzymes (API ZYM)											
Alkaline phosphatase	— ^a	1 ^b	—	1	—	1	—	—	—	—	1
Esterase (C ₄)	—	—	—	—	—	—	—	—	—	—	—
Esterase lipase (C ₈)	8	3	1	1	1	+ ^c	1	—	1	1	1
Lipase (C ₁₄)	—	—	—	—	—	—	—	—	—	—	—
Leucine aminopeptidase	3	8	1	—	1	—	—	—	1	+	+
Valine aminopeptidase	1	—	—	—	—	—	—	—	—	—	+
Cystine aminopeptidase	—	—	—	—	—	—	—	—	—	—	—
Trypsin	9	8	+	+	1	+	+	+	+	—	+
Chymotrypsin	—	—	—	—	—	—	—	—	—	—	—
Acid phosphatase	+	+	+	+	+	+	+	+	+	+	+
Phosphoamidase	+	+	2	+	+	+	+	+	+	1	+
α-Galactosidase	—	—	—	—	1	—	—	—	—	—	—
β-Galactosidase	—	—	—	—	—	—	—	—	—	—	—
β-Glucuronidase	—	—	—	—	—	—	—	—	—	—	—
α-Glucosidase	+	+	+	+	+	+	+	+	+	+	+
β-Glucosidase	—	—	—	—	+	—	—	—	—	—	—
N-Acetyl-β-glucosaminidase	—	—	1	—	—	—	1	1	1	—	—
α-Mannosidase	—	—	—	—	—	—	—	—	—	—	—
α-Fucosidase	—	—	—	—	—	—	—	—	—	—	—
Catalase	5	1	+	—	1	3	2	+	1	1	1
Nitrogenase activity <i>ex planta</i>	10	8	+	+	—	+	+	+	4	—	—

^a all strains negative.

^b numbers indicate number of positive strains.

^c all strains positive.

Discussion

Arctic rhizobia were clustered in 11 groups (Fig. 1) irrespective of their plant origin. In fact, only the isolates of groups 6, 9 and 11 originated from one plant species. All other groups included rhizobia isolated from at least two different plant species. The ability of the isolates to nodulate *A. alpinus*, *O. maydelliana* and *O. arctobia* may be explained by the close relationship between the two plant genera *Astragalus* and *Oxytropis* (several *Oxytropis* species were formerly classified as astragali)⁷ belonging to the tribe Galegeae.

The temperate legume *A. cicer* (milkvetch) which belongs to the tribe Galegeae¹⁸ was nodulated by only 33 isolates. Moreover, among the 21 strains isolated from *A. alpinus*, seven strains failed to form nodules on *A. cicer*, which is consistent with the findings of Allen and Allen¹ that astragali rhizobia, in general, are not able to nodulate all *Astragalus* species. The temperate forage legume sainfoin, *Onobrychis viciifolia*, cultivated in Western Canada and United States was nodulated by all

arctic rhizobia studied. This species belongs to the tribe of Hedysareae which is phylogenetically related to the tribe of Galegeae¹⁸. None of the 48 arctic isolates tested were able to nodulate crownvetch, *Coronilla varia*, a member of the tribe Coronilleae. It has been reported that rhizobia from sainfoin and crownvetch effectively cross nodulate²⁰. Moreover, the tribe Coronilleae was originally classified as a sub-tribe of the Hedysareae, and both have common similarities with the Galegeae¹⁸. However, based on their cross-infection patterns, the genera *Onobrychis* (sainfoin) and *Coronilla* (crownvetch) are included in two distinct nodulation groups¹².

Alfalfa (*Medicago sativa*) and clover (*Trifolium pratense*), both members of the tribe Trifolieae, were not nodulated by arctic rhizobia. The tribe Trifolieae is comprised of plant genera with advanced characters¹⁸ and rhizobia associated with them are usually specific to a few genera within this tribe²⁵.

In general, cross-inoculation patterns and boundary jumping of rhizobia cannot be explained by the phylogeny of the plants, but observations on preferred host affinities indicated evolutionary relationships in many plant bacterial associations²⁵. Although we cannot draw a complete nodulation pattern of the arctic rhizobia in relation to known cross-inoculation groups within the Leguminosae, from the present work we suggest that arctic rhizobia may have evolved along with the arctic legumes in their environment.

By using numerical analysis, similarities between strains of the arctic *Rhizobium* population varied from 65 to 96% which falls in the range reported for members of a single *Rhizobium* species¹⁹. The characteristics of the arctic strains described in this study indicate that they have some affinities with both the fast-growing and the slow-growing type of root nodule bacteria included in the Rhizobiaceae: the genera *Rhizobium* and *Bradyrhizobium*⁸. In general, all arctic isolates were acid producers, were able to use a wide variety of carbon compounds and were sensitive to tetracycline like members of the genus *Rhizobium*^{15,7,8}. Furthermore, the 27 strains belonging to groups 1 to 5 appeared to be more closely related to the genus *Rhizobium* because they grew faster on YEM agar than strains of groups 6 to 11 (Table 1). However, 39 arctic rhizobia expressed nitrogenase activity in free-living cultures and many of them grew slowly on YEM agar as would be expected for members of the genus *Bradyrhizobium*⁸. It was recently reported that the fast-growing *Bradyrhizobium japonicum* strains have common traits with the two genus *Rhizobium* and *Bradyrhizobium*²³.

Temperature is one of the factors which limits microbial growth in cold environment, but most of the bacteria isolated from arctic regions

are able to grow at low temperatures¹⁴. In general, rhizobia are tolerant to temperatures below 4°C, but very little growth occurs²⁵. It has been reported that clover rhizobia isolated from the subarctic region in Scandinavia showed a better adaptation to low temperature and grew faster at 10°C than isolates from southern areas⁶ and we found that the arctic rhizobia could grow at 5°C (Table 1), a temperature at which the reference strains of *Rhizobium* and *Bradyrhizobium* used in this study could not grow. This apparent adaptation to low temperatures of the arctic strains might confer some properties which could be advantageous over the other rhizobia specially when temperature is limiting for both bacterial growth and nitrogenase activity during cold phases of the growing season.

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References

- 1 Allen O N and Allen E K 1981 The Leguminosae. Univ. of Wisconsin Press, Madison, Wisc. 812 p.
- 2 Allen E K, Allen O N and Klebesabel L J 1964 An insight into symbiotic nitrogen-fixing plant association in Alaska. In Alaska Science Conference, Science in Alaska, Proceedings, 14, 54-63.
- 3 Antoun H, Bordeleau L M and Sauvageau R 1984 Utilization of the tricarboxylic acid cycle intermediates and symbiotic effectiveness in *Rhizobium meliloti*. Plant and Soil 77, 29-38.
- 4 Bordeleau L M, Antoun H and Lachance R A 1977 Effets des souches de *Rhizobium meliloti* et des coupes successives de la luzerne (*Medicago sativa*) sur la fixation symbiotique d'azote. Can. J. Plant Sci. 57, 433-439.
- 5 Difco 1984 Difco Manual. Tenth edition. Difco laboratories, Detroit, Michigan, 1155 p.
- 6 Ek-Jander J and Fähræus G 1971 Adaptation of *Rhizobium* to subarctic environment in Scandinavia. Plant and Soil Spec. Vol. 129-137.
- 7 Graham P H 1964 Studies on the utilization of carbohydrates and Krebs cycle intermediates by rhizobia, using an agar plate method. Antonie van Leeuwenhoek J. Microbiol. Serol. 30, 68-72.
- 8 Jordan D C 1982 Family III. Rhizobiaceae. In Bergey's Manual of Systematic Bacteriology. Volume 1. Eds. N R Krieg and J C Holt. Williams and Wilkins, pp 234-244.
- 9 Karagatzides J D, Lewis M C and Schulman H M 1985 Nitrogen fixation in the high arctic tundra at Sarcpa Lake, Northwest Territories. Can. J. Bot. 63, 974-979.
- 10 Kriss A E, Korenyako A I and Migulina V M 1941 Root nodules bacteria in arctic regions. Mikrobiol. 10, 67-73.
- 11 Layzell D B, Rochman P and Canvin D T 1984 Low root temperatures and nitrogenase activity in soybean. Can. J. Bot. 62, 965-971.
- 12 Lieberman M T, Mallory L M, Simkins S and Alexander M 1985 Numerical taxonomic analysis of cross-inoculation patterns of legumes and *Rhizobium*. Plant and Soil 84, 225-244.
- 13 Lowry O, Rosenbrough N, Farr A and Randall R 1952 Protein measurement with the Folin phenol reagent. J. Biol. chem. 193, 265-275.
- 14 Nelson L M and Parkinson D 1978 Growth characteristics of three bacteria isolates from an arctic soil. Can J. Microbiol. 24, 909-914.

- 15 Norris D O 1965 Acid production by *Rhizobium*; a unifying concept. *Plant and Soil* 22, 143–166.
- 16 Pagan J D, Child J J, Scowcroft W R and Gibson A H 1975 Nitrogen fixation by *Rhizobium* cultured on a defined medium. *Nature* 256, 406–407.
- 17 Pankhurst C E and Layzell D B 1984 The effect of bacterial strain and temperature changes on the nitrogenase activity of *Lotus pedunculatus* root nodules. *Physiol. Plant.* 62, 404–409.
- 18 Polhill R M and Raven P H 1981 *Advances in Legume Systematics. Part 1.* Eds. R M Polhill and P H Raven. 425 p.
- 19 Robert F M, Molina J A E and Schmidt E L 1982 Properties of *Rhizobium leguminosarum* isolated from various regions of Morocco. *Ann. Microbiol. (Inst. Pasteur)* 133A, 461–470.
- 20 Schreven D A Van 1972 Note on the specificity of the rhizobia of crownvetch and sainfoin. *Plant and Soil* 36, 325–330.
- 21 Smibert R M and Krieg N R 1981 General Characterization. *In Manual of Methods for General Bacteriology.* Ed. P. Gerhardt Am. Soc. Microbiol. pp 409–443.
- 22 Sneath P H A and Sokal R R 1973 *Numerical Taxonomy.* W H Freeman and Co., San Francisco.
- 23 Stowers M D and Eaglesham A R J 1984 Physiological and symbiotic characteristics of fast-growing *Rhizobium japonicum*. *Plant and Soil* 77, 3–14.
- 24 Sutton W D 1983 Nodule development and senescence. *In Nitrogen Fixation. Volume 3. Legumes.* Ed. W J Broughton. Clarendon Press, Oxford pp 144–212.
- 25 Trinick M J 1982 Biology. *In Nitrogen Fixation, Volume 2: Rhizobium.* Ed. W J Broughton. Clarendon Press. Oxford. pp 76–146.
- 26 Turner G L and Gibson A H 1980 Measurement of nitrogen fixation by indirect means. *In Methods for Evaluating Biological Nitrogen Fixation.* Ed. F J Bergersen. John Wiley and Sons, Toronto pp 111–138.
- 27 Vincent J M 1970 *A Manual for the Practical Study of Root nodule Bacteria.* IBP Handbook No. 15. Blackwell Scientific Publications, Oxford, England.