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# Growth of Indigenous *Rhizobium leguminosarum* and *Rhizobium meliloti* in Soils Amended with Organic Nutrients†

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**The ability of indigenous *Rhizobium leguminosarum* and *Rhizobium meliloti* to use organic nutrients as growth substrates in soil was assessed by indirect bacteriophage analysis. A total of 17 organic compounds, including 9 carbohydrates, 3 organic acids, and 5 amino acids, were tested (1,000 μg g$^{-1}$) in three soils with different cropping histories. Four additional soils were screened with a glucose amendment. Nutrient amendments stimulated growth of indigenous rhizobia, allowing subsequent replication of indigenous bacteriophages. Phage populations were enumerated by plating soil extracts on 19 *R. leguminosarum* and 9 *R. meliloti* indicator strains, including root nodule isolates from the soils assayed. On the basis of indirect phage analysis, all soils contained native rhizobia similar to one or more of the indicator strains, although not all indicator strains were detected in soil. All organic compounds stimulated growth of indigenous rhizobia, but the growth response varied for each rhizobial strain depending on the nutrient, the nutrient concentration, and the soil. Indigenous rhizobia readily utilized most organic compounds except phenylalanine, glycine, and aspartic acid. The ability of indigenous rhizobia to utilize a wide range of organic compounds as growth substrates in situ indicates their ability to successfully compete with other soil bacteria for nutrients in these soils.**

Legumes play an important role in agriculture because of their symbiotic relationship with nitrogen-fixing rhizobia. Inoculation of legume seeds with effective nitrogen-fixing rhizobia is a common practice in many areas of the world (6, 7, 12). In some cases, inoculation gives dramatic results if the inoculant strains are highly competitive and very active and if the soils are deficient in native rhizobia (6, 10). However, in some soils with established populations of rhizobia, the activity of inoculant strains declines over time, as they must compete with the native rhizobia for growth substrates and nodulation of host legumes (1, 5–7, 10, 12). Since indigenous rhizobia may be a barrier to successful introduction of inoculant strains (1, 6, 12), there is the need to obtain information on the distribution, population dynamics, and ecology of these rhizobia.

Indigenous or inoculant rhizobia must compete with other soil microorganisms and maintain a population base if they are to nodulate host legumes (7, 10). The reasons why some rhizobia persist and proliferate in soil whereas other strains die out are not completely understood. A variety of biological, chemical, and physical factors affect the survival of rhizobia in soil (1, 5, 7, 10, 11). Certainly, the ability to use organic compounds found in soil and in root exudates would be a competitive advantage in soil. *Rhizobium* species use a variety of organic compounds, including root exudates, as carbon and nitrogen substrates during growth as pure cultures (2, 13, 14). However, little is known concerning the growth response of rhizobia to organic nutrients in soil (11, 16). The objective of this study was to assess the ability of indigenous rhizobia to utilize organic compounds as growth substrates in soil. This was accomplished by using an indirect bacteriophage analysis procedure (4) that allowed the growth response and activity of indigenous rhizobia to be followed in situ.

(A preliminary account of this study was presented at the 13th International Society of Soil Science Congress, Hamburg, Federal Republic of Germany, 13 to 20 August, 1986.)

## MATERIALS AND METHODS

**Bacteria and growth conditions.** The 19 *Rhizobium leguminosarum* and 9 *Rhizobium meliloti* strains used in this study are listed in Table 1. Some of these bacteria were laboratory stock cultures from various geographic regions and were described previously (9), whereas other strains were isolated from plant nodules during this study. These rhizobia were isolated from legumes growing in the field soils described below. Nodules were collected and surface sterilized in a 20% bleach solution for 1 min and then rinsed in sterile distilled water 8 times. Crushed nodules served as an inoculum source that was streaked on YEM agar plates (15). Suspected rhizobial colonies were purified by repeated streaking on YEM agar plates, and putative rhizobia were identified by bacteriophage typing.

Rhizobia were grown in 25 ml (250-ml flask) of 0.5% tryptone–0.3% yeast extract (TY) broth (containing 10 mM $CaCl_2$) on a gyratory shaker (150 rpm) at 27°C. TY agar and semisolid TY agar used for bacteriophage studies contained 1.5% and 0.7% agar, respectively. All media components were products of Difco Laboratories, Detroit, Mich.

**Soils.** The soils used in this study are listed in Table 2. Bulk (i.e., 10 to 20 kg) surface soil samples (0 to 15 cm) were collected and stored (at field moisture) in polyethylene bags at 5°C. Before analysis, subsamples were sieved (<2 mm).

**Bacteriophage analysis and isolation.** The growth response of indigenous soil rhizobia to organic amendments was monitored by the indirect bacteriophage analysis procedure of Germida and Casida (4) as modified previously (3). A sample of soil (3 g) was added to a sterile glass vial (ca. 30 ml) and then amended with 0.3 ml of either sterile distilled water or the appropriate nutrient. After incubation at 27°C for 24 h, 18 ml of sterile tap water was added to the vial, which was then hand shaken for 15 s. A sample of soil suspension (10 ml) was immediately removed from the vial

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TABLE 1. *Rhizobium* strains

| Strain | Source |
|---|---|
| *R. leguminosarum* | |
| 128C52 | PBI[a] |
| 128C30 | PBI |
| 128C79 | PBI |
| SU391 | PBI |
| H100 | PBI |
| NA503-3 | PBI |
| NA502 | PBI |
| LPNI-2 | Pea nodule isolate/Laird soil |
| PNI-S-1 | Pea nodule isolate/Sutherland soil |
| PNI-S-2 | Pea nodule isolate/Sutherland soil |
| PNI-S-3 | Pea nodule isolate/Sutherland soil |
| PNI-M-1 | Pea nodule isolate/Melfort soil |
| PNI-M-2 | Pea nodule isolate/Melfort soil |
| PNI-M-3 | Pea nodule isolate/Melfort soil |
| PNI-M-4 | Pea nodule isolate/Melfort soil |
| PNI-T-1 | Pea nodule isolate/Tisdale soil |
| PNI-T-2 | Pea nodule isolate/Tisdale soil |
| PNI-W-1 | Pea nodule isolate/Waitville soil |
| PNI-TB-1 | Pea nodule isolate/Tisdale-B soil |
| *R. meliloti* | |
| ATCC 9930 | PBI |
| NRG 185 | PBI |
| NRG 118 | PBI |
| ANI-S-1 | Alfalfa nodule isolate/Sutherland soil |
| ANI-S-2 | Alfalfa nodule isolate/Sutherland soil |
| ANI-S-3 | Alfalfa nodule isolate/Sutherland soil |
| ANI-M-1 | Alfalfa nodule isolate/Melfort soil |
| ANI-M-2 | Alfalfa nodule isolate/Melfort soil |
| ANI-M-3 | Alfalfa nodule isolate/Melfort soil |

[a] PBI, Plant Biotechnology Institute.

and centrifuged for 5 min at 480 × *g*. The supernatant fluid was filtered through a membrane filter (pore size, 0.45 μm) and then assayed for bacteriophage on an appropriate indicator strain as described previously (4). The reported number of bacteriophage PFU was the average of duplicate or triplicate plate counts on triplicate soil samples. For some experiments, the number of bacteriophage detected was greater than 3,000 PFU g of soil$^{-1}$; however, for illustrative purposes in figures, the maximum number reported was 3,000.

Bacteriophage were isolated and purified and broth lysates were prepared as described previously (4). The lytic spectra of each phage isolate was determined by the spot plate method.

The efficiency of extracting bacteriophages from soils was assessed by adding phage isolates C52p3 ($10^3$ PFU g$^{-1}$) and 9930pl ($8.4 \times 10^2$ PFU g$^{-1}$) to the Laird and Melfort soils. Recovery of phages C52p3 and 9930pl from the Laird soil

TABLE 2. Soils

| Soil location[a] | Type | Cropping history[b] |
|---|---|---|
| Laird | Mollisol | Wheat to field pea |
| Sutherland | Mollisol | Wheat to field pea |
| Melfort | Alfisol | Alfalfa to field pea (past 4–5 yr) |
| Star City | Alfisol | Alfalfa (past 3 yr) |
| Tisdale | Alfisol | Alfalfa |
| Waitville | Alfisol | Rapeseed (no legume for 15 yr) |
| Tisdale-B | Alfisol | Wheat (no legume for 40 yr) |

[a] Field sites near the Saskatchewan town listed.
[b] Crop rotation order listed, e.g., to wheat field pea, was for 1984–1985; if no rotation is listed, then crop was for 1985.

immediately after addition was 173 and 75%, respectively. By 24 h, recovery had declined to 64 and 40%, and at 48 h it was 24 and 4%, respectively. The trends for the Melfort soil (60, 31, and 24% for C52p3, and 180, 98, and 19% for 9930pl) were similar. This general decline in phage recovery was also observed when $10^4$ PFU were added to these soils. Other studies observed similar trends for *Azospirillum brasilense* and *Ensifer adhaerens* phage recovery from different soils (3, 4).

**Organic nutrients.** A total of 17 organic compounds were tested as growth substrates for indigenous soil rhizobia. These included the carbohydrates (glucose, sucrose, arabinose, ribose, xylose, galactose, and cellobiose); the sugar alcohols (mannitol and glycerol); the organic acids (succinate, citrate, and malate); and the amino acids (alanine, glycine, glutamic acid, aspartic acid, and phenylalanine). These chemicals were made up as 0.5 to 1.0% (wt/vol) stock solutions in deionized water and then filter sterilized through a 0.3-μm-pore-size membrane filter.

## RESULTS

**Response of *R. meliloti* and phage 9930pl in sterile sand to nutrient amendment.** A preliminary, suitably replicated experiment was conducted to monitor the relationship between host cell multiplication and phage replication. Host cells and phage were added to sterile sand at a ratio of approximately 100:1 and after one day of incubation received an 800-μg nutrient amendment (0.3 ml of a 0.5% tryptone and 0.3% yeast extract solution). Phage replication paralleled host cell multiplication but was delayed by about 24 h (Fig. 1). The phage burst detected was large and similar to that observed for broth cultures. These results were expected for this sand system which lacked organisms to compete for nutrients and soil particles (e.g., clays) which adsorb phage. Similar studies with *A. brasilense* and its phage in natural soil indicate a 10,000-fold increase in phage PFU corresponding to a 10-fold increase in host CFU (3).

**Response of indigenous soil rhizobiophage to nutrient amendment; effect of nutrient concentration and incubation period.** The effect of glucose concentration on the indigenous phage response (IPR) for *R. leguminosarum* (i.e., 128C52-like rhizobia) in the Laird soil is shown in Fig. 2. Increasing the glucose concentration 10-fold (i.e., from 100 to 1,000 μg g of soil$^{-1}$) resulted in more than a 30-fold increase in the detectable phage population. A 1,000-μg glucose amendment increased the phage population from an initial, undetectable

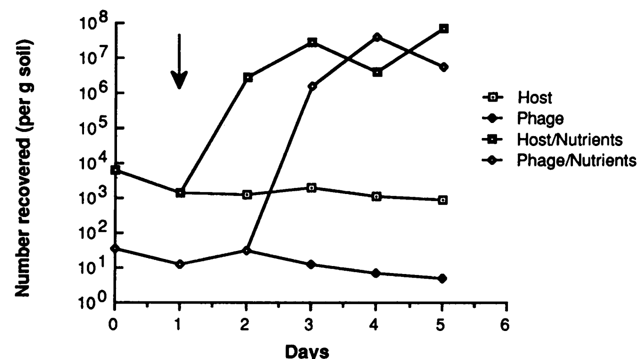


FIG. 1. Growth response of *R. meliloti* ATCC 9930 and its phage 9930pl in sterile sand to an 800-μg nutrient amendment (arrow) per gram of sand.

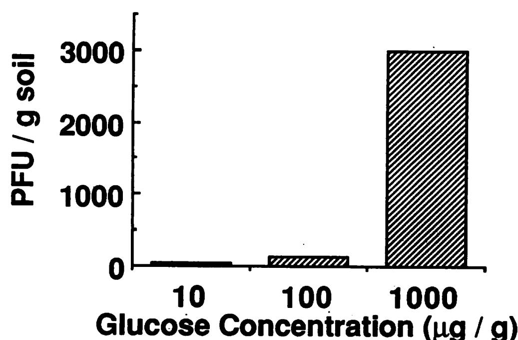


FIG. 2. Effect of glucose concentration on the indigenous phage response for *R. leguminosarum* 128C52 in the Laird soil.

level to greater than 3,000 PFU g of soil$^{-1}$ by 24 h. For example, in the Laird soil the number of PFU detected for strain 128C52 was $3.2 \times 10^3$ PFU g of soil$^{-1}$. In the Sutherland soil amended with glucose, the numbers of PFU detected for *R. leguminosarum* 128C52 and *R. meliloti* 9930 were $3.7 \times 10^3$ and $7.5 \times 10^3$, respectively. Depending on the nutrient amendment, the IPR peaked between 24 to 48 h and then declined over the next 48 h to approximately 1,000 PFU g of soil$^{-1}$. In subsequent experiments, all nutrients were tested at 1,000 μg g of soil$^{-1}$ and the IPR was measured at 24 h.

**Response of indigenous rhizobia in Laird, Melfort, and Sutherland soils to nutrient amendments. (i) Carbohydrate amendments.** The growth responses (measured as IPR) of indigenous *R. leguminosarum* and *R. meliloti* strains to the carbohydrates glucose, sucrose, ribose, and cellobiose were illustrated, as they represent compounds of varying complexity and because similar responses were elicited with arabinose, xylose, and galactose (Fig. 3). In the Laird soil, indigenous *R. leguminosarum* strains (similar to indicator strain 128C52) were the most active and readily utilized the carbohydrate amendments. Thus, the largest detectable IPR was for indicator strain 128C52. Surprisingly, low numbers of phages were detected for the indicator strain LPNI-2 even though this organism was one of the rhizobial isolates obtained from nodules of *Pisum sativum* grown on this Laird soil. It would seem, therefore, that in these soil samples the indigenous LPNI-2 rhizobia did not use exogenous nutrients as well as other native strains. It should be pointed out, however, that the distribution and density of specific host cells (e.g., LPNI-2) will effect the IPR obtained. On the basis of the IPR, this Laird soil lacked indigenous *R. meliloti* strains (i.e., ATCC 9930-like bacteria).

The IPR profile for the Melfort soil was almost the reverse of that observed for the Laird soil (Fig. 3). This soil contained active populations of indigenous *R. meliloti* (i.e., ATCC 9930-like bacteria) and these organisms grew on added nutrients better than the indigenous *R. leguminosarum* strains did. The *R. meliloti* strains in this soil responded to all carbohydrates tested, although the response varied somewhat for each compound. In contrast, low numbers of phages, comparable to the level obtained with a 10- to 100-μg nutrient amendment, were detected for indigenous *R. leguminosarum* 128C52 and LPNI-2-like rhizobia. Nevertheless, on the basis of the IPR results, at least these three indigenous rhizobium strains were detected in this soil.

Amendment of the Sutherland soil with carbohydrates stimulated the growth of indigenous rhizobia such that an IPR was detected for all three indicator strains (Fig. 3). In this soil, the indigenous rhizobia appeared to be equally active, as the IPR was similar for all three indicator strains. In all three soils, there was no overwhelming preference for a particular carbohydrate, nor was an IPR detected in any soil when water, potassium nitrate, ammonium chloride, or urea was tested.

**(ii) Organic acid and sugar alcohol amendments.** The growth responses of indigenous *R. leguminosarum* and *R. meliloti* to succinic, critic, or malic acids and to mannitol or glycerol amendments were similar to those observed for carbohydrates (Fig. 4). Thus, the Laird soil contained *R. leguminosarum* (i.e., 128C52 and LPNI-2-like strains) but not ATCC 9930-like *R. meliloti*, the Melfort soil contained both *R. leguminosarum* and *R. meliloti* strains with the latter being more active, and the Sutherland soil contained rhizobia similar to all three indicator strains. Notable growth responses to certain nutrients included the response of indigenous LPNI-2 strains to mannitol and succinic acid in the Laird soil and the poor response of indigenous ATCC 9930-like strains to citric acid in the Melfort soil.

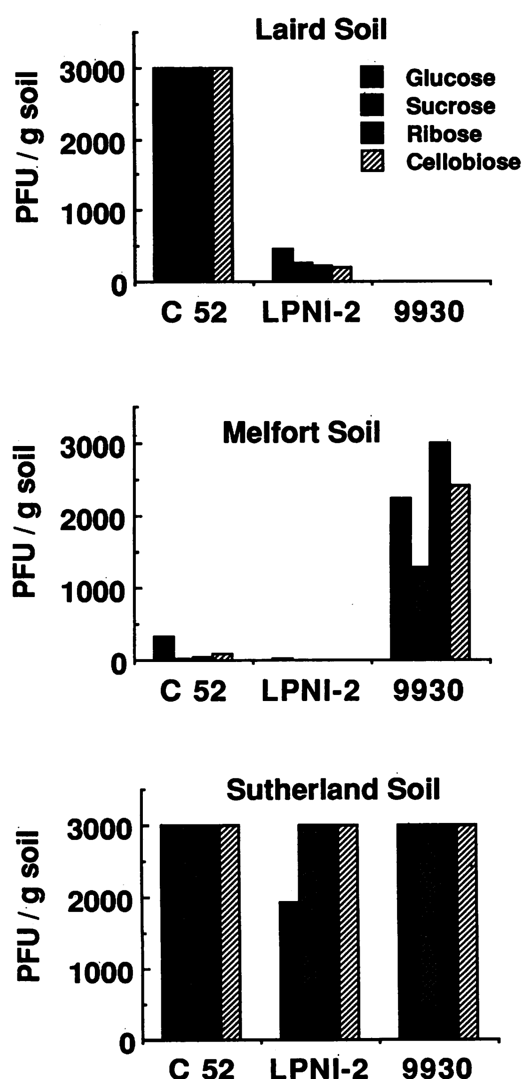


FIG. 3. Indigenous phage response for *R. leguminosarum* 128C52, *R. leguminosarum* LPNI-2, and *R. meliloti* ATCC 9930 strains in soils amended with 1,000 μg of carbohydrate g of soil$^{-1}$.

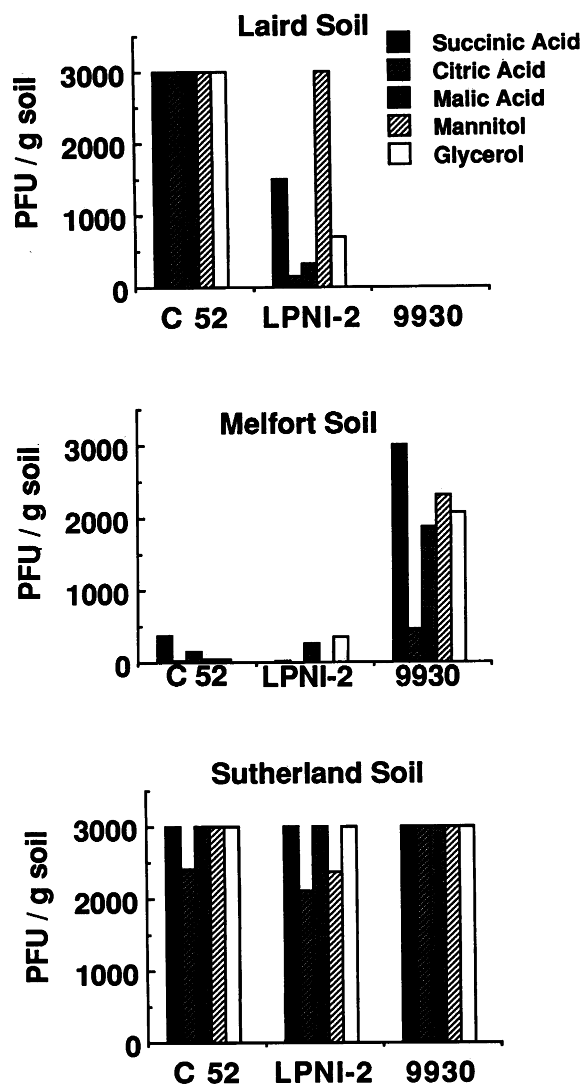


FIG. 4. Indigenous phage response for *R. leguminosarum* 128C52, *R. leguminosarum* LPNI-2, and *R. meliloti* ATCC 9930 strains in soils amended with 1,000 μg of organic acid or sugar alcohol g of soil$^{-1}$.

**(iii) Amino acid amendments.** Growth responses of indigenous rhizobia to alanine, glycine, glutamic acid, aspartic acid, or phenylalanine amendments were similar to those for other nutrients with respect to the occurrence of a specific rhizobium strain in a given soil (e.g., Laird soil contained only 128C52 and LPNI-2 like rhizobia) (Fig. 5). However, utilization of amino acids as growth substrates was more specific than for the other nutrients tested. For example, alanine and glutamic acid were readily utilized as growth substrates by most indigenous rhizobia in all three soils, whereas glycine and phenylalanine usually were poor growth substrates (Fig. 5). In many cases, aspartic acid was also a poor growth substrate. Alanine and glycine stimulated growth of indigenous LPNI-2 strains in the Melfort soil. This was surprising, since *R. leguminosarum* was not very active in the Melfort soil amended with other nutrients.

**Isolation of *Rhizobium* bacteriophages and typing of *Rhizobium* isolates.** Bacteriophages active against different *R. leguminosarum* and *R. meliloti* species were isolated from various soils. The lytic spectra of five selected *R. leguminosarum* phage isolates are given in Table 3. Many other phage isolates were obtained but these were not listed, as they exhibited lytic spectra similar to those of the phages in Table 3. Phage isolates were placed in three groups. One group, (e.g., C52p2) replicated on all of the *R. leguminosarum* strains tested. The second group (e.g., H100p1) replicated on host strains exhibiting a phage sensitivity pattern similar to that of strain 128C52 (i.e., only on strains not attacked by LPNp1 and LPNp2 phages). The third group (e.g., LPNp1) replicated on host strains similar to LPNI-2. A notable difference between these phage groups was plaque characteristics on host cell lawns. Group 3 phages exhibited fuzzy, diffuse plaques that took several days to develop, whereas group 1 and 2 phages were characterized by distinct plaques that developed within 24 h.

On the basis of phage lytic spectra, the *R. leguminosarum* strains in Table 1 were placed into three distinct phage types. In practice, it was possible to identify the activity of two distinct groups of *R. leguminosarum* in soil, i.e., 128C52-like

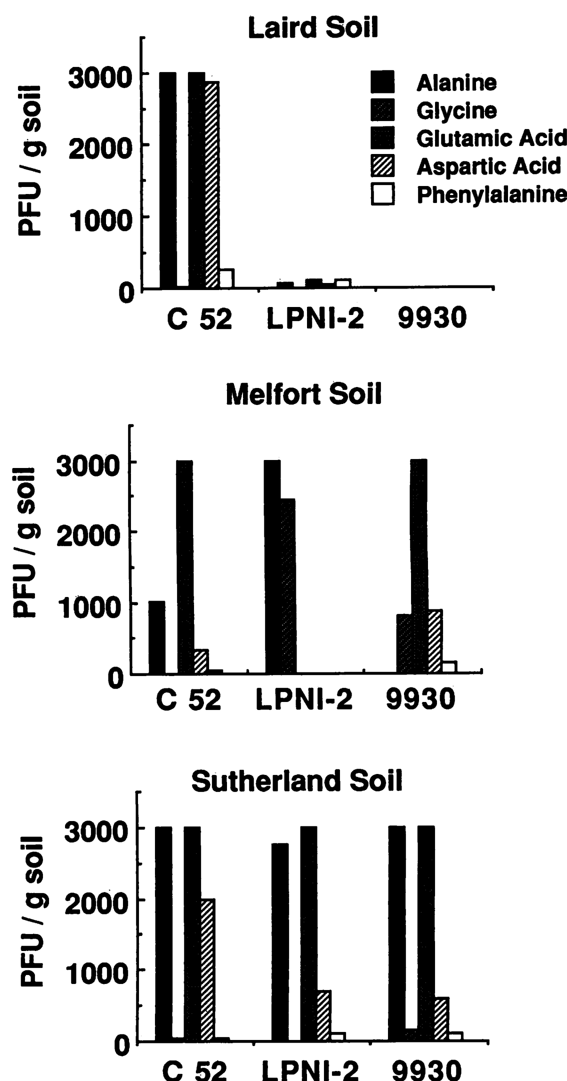


FIG. 5. Indigenous phage response for *R. leguminosarum* 128C52, *R. leguminosarum* LPNI-2, and *R. meliloti* ATCC 9930 strains in soils amended with 1,000 μg of amino acid g of soil$^{-1}$.

TABLE 3. Lytic spectra of five *R. leguminosarum* bacteriophage isolates typed against various *R. leguminosarum* strains

| Host | Lytic reaction of phage isolate[a]: | | | | |
|---|---|---|---|---|---|
| | C52p2 | C52p3 | H100p1 | LPNp1 | LPNp2 |
| 128C52 | ++++ | ++++ | ++++ | – | – |
| NA503-3 | + | + | ++++ | – | – |
| PNI-T2 | ++++ | ++++ | +++ | – | – |
| PNI-S-2 | ++++ | + | + | – | – |
| H100 | ++++ | – | ++++ | – | – |
| PNI-M-3 | + | – | + | – | – |
| LPNI-2 | +++ | – | – | ++++ | ++++ |
| PNI-T-1 | +++ | – | – | ++++ | ++++ |
| PNI-TB-1 | +++ | – | – | ++++ | ++++ |
| PNI-M-1 | + | – | – | ++++ | ++++ |

[a] Phage isolates were coded according to the original host on which the phage was isolated. For example, C52p2 is isolate no. 2 of phage isolated on strain 128C52. Lytic reactions were as follows: ++++, confluent lysis on the propagating strain within 24 h; +++, lysis, but not confluent within 24 h; +, faint lysis within 24 h; –, no lysis.

and LPNI-2-like rhizobia. This was possible because of the plaque characteristics of the phage, time for plaque development, and the relative number of PFU detected after amendments.

Several phages of *R. meliloti* 9930 were isolated from soil, and these phages exhibited similar lytic spectra against all of the *R. meliloti* nodule isolates. Strains NRG 118 and 185 were not lysed by these phages.

All of the *R. leguminosarum* and *R. meliloti* phage isolates were specific for rhizobia. None of these isolates formed plaques on laboratory cultures of *Arthrobacter globiformis*, *Bacillus subtilis*, or *Pseudomonas synxantha*. In addition, 54 bacterial isolates, randomly selected from soil dilution spread plates of the Laird and Sutherland soils, were tested for sensitivity to the rhizobium phage isolates. None of these isolates were attacked.

**Indigenous rhizobial populations in soils.** Soils were amended with glucose and screened for certain populations of indigenous rhizobia by the indirect phage analysis procedure. Native rhizobia were detected in all soils, even those that had not been cropped to a legume for 15 to 40 years (Table 4). An interesting observation was that laboratory indicator strains were so prevalent in soil. It was surprising that the Tisdale soil lacked detectable populations of *R. meliloti* even though it was recently cropped to alfalfa. However, the IPR detected was limited by the choice of the three indicator strains used for the assay.

The *R. leguminosarum* IPR for the Sutherland and Melfort soils are shown in Fig. 6a. The indicator strains used to assay the IPR were isolated from these soils. The isolates PNI-S-1, -S-2, and -S-3, and PNI-M-3 and M-4 exhibited a phage sensitivity pattern similar to that of 128C52, whereas the isolates PNI-M-1 and M-2 were similar to LPNI-2. The IPR indicated that some of these strains were more active than other strains and that the occurrence of some strains was limited to the soil from which they were isolated. The *R. meliloti* IPR for these soils is shown in Fig. 6b. Again there were different IPR patterns for specific strains, although in this case the isolates were present in both soils.

**Occurrence of *R. meliloti* ATCC 9930 in Laird soil?** On the basis of the failure to elicit an IPR for *R. meliloti* in the Laird soil (Fig. 3), it would appear that this soil lacked these rhizobia. However, the failure to elicite a phage response for ATCC 9930 might be due to a lack of *R. meliloti* phage in this soil, a lack of host cells to support phage replication in soil, or a lack of both phage and host cells. To test these hypotheses, Laird soil was inoculated with either ATCC 9930 host cells ($10^7$ CFU g of soil$^{-1}$), a phage (9930 p1; 100 PFU g of soil$^{-1}$) active against ATCC 9930 host cells, or both host cells and phage. This treated soil was then amended with 1,000 μg glucose g soil$^{-1}$ (Fig. 7). Phage replication occurred only when both host cells and phage

TABLE 4. Occurrence of indigenous *R. leguminosarum* and *R. meliloti* populations in seven soils as determined by indirect phage analysis

| Host (strain) | Indigenous phage response[a] for soil: | | | | | | |
|---|---|---|---|---|---|---|---|
| | Laird | Sutherland | Melfort | Star City | Tisdale | Waitville | Tisdale-B |
| *R. leguminosarum* | | | | | | | |
| 128C52 | + | + | – | + | + | + | + |
| LPNI-2 | + | + | + | + | – | + | – |
| PNI-S-1 | nt | + | – | nt | nt | nt | nt |
| *R. meliloti* | | | | | | | |
| ATCC 9930 | – | + | + | + | – | – | – |
| NRG 185 | – | – | – | – | – | – | – |
| NRG 118 | – | – | – | – | – | – | – |
| ANI-S-1 | – | + | + | nt | nt | + | nt |

[a] Soil was amended with 1,000 μg of glucose g of soil$^{-1}$. +, Positive phage response; –, no phage response; nt, not tested.

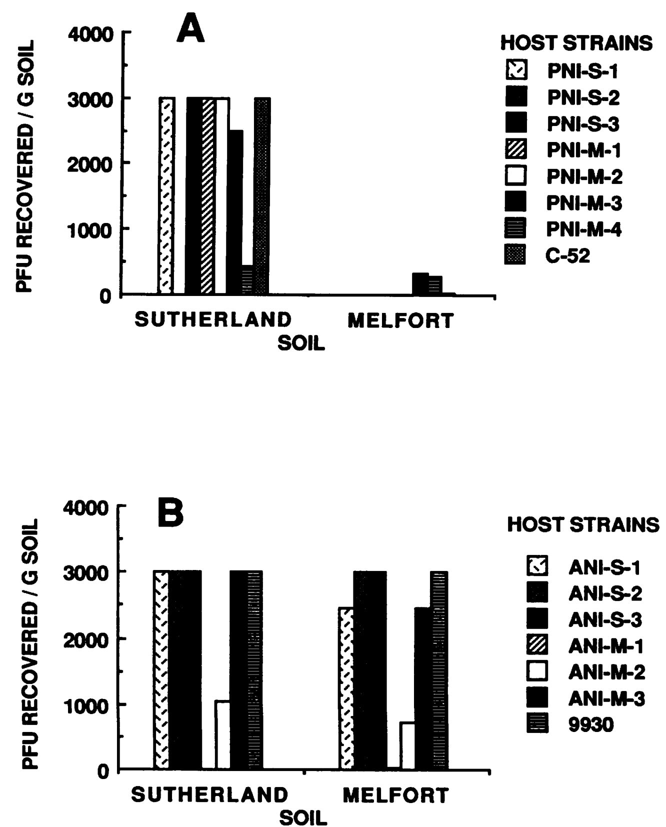


FIG. 6. Indigenous phage response profile for root nodule isolates in the Melfort and Sutherland soils amended with 1,000 μg of glucose g of soil$^{-1}$. Panels: A, *R. leguminosarum*; B, *R. meliloti*. Host strains are identified in Table 1.

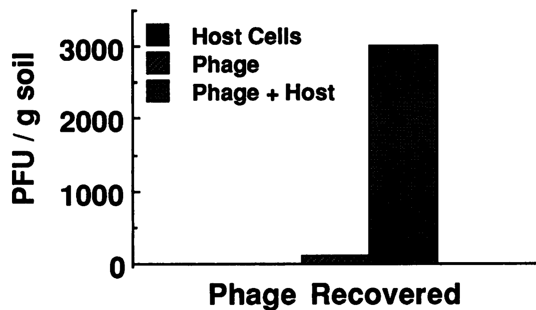


FIG. 7. Recovery of phage from Laird soils inoculated with *R. meliloti* ATCC 9930 cells, ATCC 9930 cells and phage 9930pl, or phage 9930pl, and then treated with 1,000 μg of glucose g of soil$^{-1}$. Phage recovery was determined after 24 h.

were added, indicating that the Laird soil lacked indigenous ATCC 9930 host cells and appropriate phage.

## DISCUSSION

The growth of indigenous *R. leguminosarum* and *R. meliloti* strains was stimulated by specific organic nutrient amendments to soil. During growth of these bacteria, specific indigenous bacteriophage attacked the multiplying host cells in soil and replicated. This IPR was detected with appropriate indicator strains and provided a relative index of the activity of the indigenous soil rhizobia. Previous studies demonstrated how indirect phage analysis can be used to follow the activity of bacterial predators in soil (4) or to study host-phage population dynamics in soil (3). The present study shows that the relative activity of individual members of bacterial populations in soil (i.e., rhizobia) can be monitored by using several indicator strains. Thus, the relative in situ growth responses of several native *R. leguminosarum* and *R. meliloti* strains to organic nutrient amendments were monitored in soil.

All 17 organic compounds stimulated growth of indigenous *R. leguminosarum* and *R. meliloti* to some extent. The growth response of each indigenous rhizobial strain varied depending on the nutrient and the soil. For example, when carbohydrates were the growth substrates (Fig. 3), the activity of *R. leguminosarum* 128C52 was very high in the Laird soil but almost absent in the Melfort soil. On the other hand, the activity of strain 128C52 was stimulated to an equal extent in both soils by certain amino acid amendments (Fig. 4). These results were not unexpected because populations of native rhizobia in soils are known to contain a variety of species and strains within species (12). It is conceivable, therefore, that one strain responded to carbohydrates whereas another strain responded to amino acids. It should be pointed out that the IPR profile of a given soil will reflect the activity of a consortium of rhizobial strains in that soil which are similar to the indicator strains used for the IPR assay. In general, the indigenous rhizobia readily utilized all organic compounds except phenylalanine, glycine, and aspartic acid. Finally, water and inorganic nitrogen amendments did not stimulate growth of indigenous rhizobia, indicating that carbon and energy sources were more limiting in soil.

Little is known about the ability of rhizobia to utilize organic nutrients in soil. Pena-Cabriales and Alexander (11) studied the growth of small populations of *R. meliloti*, *Rhizobium phaseoli*, *Rhizobium japonicum*, and cowpea rhizobia inoculated into soil. They found that 0.5% and 1.0% mannitol (i.e., 5,000 to 10,000 μg g of soil$^{-1}$) was required to support the growth of these added rhizobia. On the basis of this and other evidence, they concluded that competition from indigenous soil bacteria prevented growth of the added rhizobia. In the present study, nutrient concentration significantly influenced the IPR for soil rhizobia. Low levels of nutrients (i.e., 10 to 100 μg g of soil$^{-1}$) had little effect on the activity of native *R. leguminosarum* and *R. meliloti*, whereas 1,000-μg amendments were usually quite stimulatory. Thus, my results show that indigenous rhizobia are very competitive (i.e., grow on 1,000-μg nutrient amendments) and readily utilize a variety of organic compounds as growth substrates in soil. A recent study done by using a direct immunofluorescence assay supports this conclusion (16).

The Laird, Melfort, and Sutherland soils contained indigenous rhizobia, but each soil exhibited a unique IPR profile. Thus, the Laird soil contained *R. leguminosarum* strains but not ATCC 9930-like *R. meliloti*, the Melfort soil contained both *R. leguminosarum* and *R. meliloti* with the latter being more active, and the Sutherland soil contained both groups of rhizobia. To some extent, the unique IPR profiles can be explained by previous cropping history. For example, the crop rotation on the Laird and Sutherland soils was from wheat to pea, and both had been cropped to field pea during the year they were collected. This would explain the high activity of *R. leguminosarum* in these soils. The Laird soil had never been cropped to alfalfa and thus lacked native ATCC 9930-like *R. meliloti* strains. The Melfort soil was cropped to field pea during the year of collection but had been cropped to alfalfa during the preceeding years. Thus, there might have been ample time for the *R. meliloti* population to become established, whereas the *R. leguminosarum* populations would be relatively new and might be less abundant.

All of the soils assayed contained native rhizobia, even soils that had not been cropped to a legume for a long period of time. Similar findings have been reported in other studies and are usually attributed to wild native legumes (1, 10). In the present study, some soils lacked specific *Rhizobium* spp. or strains, an example being the absence of *R. meliloti* ATCC 9930 from the Laird soil. This might be explained by past cropping history. In some cases, the past cropping history might not be known or the results of the IPR might be unclear. In such cases, it would be advisable to check the soil for the presence of both indigenous host cells and phage. For the Laird soil, simply adding host cells and nutrients demonstrated that phage capable of infecting these host cells were absent. Adding just phage and nutrients demonstrated that susceptible host cells were absent from the soil. Information on the presence or absence of specific rhizobia and their phages in soil is useful, as it identifies soils that can be used to study the ecology and population dynamics of introduced rhizobia.

Bromfield et al. (1) examined rhizobial isolates that occupied alfalfa root nodules. The isolates were separated into 55 to 65 unique phage types (i.e., strains). These workers also found a field site where two phage types dominated and seven others occurred frequently. Thus, many different indigenous rhizobium strains exhibiting unique phage sensitivity patterns occur in soil and support populations of specific phages. In my study, the presence of distinct rhizobiophages was related to the activity of specific rhizobium strains. The use of a large number of unique (based on phage typing) rhizobia to determine the IPR profile of a soil would

provide considerable information on the ecology of indigenous rhizobia and their ability to use organic nutrients.

Bacteriophage have been successfully employed as an indirect indicator of bacterial activity in soil during this and other investigations (3, 4). This procedure is rapid and highly specific, allows study of indigenous bacteria that have not been or can not be isolated from natural environments, and provides a relative index of activity. Additional studies are required to assess the relationship between the IPR profile of a soil and the nodulation activity of indigenous rhizobia. This would allow for rapid screening of soils to predict the success or failure of an inoculant.

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