A Selective Medium for the Isolation and Quantification of 
Bradyrhizobium japonicum and Bradyrhizobium elkanii Strains 
from Soils and Inoculants†

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The ecological examination of members of the family Rhizobiaceae has been hampered by the lack of a 
selective medium for isolation of root nodule bacteria from soil. A novel non-antibiotic-containing medium has 
been developed which allows selective isolation of Bradyrhizobium japonicum and B. elkanii strains from soil and 
inoculants. The medium, BJSM, is based on the resistance of B. japonicum and B. elkanii strains to more than 
40 μg of the metals ions Zn²⁺ and Co²⁺ per ml. BJSM does not allow growth of Rhizobium sp. strains. We used 
BJSM to isolate bacteria from a Hubbard soil and from several commercially prepared soybean inoculants. 
Ninety-eight percent of the isolates obtained from Hubbard soil nodulated Glycine max cv. Kasota, and between 
55 and 95% of the isolates from the commercial inoculants had the ability to nodulate soybeans. Numbers of 
bradyrhizobia obtained by using BJSM, strain-specific fluorescent antibodies, and the most-probable-number 
plant infection assay indicated that the three techniques were comparable in quantifying B. japonicum strains 
in soils and inoculants, although most-probable-number counts were generally 0.5 order of magnitude greater 
than those obtained by using BJSM. Results of our studies indicate that BJSM is useful for direct isolation and 
quantification of B. japonicum and B. elkanii from natural soils and inoculants. This medium may prove to be 
an important tool for autecological and enumeration studies of diverse populations of bradyrhizobia and as a 
quality control method for soybean inoculants.

Ecological studies of members of the family Rhizobiaceae require the quantification and isolation of indigenous and 
applied inoculant strains. Such studies have been hampered by the lack of a selective medium for the direct isolation and 
accurate enumeration of root nodule bacteria from soils. Present isolation methodology requires the use of a trap host, 
which biases recovered isolates to the most competitive strains which have the ability to nodulate a given legume genotype. 
Current enumeration methodologies include the quantitative immunofluorescence technique (22), the plant infection most- 
probable-number (MPN) assay (27, 31), and direct plating on antibiotic-containing laboratory growth media (19, 27). While 
all three techniques provide estimates of cell numbers, they each have limitations. While the plant infection MPN assay has 
been a method of choice among many researchers, the technique is expensive and time consuming and is predicated on 
the hypothesis that a single bacterium is capable of forming a nodule. In addition, the technique is sensitive to the host 
species (or genotype) and growth system used (31). The immunofluorescence technique is also frequently used to 
quantify rhizobia in soils and inoculants. This methodology, however, tends to overestimate population sizes and is subject 
to problems associated with overall sensitivity, the optimal dispersal and flocculation of bacteria from different soil types, 
and the availability of specific, non-cross-reactive antibodies. Moreover, with the exception of the MPN assay, current 
enumeration methodologies are generally limited to the quantification of single strains within a species of legume-nodulating 
bacteria.

Some selective agents have been used for the recovery of Rhizobium and Bradyrhizobium spp. from soils and inoculants. 
The selective agents used have included antibiotics, dyes, and metabolic inhibitors (4, 10, 28–30). The use of antibiotics as 
selective agents has not proven to be particularly effective, since even strains within a given species group can have diverse 
resistances to many different antibiotics. Pattison and Skinner (18) reported the formulation of a selective medium for 
rhizobia that contained pentachloronitrobenzene (PCNB), brilliant green (BG), sodium azide, crystal violet, and penicillin. 
Barber (1) subsequently modified the concentrations of these inhibitors to make the medium more selective for R. 
meliloti.

Metal tolerance among soil microorganisms has been extensively 
studied (5, 7–9, 11, 25). High-level resistance to zinc, cobalt, and cadmium has been well studied in Klebsiella spp. 
(2) and Alcaligenes eutrophus (6, 14–17, 29). Despite the presence of metal-contaminated soils, there have been few 
reports on metal and metalloid resistance in rhizobia. Previously, Kinkle et al. (13) examined two genera of soybean- 
nodulating rhizobia for levels of resistance to eight different metals. Marked differences in levels of resistance to several 
heavy metals were found, even for different rhizobial strains within a single species group. Moreover, B. japonicum strains 
were found to be more resistant to several heavy metals than were the several Rhizobium sp. strains examined.

The objectives of the current study were to examine the heavy metal resistance levels of various groups of bradyrhizobia 
and to develop a heavy-metal-based, non-antibiotic-containing, selective medium for the direct isolation and quantification 
of B. japonicum strains from agricultural soils.

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B. japonicum USDA 4, USDA 38, USDA 62, USDA 110, USDA 122, USDA 123, USDA 127, USDA 129, USDA 135
R. tropici CIAT 899

<table>
<thead>
<tr>
<th>Strain(s)</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>B. japonicum USDA 4, USDA 38, USDA 62, USDA 110, USDA 122, USDA 123, USDA 127, USDA 129, USDA 135</td>
<td>1</td>
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<td>B. elkanii USDA 46, USDA 61, USDA 76, USDA 94, USDA 130</td>
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<tr>
<td>R. etli Tal 182, Tal 1383</td>
<td>2</td>
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<td>R. fredii USDA 191, USDA 205, USDA 257</td>
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<tr>
<td>HII 103</td>
<td>3</td>
</tr>
<tr>
<td>R. leguminosarum bv. trifolii Tal 1820, Tal 1824</td>
<td>2</td>
</tr>
<tr>
<td>R. leguminosarum bv. viciae Tal 634, Tal 640</td>
<td>2</td>
</tr>
<tr>
<td>R. meliloti Tal 380, Tal 1372</td>
<td>2</td>
</tr>
<tr>
<td>102FF34</td>
<td>4</td>
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<tr>
<td>R. tropici CIAT 899</td>
<td>5</td>
</tr>
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</table>


MATERIALS AND METHODS

Strains and media used. The *Rhizobium* and *Bradyrhizobium* strains used in this study and their sources are listed in Table 1. Strains were routinely grown and maintained on AG medium (21) at 28°C.

Soils and inoculants. Commercially prepared soybean inoculant I was a peat-based inoculant obtained from Lipha Tech Inc. (Milwaukee, Wis.), inoculant II was a peat-based soybean inoculant from Urbana Laboratories (St. Joseph, Mo.), and inoculant III was a liquid soybean inoculant from Lipha Tech Inc. Monmouth fine sandy loam soil (Typic Hapludults) was obtained in Maryland and contained fewer than 10 B. *japonicum* organisms per g of soil as determined by the plant infection MPN method (27, 31). Hubbard loamy sand soil (Udorthentic Haploboroll) was obtained from the University of Minnesota Agricultural Experiment Station in Becker. The soil has been used for soybean cultivation.

Determination of resistance to heavy metals and inhibitors of fungi and gram-positive bacteria. *Rhizobium* and *Bradyrhizobium* strains were grown in AG broth medium at 28°C to the late log phase and serially diluted in 0.85% NaCl, and dilutions were spread plated or streaked onto the surface of metal-amended or unamended AG agar medium. The metals used were added as ZnCl₂, CrCl₃·6H₂O, CuCl₂, CdCl₂·2H₂O, PbCl₂, HgCl₂, MoO₃, and NiCl₂. Suspension of each metal ion were prepared at 10 g/liter in distilled water, except for Pb²⁺, which was prepared as a 5-g/liter stock solution. All metal solutions were filter sterilized before addition to sterile and molten media. Plates were amended with either one or two of the metals. Each strain was tested on different concentrations of all eight metals. All plates were replicated at least three times and incubated at 28°C. Plates were routinely scored after 10 to 12 days of growth. MICs are presented as micrograms of metal ions per milliliter. Percent relative growth efficiency of *B. japonicum* and *B. elkanii* strains was determined in liquid AG medium supplemented with various concentrations of metals. Growth was quantified after 5 days of incubation at 28°C by measuring optical density at 590 nm. Resistance to PCNB, cycloheximide, and BG was tested by spread plating dilutions of AG-grown *B. japonicum* cultures onto AG agar medium amended with several concentrations of each inhibitor. Plates were incubated at 28°C, and viable cell numbers on duplicate plates were determined after 10 days of growth.

Soil inoculations. Mid-log-phase, AG-grown cultures of strains of rhizobia were harvested by centrifugation at 10,000 × g for 10 min at 4°C, washed in 0.85% NaCl solution, and centrifuged at 10,000 × g for 10 min at 4°C. Cell pellets were suspended in 10-fold-diluted HM salts solution (21) and added to soils (10 g) to achieve initial cell numbers of 10⁵ to 10⁷ rhizobia per g of soil. The final moisture content of the soils was adjusted to approximately field capacity. Soils were incubated in sterile 150-ml broad-mouth bottles at 28°C for 7 days (13).

Extraction of rhizobia from soils and inoculants. Rhizobia were extracted from soils and inoculants by a modification of the gelatin-ammonium phosphate method of Kingsley and Bohlool (12). Ten-gm aliquots of soils and inoculants were extracted in 95 ml of gelatin-ammonium phosphate solution containing 0.5 ml of Tween 80 and 0.1 ml of silicone antifoam AF72 (General Electric Co., Waterford, N.J.). Soil and inoculant suspensions were shaken on a wrist action shaker for 30 min and allowed to settle for an additional 30 min. The upper aqueous phase was removed to a sterile test tube, and serial dilutions were used for plant infection MPN assays (27, 31), direct quantitative fluorescent-antibody (FA) enumeration (12, 22) and plating onto the various selective media.

Composition of *Bradyrhizobium* selective medium (BJSM). The *B. japonicum* selective medium used consisted of AG medium supplemented with 1.0 μg of BG per ml, 500 μg of PCNB per ml, 83 μg of ZnCl₂ per ml, and 88 μg of CoCl₂ per ml. PCNB was prepared and added to the medium as previously described (1, 18). The BG, heavy metals, and cycloheximide were added to the sterile and molten medium as filter-stereiled stocks. Plant infection assays. Plant infection assays were done in plastic growth pouches as described by Somasegaran and Hoben (27). Each pouch contained two plants. Bacteria isolated on BJS from inoculated and un inoculated field soils and inoculants were tested for the ability to nodulate soybean plants. Plant nodulation tests were done with surface-sterilized (27) soybean seeds (Glycine max cv. Kasota). Plants were watered with N-free plant nutrient solution (3). Individual small, white colonies were picked from BJS and grown for 3 days in AG medium at 28°C, and 1-ml aliquots were inoculated onto pregerminated seedlings. Positive (inoculated with *B. japonicum* USDA 110) and negative (inoculated with blank AG broth) controls were included. MPN analyses were done with four replicates as previously described (27). Inoculated seedlings were incubated in a growth chamber at 25°C with a 16-h photoperiod for 4 weeks.

Direct quantitative immunofluorescence assay. Bradyrhizobia in extracts of soils and inoculants were quantified by direct immunofluorescence microscopy as previously described (12, 22). Strain-specific FAs were prepared as previously described (22).

RESULTS AND DISCUSSION

Resistance of bradyrhizobia and rhizobia to heavy metals. We examined whether heavy metals could be used as selective agents for the direct isolation of *B. japonicum* and *B. elkanii* strains from soils and inoculants. Initially, five strains of bradyrhizobia (representing type members of five serogroups)
and eight strains of rhizobia were tested for intrinsic resistance to eight heavy metals. With few exceptions, the B. japonicum and B. elkanii strains tested were resistant to relatively high concentrations of most of the metals (Table 2). Our results are in general agreement with those reported by Kinkle et al. (13). USDA 123 and USDA 127 proved to be the least tolerant B. japonicum strains tested. The MICs of Mo\(^{7+}\) were the highest (1,600 to 3,200 \(\mu g/ml\)) for the bradyrhizobia tested. For B. elkanii USDA 61, the MICs of seven of the eight tested metals were the highest. Tolerance to Zn\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\), and Cd\(^{2+}\) varied significantly among strains USDA 61, USDA 110, USDA 122, USDA 123, and USDA 127. Tolerance to Cd\(^{2+}\) also varied greatly among the bradyrhizobia tested, with MICs ranging from <20 to 80 \(\mu g/ml\). All of the bradyrhizobia tested were sensitive to Hg\(^{2+}\), with MICs of 1 \(\mu g/ml\) or less.

To be effective selective agents for the bradyrhizobia, heavy metals must inhibit the growth of the phylogenetically related Rhizobium strains. Consequently, intrinsic resistance to heavy metals was also examined among several diverse Rhizobium species: R. leguminosarum bv. vicieae Tal 634 and Tal 640, R. meliloti Tal 380 and Tal 1372, R. etli Tal 182 and Tal 1383, and R. leguminosarum bv. trifolii Tal 1820 and Tal 1824. For most of the Rhizobium sp. strains examined, the MICs of all of the tested metals were nearly identical, with the exception of those of Mo (Table 3). In contrast to the bradyrhizobia, the Rhizobium strains had lower MICs of Zn\(^{2+}\), Co\(^{2+}\), and Cd\(^{2+}\) (Table 3). Low MICs of Cu, Al, Ni, Zn, and Cd have also been previously reported for 11 R. fredii strains (13). Since Bradyrhizobium strains were uniformly more resistant to Zn\(^{2+}\) and Co\(^{2+}\) than were Rhizobium strains, our results suggested that these heavy metals might be useful as selective agents for the direct isolation of bradyrhizobia from soils.

However, since Zn\(^{2+}\) MICs varied widely among strains of bradyrhizobia in plate assays, we more precisely evaluated the impact of Zn\(^{2+}\) concentration on the growth of B. elkanii USDA 61, B. japonicum USDA 110 and USDA 122, and R. meliloti Tal 380 growing in liquid culture (Fig. 1). R. meliloti Tal 380 had little growth when the Zn\(^{2+}\) concentration was greater than 40 \(\mu g/ml\) and less than 25% of the growth of the metal-unamended control medium when the Zn\(^{2+}\) concentration reached 10 \(\mu g/ml\). In contrast, the three Bradyrhizobium strains tested (USDA 122, USDA 110, and USDA 61) had between 78 and 90% of the growth of the control culture when the Zn\(^{2+}\) concentration reached 100 \(\mu g/ml\). B. japonicum USDA 122 and USDA 110 failed to grow adequately at medium Zn\(^{2+}\) concentrations higher than 250 \(\mu g/ml\). The B. elkanii strain tested, USDA 61, proved to be very tolerant to high Zn\(^{2+}\) concentrations and had relatively good growth even when Zn\(^{2+}\) reached 350 \(\mu g/ml\) in liquid medium (Fig. 1).

### TABLE 2. Tolerance of B. japonicum strains to heavy metals

<table>
<thead>
<tr>
<th>Strain</th>
<th>USDA 61</th>
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<th>USDA 122</th>
<th>USDA 123</th>
<th>USDA 127</th>
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<tr>
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<td>Zn</td>
<td>Co</td>
<td>Cu</td>
<td>Cd</td>
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<tr>
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<td>480</td>
<td>160</td>
<td>80</td>
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<td>80</td>
<td>80</td>
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<td>640</td>
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<td>640</td>
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<tr>
<td>USDA 123</td>
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<td>80</td>
<td>40</td>
<td>&lt;20</td>
<td>480</td>
</tr>
<tr>
<td>USDA 127</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>&lt;20</td>
<td>480</td>
</tr>
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### TABLE 3. Tolerance of Rhizobium strains for heavy metals

<table>
<thead>
<tr>
<th>Strain</th>
<th>Host</th>
<th>MIC ((\mu g/ml)) of:</th>
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<tbody>
<tr>
<td></td>
<td>Zn</td>
<td>Co</td>
</tr>
<tr>
<td>Tal 634</td>
<td>LH(^a)</td>
<td>40</td>
</tr>
<tr>
<td>Tal 640</td>
<td>LW(^a)</td>
<td>20</td>
</tr>
<tr>
<td>Tal 380</td>
<td>MS(^a)</td>
<td>40</td>
</tr>
<tr>
<td>Tal 1372</td>
<td>MS</td>
<td>40</td>
</tr>
<tr>
<td>Tal 182</td>
<td>PV(^d)</td>
<td>40</td>
</tr>
<tr>
<td>Tal 1383</td>
<td>PV</td>
<td>40</td>
</tr>
<tr>
<td>Tal 1820</td>
<td>TR(^c)</td>
<td>40</td>
</tr>
<tr>
<td>Tal 1824</td>
<td>TP(^f)</td>
<td>40</td>
</tr>
</tbody>
</table>

\(^a\) LH, Lathyrus hirsutus.
\(^b\) LW, Lens culinaris.
\(^c\) MS, Medicago sativa.
\(^d\) PV, Phaseolus vulgaris.
\(^e\) TR, Trifolium repens.
\(^f\) TP, Trifolium pratense.

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**FIG. 1. Growth of Rhizobium and Bradyrhizobium strains in AG liquid medium containing various concentrations of Zn\(^{2+}\).** Symbols: ●, USDA 61; ▼, USDA 110; ▪, USDA 122; □, Tal 380.
TABLE 4. Enumeration of *B. japonicum* and *B. elkanii* strains from soils and inoculants with selective media and the MPN plant infection assay

<table>
<thead>
<tr>
<th>Source</th>
<th>No. of bacteria on following medium:</th>
<th>No. of bacteria determined by plant infection</th>
</tr>
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<tr>
<td></td>
<td>AG</td>
<td>Zn²⁺ + Co³⁺</td>
</tr>
<tr>
<td>Strains added to</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monmouth soil</td>
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</tr>
<tr>
<td>USDA 61₄</td>
<td>9.0 × 10⁶</td>
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<tr>
<td>USDA 110</td>
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<td>1.9 × 10⁷</td>
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<td>USDA 122</td>
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<td>6.0 × 10⁷</td>
<td>1.3 × 10⁷</td>
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<td>USDA 127</td>
<td>1.6 × 10⁷</td>
<td>8.7 × 10⁶</td>
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<tr>
<td>Inoculants⁵</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4.9 × 10⁶</td>
<td>9.0 × 10⁶</td>
</tr>
<tr>
<td>II</td>
<td>2.1 × 10⁷</td>
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<tr>
<td>III</td>
<td>7.0 × 10⁷</td>
<td>ND</td>
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<tr>
<td>Soils⁶</td>
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</tr>
<tr>
<td>Hubbard</td>
<td>9.0 × 10⁶</td>
<td>8 × 10⁵</td>
</tr>
<tr>
<td>Monmouth</td>
<td>1.0 × 10⁷</td>
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</table>

*The media used were AG medium and AG medium amended with 40 μg of Zn²⁺ per ml and 40 μg of Co³⁺ per ml. BJSME is AG medium amended with 40 μg of Zn²⁺ per ml, 40 μg of Co³⁺ per ml, 1.0 μg of BG per ml, and 500 μg of PCNB per ml.

† The *B. japonicum* strains were inoculated into Monmouth soil and incubated for 7 days at 28°C.

‡ Values are means of replicated plates (n = 2) or growth pouches (n = 4) and are expressed as numbers of bacterial cells per g of soil.

§ ND, not determined.

‖ Commercial inoculants were used. Inoculant I was Lipha Tech pea based. Inoculant II was Urbana Laboratories Research Seeds pea based. Inoculant III was Lipha Tech solid inoculant.

§ Soils were uninoculated.

and 500 μg of PCNB per ml. We named this medium BJSME, for *B. japonicum* selective medium. These inhibitors have been previously used in selective media for other strains of rhizobia (1, 18). The selective agents effectively inhibited the growth of contaminants (the average number of contaminants ranged from 2 to 5 colonies per plate at the 10⁻¹ soil dilution for Montmorency soil). Following recovery from Monmouth soil, viable cell counts of strains USDA 61 and USDA 110 were seemingly unaffected by the inhibitors (Table 4), while viable counts of *B. japonicum* USDA 122 were slightly reduced relative to those in the same medium without the inhibitors. Since BJSME was completely inhibitory for the growth of *B. japonicum* USDA 123 and USDA 127, the results in Table 4 suggested that BJSME selectively recovered bacteria in different serological groups.

BJSME was also tested for the ability to recover bacteria from commercially prepared soybean inoculants and Hubbard soil (Table 4). While the Hubbard soil contained 9.0 × 10⁶ total bacteria per g of soil (on AG medium), BJSME recovered about 10⁴ bacteria per g of soil (these were tentatively called bradyrhizobia [see below]). BJSME also allowed for the isolation of 9.0 × 10⁷ and 1.8 × 10⁷ bacteria per g of peat from the Lipha Tech (formally Nitragin Co.) and Urbana Laboratories peat-based inoculants, respectively.

To determine whether other bradyrhizobia were capable of growing on the selective medium, we streak inoculated BJSME with AG-grown cultures of nine additional serologically distinct *B. japonicum* and *B. elkanii* strains. Although BJSME allowed growth of *B. japonicum* USDA 4, USDA 38, and USDA 62, it inhibited the growth of strains USDA 129 and USDA 135. With the exception of strain USDA 135, all of the inhibited strains belong to serocluster 123 (23). All of the *B. elkanii* strains tested (USDA 46, USDA 76, USDA 94, and USDA 130) grew on BJSME. BJSME failed to support the growth of the fast-growing, soybean-nodulating *R. fredii* strains USDA 191, USDA 205, USDA 257, and HH 103, indicating that the ability to nodulate soybeans is not directly correlated with the ability to grow on the selective medium. Other *Rhizobium* species, *R. tropici* CIAT 899 and *R. melliloti* 102F34, also failed to grow on BJSME. Our results indicated that BJSME is capable of supporting the growth of a majority of *Bradyrhizobium* serogroup strains found in soils within the United States.

Selective inhibition of the growth of *B. japonicum* USDA 123 and USDA 127 on BJSME appears to be due to the sensitivity of these strains to the relatively high concentrations of the inhibitors used (Table 5). The AG medium amended with BG at a concentration greater than or equal to 1.0 μg/ml completely inhibited the growth of strain USDA 123 and reduced viable counts of strain USDA 127 by about 1 order of magnitude relative to unamended AG medium. Similarly, PCNB at 500 μg/ml (the concentration used in BJSME) reduced the viable plate counts of USDA 123 and USDA 127 by about 1 order of magnitude relative to unamended AG medium (Table 5). Maximum cell counts for both strains were obtained at BG and PCNB concentrations of 0.5 and 250 μg/ml, respectively. Cycloheximide, at 200 μg/ml, was a useful anti-fungal substitute for PCNB and allowed isolation of about 100-fold more USDA 123 colonies than did BJSME (data not presented). Results of these studies indicate that BJSME could be modified to include lower concentrations of BG (0.5 μg/ml) and PCNB or substitution of the fungal inhibitor cycloheximide (100 to 150 μg/ml) if greater numbers of serocluster 123 strains were to be isolated.

Comparison of enumeration methodologies. Table 4 also shows the comparison of bacterial counts (expressed as numbers of bacteria per gram of soil) recovered from Monmouth soil individually spike inoculated with *B. japonicum* USDA 61, USDA 110, and USDA 122; uninoculated Hubbard soil; and three commercially prepared soybean inoculants by using the MPN plant infection assay and plate counts on BJSME. In most cases, the MPN counts of *B. japonicum* were about 3 to 10 times greater, but usually about 0.5 order of magnitude higher, than those obtained with BJSME. For USDA 110 and the Urbana Laboratories peat inoculant, MPN values were nearly identical to those determined with BJSME. In most instances, as expected, total bacterial counts on AG medium were greater than MPN or BJSME counts. Nevertheless, MPN counts were generally in close agreement with the bacterial counts obtained with BJSME, with the exception of those obtained with the Lipha Tech peat-based inoculant.
TABLE 6. Comparison of BJSM, the FA technique, and the MPN plant infection method for enumeration of *B. japonicum* USDA 110 recovered from Monmouth soil

<table>
<thead>
<tr>
<th>Incubation time (days)</th>
<th>No. of cells/g determined by:</th>
<th>BJSM plate count</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>FA count</td>
<td>MPN plant infection assay</td>
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<tr>
<td>0</td>
<td>$1.5 \times 10^5$</td>
<td>$2.3 \times 10^4$</td>
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<tr>
<td>7</td>
<td>$1.9 \times 10^6$</td>
<td>$1.3 \times 10^4$</td>
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<tr>
<td>Monmouth soil</td>
<td>0</td>
<td>$2.9 \times 10^1$</td>
</tr>
</tbody>
</table>

* The values shown are means of duplicate samples.

A comparison of bacterial counts of strain USDA 110 from spiked Monmouth soil obtained with the FA method, the MPN plant infection assay, and BJSM plate counts is shown in Table 6. While the numbers of strain USDA 110 obtained with the FA technique are generally a little higher than those obtained with the other two methods, the plant infection MPN assay and BJSM plate counting results were virtually identical at both time points. The differences between plate and FA counts have been noted previously (12) and could be due to problems associated with plating efficiencies on the selective medium or the FA counting of dead or dying bacteria.

**Nodulation ability of soil and inoculant bacteria recovered on BJSM.** Small, whitish colonies obtained from soils and inoculants on BJSM were tested for the ability to nodulate soybeans (Table 7). All of the bacteria recovered on BJSM from *B. japonicum*-spiked Monmouth soil were capable of nodulating soybean plants. Moreover, since 98% of the randomly selected bacterial colonies (49 of 50) recovered from Hubbard soil on BJSM were also able to nodulate soybean plants, our results indicated that BJSM was capable of effectively isolating about $10^6$ soybean-nodulating bacteria per g of soil among the total of $9 \times 10^6$ bacteria per g of soil. Moreover, our results indicated that contrary to what was reported for *R. leguminosarum* soil isolates (24, 26), *B. japonicum* strains isolated from soils are relatively stable for nodulation ability.

High percentages of soybean-nodulating bacteria were also recovered from the Lipha Tech Company peat- and liquid-based inoculants (Table 7), suggesting that BJSM is useful for quantifying bradyrhizobia in commercial and laboratory inoculants. However, only 55% of the BJSM-recovered colonies from the Urbana Laboratories peat-based inoculant had the ability to nodulate soybean plants. The exact reason for the low level of nodulation ability among the bacteria recovered from the Urbana Laboratories inoculant is unknown. One plausible explanation is that the peat carrier used contains bacteria (or a bacterium) that are highly resistant to the selective agents used and morphologically resemble *B. japonicum* colonies growing on BJSM.

Taken together, the results of our studies indicate that non-antibiotic-containing BJSM is useful for the direct isolation of *B. japonicum* and *B. elkanii* from natural soils and legume inoculants and that this medium may be an important tool for autecological studies. In addition, since BJSM can be used to isolate and quantify bradyrhizobia irrespectively of serological group or resistance to antibiotics or phage, the medium will allow ecological examination of diverse populations of bradyrhizobia at the genus level. Moreover, since plant infection MPN assays are expensive and time consuming and often delay acquisition of results when used in large-scale studies and inoculant quality control programs, the newly described *Bradyrhizobiunum selective medium* could prove to be the method of choice for cheap and relatively rapid enumeration of bradyrhizobia in soils and inoculants.

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**ADDITION IN PROOF**

After this paper was accepted, we learned of another reported medium for the selective isolation of *B. japonicum* from soils (R. R. Gault and E. A. Schwinghamer, Soil Biol. Biochem. 25:1161–1166, 1993).

**REFERENCES**