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Laboratory Prescreening of *Bradyrhizobium japonicum* for Acid pH Tolerance to Predict Their Survival in Acid Soils

ARIEF INDRASUMUNAR[1*] & P. J. DART[2]

[1]*Research Institute for Food Crops Biotechnology, Jalan Tentara Pelajar No. 3A, Bogor 16111*
[2]*School of Land and Food, The University of Queensland, St. Lucia, Australia*

Introduced *Bradyrhizobium japonicum* strains are often unable to tolerate acid soil stress factors in a new environment. There is a need to improve the survival of *B. japonicum* in acid soils. This experiment was conducted to study the selectivity of four agar media in screening acid tolerant strains. Sixteen strains of *B. japonicum* were stab inoculated onto four screening media at five pH levels (3.8, 4.2, 4.5, 5.0, and 6.8) and the growth of each strain was observed and scored everyday. To test the usefulness of this screening method, *B. japonicum* strains were inoculated to two sterile acid soils which differ in chemical properties. The survival of each strain was determined at days 1, 8, 18, and 28 after inoculation. The results showed that the selectivity of each screening medium was different, where the more acid stress factors incorporated to that medium the more selective was that medium. The relationship between screening in acidic agar media and survival capability in sterile acid soils was quite well established, where the strains that survived well or poorly in sterile soils were those that were identified as acid tolerant or acid sensitive strains in acidic agar media. Results from this experiment confirm that laboratory prescreening of *B. japonicum* for acid, Al and Mn tolerance using acidic agar media was successful in selecting strains which were tolerant in low pH soils, and those which were less so.

Key word: laboratory prescreening, *Bradyrhizobium japonicum*, screening media, acid soil

The survival of an introduced effective strain of *Bradyrhizobium japonicum* may be adversely affected by the levels of soil pH and competition for nodulation by naturally occuring (acid tolerant) bradyrhizobia. It is important therefore to be able to select effective and competitive strains that are tolerant of acid soil.

The use of liquid media for pre-selection of rhizobia tolerant of acid soil stresses such as high Al, low P, and low pH had been reported (Date & Halliday 1979, Keyser & Munns 1979). The level of tolerance was measured by the level of turbidity. Changes in medium pH from the metabolism of rhizobia have led to inaccurate classification of acid tolerant rhizobia. Keyser and Munns (1979) attempted to avoid changes of the medium pH by using low inoculum potentials. Richardson and Simpson (1989) showed that supplementation of 5 M P into the medium prevented shifts in pH of the medium cultured with clover rhizobia. Date and Halliday (1979) screened *Stylosanthes* rhizobia and showed that arabinose or galactose when substituted for mannitol prevented the formation of alkali in broth culture. These works were adopted by other researchers (Ayanaba *et al.* 1983, Wood & Cooper 1985, Wood & Shepherd 1987) to prevent rhizobia from raising the medium pH to enhance a strain's growth.

Ayanaba *et al.* (1983) developed an agar plate medium based on Keyser and Munns (1979) basal constituents together with a pH indicator. They characterized isolates of *Bradyrhizobium* and *Rhizobium* sp. into groups of strains tolerant and sensitive to Al and low pH. Gemell *et al.* (1993) modified Ayanaba *et al.* (1983) medium by adding more acidity stress factors into the medium to screen *Rhizobium trifolii*. They lowered the Ca level from 300 µM to 50 µM and increased Mn level from 1.0 µM to 1000 µM. They found that this medium was more selective than Keyser and Munns (1979) and Ayanaba *et al.* (1983) medium in identifying the tolerance of *R. melilotii* to soil acidity related stress.

This experiment examines the hypothesis that effective bradyrhizobia tolerant of soil acidity factors can be selected by screening strains that grow on laboratory media which simulates acid soil conditions. Further, that differences between strains tolerant and sensitive to acidity factors can be demonstrated in the field, thus validating the laboratory method as a means for selecting bradyrhizobia for inoculation trials in acidic soils. The first experiment was conducted to investigate the effect of cation imbalance associated with acid soils on the growth of *B. japonicum*. The second experiment was conducted to test the potential use of screening technique with acidic agar media to measure the survival capability of *B. japonicum* strains in sterile acid soils.

MATERIALS AND METHODS

Screening of Bradyrhizobia in Acidic Agar Media. The growth of 16 bradyrhizobia strains was compared on four different media: (i) Medium of Keyser and Munns (1979); (ii) Modified medium of Date and Halliday (1979); (iii) Medium of Ayanaba *et al.* (1983); and (iv) Medium of Gemell *et al.* (1993). The Keyser and Munns basal medium consists of 300 µM $MgSO_4.7H_2O$, 300 µM $CaCl_2$, 100 µM Fe EDTA, 10 µM KCl, 1 µM $MnCl_2.4H_2O$, 0.4 µM $ZnSO_4.7H_2O$, 0.1 µM $CuCl_2.2H_2O$, 0.02 µM

* Penulis untuk korespondensi; Tel. 62. 251. 337975, Faks. 62. 251. 338820, E-mail: rifcb@indo.net.id

$Na_2MoO_4.2H_2O$, 0.02 µM $Co(NO_3)_2.6H_2O$, 500 µM KH_2PO_4, 500 µM K_2HPO_4, 10 g Mannitol, 1.1 g Na glutamate, 0.005% bromocresol green or bromothymol blue, 15 g agar, and 1 L distilled water. In the modified Date and Haliday medium, concentrations of KH_2PO_4 and K_2HPO_4 were adjusted to 5 and 0 µM and that of Na glutamate to 1.8 g. Arabinose and Galactose were added at 5 g L^{-1}. In the Ayanaba *et al.* medium, $KAl(SO_4)_2$ was added at 50 µM. In the Gemell *et al.* medium, concentration of $CaCl_2$ was adjusted to 50 µM and that of $MnCl_2.4H_2O$ to 1000 µM. Biotin and Thiamin were also added to Gemell et al. medium at 0.1 mg and 1.0 mg. The pH of the medium was adjusted 1 day before use (pH 3.8, 4.2, 4.5, 5.0, and 6.8). Al as $KAl(SO_4)_2$ was filter sterilized (0.22µm) before added to the agar medium held at 50°C. Bromocresol green was used as pH indicator at pH 3.8 to 5.0, while bromothymol blue was used at pH 6.8.

All strains were sub-cultured onto YMA plate and incubated for 7 days at 28°C before stab inoculated 15 times into the screening medium. Growth of bradyrhizobia was observed everyday and measured using a numerical rating of 0 for "no growth", 1 for "sparse", 2 for "good", and 3 for "very good growth" and any change in pH was noted. The inoculum potential of rhizobia applied was determined for two representative strains with different colony types, FCB 152 (raised pinpoint cream/white colony) and FCB 166 (flat watery gummy colony) by counting the number of rhizobia in the YMA at the first, third and 15th stab. A portion of agar (about 1 cm^3) from each inoculation point was immediately excised and homogenized in sterile water, serially diluted and plated (spread plate and drop plate) on YMA and incubated for 7 days at 28°C.

Survival Capability of *B. japonicum* in Acid Soils. Two acid soils (types 1 and 2) with different chemical properties were ground, sieved to pass 2 mm and packed into a polyethylene bag (100g $packet^{-1}$). Soils were then sterilized using γ- irradiation at 5.0 Mrad. Seven day-old broth cultures of each *Bradyrhizobium* strain were diluted to bring the cell density to 10^4 colony forming unit (cfu) mL^{-1}. Twenty-two mL of diluted broth culture was injected into soils 1 and 2 and replicated 2 times. Since soil 2 required more water to reach its field capacity, 10 mL of sterile deionized water was added to this soil. Inoculated soils were then hand mixed and incubated at 28°C and the number of cells was counted at days 1, 8, 18 and 28 after inoculation using drop and spread plate method. The number of cells at day 0 was calculated based on the number of cells inoculated (established by plate counts of the diluted broth). In each counting, 10 g of inoculated soils were taken and serially diluted (10 fold) using ¼ strength Fahraeus media (Fahraeus 1957).

RESULTS

Screening of *Bradyrhizobia* in Acidic Agar Media. Table 1 shows the inoculum potential of FCB 152 (raise

Tabel 1. Number of viable rhizobia (x 10^3) inoculated into acid medium (mean of four replications)

Bradyrhizobium strains	Point of inoculation		
	1st	3rd	15th
FCB 152	106	25	2
FCB 166	153	38	1

pinpoint cream/white colony) and FCB 166 (flat watery gummy colony) at the 1st, 3rd and 15th stabs.

Growth of each strain at a particular pH was recorded as positive if rhizobia grew at or beyond the third stab. Growth at the first two stab points into the agar were not scored as positive because residual cells of rhizobia from the inoculation were often evident in sufficient quantity on the agar to alter the surface pH.

All strains grew well on Keyser and Munns (1979) medium at pH 6.8. Lowering the pH to 5.0, 4.5, and 4.2 reduced the growth of 7 strains, while the growth of 9 strains was not affected. All strains produced alkali when grown at pH 4.2 to 6.8; when the pH was initially very acid (pH 3.8) only 6 strains increased medium pH. Four strains (FCB129, FCB131, FCB26, and FCB152) were found to be very tolerant of low pH in this medium, since their growth was not affected at pH 3.8. Three strains (FCB110, FCB61, and FCB146) were very sensitive to low pH and no growth was found at pH 3.8.

Adding more acid stress factors (low P: 5 µM P) medium (Date & Halliday 1979) reduced the growth score of all strains. Three strains (FCB146, FCB179, and FCB189) were particularly sensitive to low P even at pH 6.8. The combination of low pH and low P made this medium more selective than Keyser and Munns (1979) medium. Reducing the pH to 4.5 eliminated the growth of 4 and to 4.2 of 9 strains; no strains were able to grow at pH 3.8. The use of arabinose or galactose as substitute for mannitol did not prevent pH change of the medium. All strains changed the initial pH of the medium, ten strains produced acid and 6 produced alkali. Seven strains were found tolerant of the combination of low pH and low P. These strains were still able to grow at pH 4.2 and 5 µM P.

Increasing the selection pressure by adding 50 µM Al [Ayanaba *et al.* (1983) medium] reduced the mean growth score of 7 strains, while the growth score of 9 other strains were not affected. The combination of low pH, low P and high Al reduced the growth of 14 strains at pH 4.2. Eleven strains were not able to grow, three grew poorly, while 2 strains (FCB26 and FCB206) were not affected by the combination of acid stress factors at this pH. No strains grew at pH 3.8. Similar to medium Date and Haliday (1979) ten strains produced acid and 6 produced alkali.

Reducing Ca to 50 µM and increasing Mn to 1000 µM [Gemell *et al.* (1993) medium] reduced the mean growth score of 8 strains but did not reduce the growth of the other 8 strains. Strain FCB26 was tolerant of all combinations of acid stress factors tested, its growth was not affected by acid stress factors even at pH 4.2. At pH 4.2 five other

strains grew poorly and the remaining 10 strains were not able to grow. No strain was able to grow at pH 3.8. Four strains (FCB 61, FCB 179, FCB 146 and FCB 189) that were found as acid producer in the Date and Halliday (1979) and Ayanaba (1983) media became alkali producer in Gemell *et al.* (1993) medium.

The average growth score of *B. japonicum* strains in four acid media (Table 2) was used to classify strains into three groups. Strains with growth score 1.0 were classified as "presumptive sensitive", strains with growth score 1.0 to 1.5 were classified as "presumptive moderately tolerant", while those with growth score > 1.5 were classified "presumptive tolerant".

Table 2. Average growth score of 16 strains of *Bradyrhizobium japonicum* across a range of media pH for four acid media types, after incubation for 8 days at 28°C.

Strains	Keyser & Munns (1979)	Date & Halliday (1979)	Ayanaba *et al.* (1983)	Gemell *et al.* (1993)	Average score
Acid sensitive					
FCB 61	1.2	0.8	0.4	0.6	0.8
FCB 179	1.2	0.4	0.8	0.8	0.8
FCB 110	1.2	0.8	0.8	0.4	0.8
FCB 146	1.4	0.8	0.6	0.8	0.9
Moderate tolerant					
FCB 189	1.4	1.2	1.2	0.8	1.2
FCB 166	1.8	1.0	1.2	1.6	1.4
FCB 230	2.0	1.4	1.0	1.6	1.5
Acid tolerant					
CB 1809	2.6	1.8	1.8	2	2.0
CB 2940	2.6	1.8	1.8	1.6	2.0
FCB 44	2.6	2.0	1.8	1.6	2.1
FCB 206	2.6	2.2	2.4	1.8	2.2
FCB 34	2.6	2.4	2.2	1.6	2.3
FCB 152	3.0	2.0	1.8	2.0	2.2
FCB 131	3.0	1.8	2.0	1.6	2.1
FCB 129	3.0	2.2	2.0	1.8	2.3
FCB 26	3.0	2.4	2.4	2.4	2.6

Growth of bradyrhizobia was measured using a numerical rating of: 0= no growth, 1= sparse growth, 2= good growth, 3= very good growth

The poorly growing strains at low pH were common media (Table 3). At pH 4.5 the growth of acid sensitive and moderately tolerant strains were markedly decreased, while the acid tolerant strains were not so affected. Reducing the pH to 4.2 markedly decreased the growth of all strains except the acid tolerant strains in Keyser and Munns (1979) medium. At pH 3.8, *B. japonicum* strains were only able to grow in Keyser and Munns (1979) medium, while in the other 3 media, no single strains were able to grow at this pH.

Table 3. Average growth score of *B. japonicum* across a range of their acid sensitivity group in four media.

Screening medium	Strains	pH					Average Score
		3.8	4.2	4.5	5.0	6.8	
Keyser & Munns (1979)	Acid sensitive	0.3	1.0	1.0	1.3	2.8	1.3
	Moderately tolerant	1.3	2.0	1.3	1.3	2.3	1.6
	Tolerant	1.9	3.0	3.0	3.0	3.0	2.8
Modified Date & Halliday (1979)	Acid sensitive	0.0	0.0	0.0	1.3	2.3	0.7
	Moderately tolerant	0.0	0.0	1.3	2.0	2.7	1.2
	Tolerant	0.0	1.6	2.9	3.0	3.0	2.1
Ayanaba *et al.* (1983)	Acid sensitive	0.0	0.0	0.3	1.3	1.8	0.7
	Moderately tolerant	0.0	0.0	1.0	2.0	2.7	1.1
	Tolerant	0.0	1.1	3.0	2.0	3.0	2.0
Gemell *et al.* (1993)	Acid sensitive	0.0	0.0	0.3	1.0	2.0	0.7
	Moderately tolerant	0.0	0.7	1.3	2.0	2.7	1.3
	Tolerant	0.0	0.8	2.3	2.9	3.0	1.8

* Growth of bradyrhizobia was measured using a numerical rating of: 0 for no growth; 1 for sparse growth; 2 for good growth; 3 for very good growth

Figure 1 shows that low P was the most important acid stress factor affecting the growth of *Bradyrhizobium* strains tested in this experiment. Compared to Keyser and Munns (1979) medium, low P in the medium of Date and Halliday (1979) reduced the average growth score of all strains tested by 0.64 (from 2.20 to 1.56). The addition of Al into Ayanaba *et al.* (1983) medium reduced the average growth score by only 0.05 (from 1.56 to 1.51). Adding more acid stress factors (low Ca and high Mn) in Gemell *et al.* (1993) medium caused further reduction in average growth score by 0.08 (from 1.51 to 1.48).

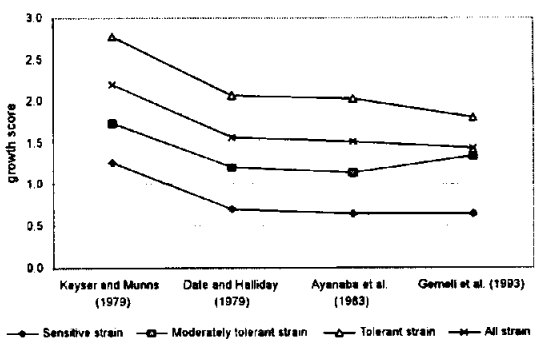

Figure 1. Growth score of bradyrhizobia strains in four acid media
* All strains is the average of growth score across all *Bradyrhizobium japonicum* strains

Correlation coefficients were used to indicate the strength of the relationships between each screening medium. There were significant linear relationships between each screening medium. Correlation among the first three

screening media (Keyser & Munns 1979, Date & Halliday 1979, Ayanaba *et al.* 1983) was high with r^2 more than 0.90. The correlation between Gemell *et al.* (1993) medium with the other media was also high with r^2 ranging from 0.85 to 0.89. The lower correlation with the Gemell *et al.* (1993) screening medium might be caused by the increased growth score of 2 moderately tolerant strains in this medium compared to Date and Halliday (1979) and Ayanaba *et al.* (1983) media. Some rhizobia strains need vitamins for their growth (Somasegaran & Hoben 1994). The addition of biotin and thiamin in this medium might have increased the growth of those 2 strains.

Survival Capability of *B. japonicum* in Acid Soils. *Bradyrhizobium* strains were inoculated into acid soils at similar numbers. The calculated number (Log $_{10}$) of bradyrhizobia cells at inoculation time (established by plate counts of the diluted broth) were between 4.74 to 4.99 in soil 1 and 4.03 to 4.58 in soil 2. Bradyrhizobia numbers in soil 2 were initially significantly smaller than in soil 1, because soil 2 need more water addition than soil 1 to reach its field capacity.

At day 1, the population of all strains in both soils was reduced, less in soil 2 than in soil 1. The viable cell number of all strains decreased in soil 1. By contrast, in soil 2, 11 strains maintained their original populations while the other 5 strains decreased. The cell number of acid sensitive strain FCB 61 decreased from 4.90 to 3.20 in soil 1 and from 4.24 to 3.42 in soil 2. Cells of the other acid sensitive strain FCB 110 also died, but less markedly than for FCB 61. The populations of two moderately tolerant strains FCB 166 and FCB 230 and two tolerant strains FCB 206 and FCB 152 also decreased in both soils.

At day 8, there was a further decrease in viable number of all strains in both soils except for acid tolerant strain FCB 26. In both soils, the survival of 4 acid sensitive strains (FCB 61, FCB 179, FCB 110 and FCB 146) and 1 moderately tolerant strain (FCB 230) decreased significantly to less than 100 cells g^{-1} soil, while the other strains were still able to maintain their viable populations around 10^3 cells g^{-1} soil. Interestingly, three acid tolerant strains FCB 129, FCB 131 and FCB 26 were found to be tolerant of both acid soils. They maintained their viable number around 10^4 cells g^{-1} soil.

At day 18, only 2 acid tolerant strains FCB 34 and FCB 26 were able to survive in both acid soils, while the other strains were undetectable. Strain FCB 34 only survived in soil 2 with around 10^3 cells g^{-1}, while FCB 26 still survived well in both soils. In soil 1, the viable number of FCB 26 decreased from 10^4 at day 8 to less than 100 cells g^{-1} soil. By contrast, in soil 2, this strain was able to maintain its viable number at more than 10^4 cells g^{-1}. At 28 days after inoculation, only strain FCB 26 was tolerant of acid soil stress factors. This strain still maintained a viable number more than 100 cells g^{1} soil in soil 1, while in soil 2 its viable number even increased from $\log_{10}$ 4.25 at day 18 to 4.52.

Figure 2 shows that the survival of acid tolerant strain was greater than that of moderately tolerant and acid sensitive strains. Therefore, there was a quite good correlation between screening in acidic agar medium and in acid soils (Figure 3). Thus, it appears that the strains more tolerant of acidity and Al in culture are also better able to survive in sterile acid soils.

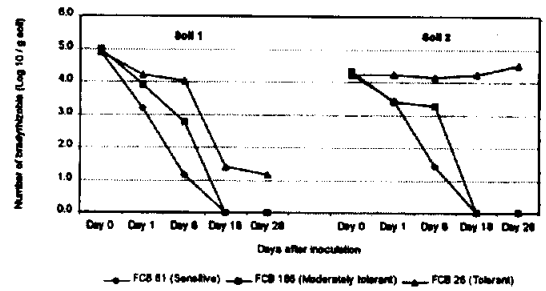

Figure 2. Changes in population of *Bradyrhizobium* strains in acid soils 1 and 3.

DISCUSSION

Screening of Bradyrhizobia in Acidic Agar Media. The simplicity of the stab inoculation technique makes it possible to study the growth of numerous strains over a range of low inoculum potentials on agar (Gemell 1991). This is more practical than using liquid medium (Keyser & Munns 1979, Bromfield & Jones 1980, Wood & Sheperd 1987) or preparing liquid medium cultures prior to screening for growth on acidified agar medium (Ayanaba *et al.* 1983).

The number of viable bradyrhizobia inoculated into acid medium were 10^5, 10^4 and 10^3 at the first, third and 15th stab respectively. The importance of low inoculum potential in assessing the acid tolerance of strains has been reported by Keyser and Munns (1979) and Wood and Cooper (1988a). Wood and Cooper for example showed two strains (one acid sensitive, the other acid tolerant) grow at the same rate at pH 5.5, but at pH 4.5 the acid sensitive strain grew only with an inoculum potential of 10^3 or more cells.

All 16 strains which grew at pH 4.2 to 6.8 on Keyser and Munns (1979) medium were alkali producers. At pH 3.8, 13 strains were able to grow with 6 strains increasing the medium pH, and 7 strains did not alter the pH. The excess of alkali production by the growth of bradyrhizobia may exceeded the buffering capacity of the medium, and resulted in the pH shifts. Rhicardson and Simpson (1989) found shifts from the initial pH of a medium based on that of Keyser and Munns (1979) but only after substantial growth of rhizobia had occurred or at high concentrations of P (100 and 1000 μM P) which was associated with increased numbers of rhizobia. High P in this medium may have contributed to increased cell growth and the utilization of more mannitol resulting in the pH change.

Substituting arabinose and galactose for mannitol, but including other mineral constraints in the other three media, did not prevent the pH change associated with the growth

of bradyrhizobia at an inoculum potential of 1.04 x 10^3 to 37.5 x 10^3 cfu at the point of inoculation. This finding differs from those of Date and Halliday (1979) for *Stylosanthes* rhizobia and Gemell *et al.* (1993) for *R. leguminosarum* bv. *trifolii*. Using an inoculum density of 10^3-10^4 cfu mL^{-1} (Date & Halliday 1979) and 10^4-10^5 cfu mL^{-1} (Gemell *et al.* 1993), no strain tested altered the pH of the medium which included arabinose and galactose at low pH. However, Ayanaba *et al.* (1983) and Wood and Cooper (1988b) found contrary results in liquid medium containing arabinose and galactose with an increase in pH when the cell density reach 10^6-10^7 cfu mL^{-1} from the inoculum level of 10^2-10^5 cfu mL^{-1}. Wood and Cooper suggested that when population of rhizobia in liquid medium exceeds approximately 10^7 cfu mL^{-1}, excess acid or alkali production exceeds the buffering capacity of the medium. The sodium glutamate used in these media served not only as a pH buffer, but also as an N source for the cells, and possibly also a carbon and energy source (Wood & Cooper 1988b). Therefore, as the cells grow they reduced the pH buffering capacity of the medium and increased the input of acid or alkali, and when the population density reached 10^7 cfu mL^{-1} the buffering capacity was exceeded and the pH value changed.

All four strains which were classified as acid sensitive, 2 moderately tolerant strains and 4 acid tolerant strains were acid producers in Date and Halliday (1979) and Ayanaba *et al.* (1983) media, while one moderately tolerant and five acid tolerant strains were alkali producers. The 4 acid sensitive strains which were acid producers in Date and Halliday (1979) and Ayanaba *et al.* (1983) media became alkali producers in Gemell *et al.* (1993) medium. These results suggest that there was no correlation between acid or alkali production by bradyrhizobia and their tolerance of acidity. Wood and Cooper (1988b) screened *R. trifolii* in liquid medium containing glutamate with combinations of galactose or arabinose and found that pH changes depended on the source of sugar. By contrast Cooper (1982) who tested 27 acid and alkali producing *Lotus* strains for growth in broth at pH 4.6 found that alkali producers were sensitive to acidity while the acid producers were tolerant. Therefore, there does not appear to be any consistent relationship between tolerance of acidity and alkali production by rhizobia.

Comparison among four screening medium shows that the more acid stress factors incorporated into screening medium the more discriminating was that medium (Figure 1). Adding the acid stress factors of low P and added Al into the medium (Date & Halliday 1979, Ayanaba *et al.* 1983) reduced the growth of all strains. The growth score of acid sensitive and tolerant but not moderately tolerant strains decreased further following addition of more acid stress factors (low Ca and high Mn) in the Gemell *et al.* (1993) medium.

Survival Capability of *B. japonicum* in Acid Soils. The development of laboratory procedures for testing the behavior of rhizobia strains in soil would be a useful screen. The results presented here indicate that survival of *B. japonicum* strains in two acid soils can be predicted from their ability to grow in defined, acidified agar medium. Thus, the strains that survived well in sterilized soils of pH 4.24 and 4.35 were those that were identified as acid tolerant in acidified agar medium. Figure 3 shows that. *Bradyrhizobium* strains that were identified as acid tolerant in acidified medium had significantly greater survival in acid soils than the acid sensitive strains. In addition, the most acid tolerant (FCB 26) and the most acid sensitive (FCB 61) strains in acidified media were also found to be the most tolerant and the most sensitive strains in acid soils.

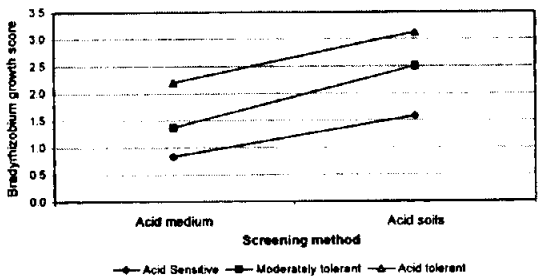

Figure 3. Relationship between screening in acidified agar medium and inacid soil.

Norris (1965) suggested that the survival of rhizobia in acid soils is inversely related to the amount of acid produced, i.e. those rhizobia that produce the least acid should survive best in acid soils. However, acid production depends on the substrate available for slow growing rhizobia which are normally considered to increase the pH of the medium (Tan & Broughton 1981). In the present experiment, the overall pH of both soils increased from an initial pH of 4.24 to 4.46 in soil 1 and from 4.35 to 4.43 in soil 2. This increase did not help the survival of most strains because this pH is still very acid (< 4.5). In Keyser and Munns (1979) medium all strains were still able to grow at pH 4.2, but in other media when more acid soil stress factors were applied, only some acid tolerant strains were able to grow at a pH less than 4.5. The combination of low pH, low Ca and high Al and Mn might have caused the low survival of most strains in both soils. In addition, the multiplication and survival of rhizobia can also be affected by microhabitats in soils. These can have a higher or lower pH than overall soil pH. The lower survival of *Bradyrhizobium* strains in soil 1 than in soil 2 might be caused by the lower pH, Ca and Mg contents of soil 1 than soil 2.

This experiment confirm that laboratory prescreening of *Bradyrhizobium* for acid, Al and Mn tolerance in acidic agar media was successful in selecting strains which were tolerant in low pH soils, and those which were less so.

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