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Selecting *Rhizobium phaseoli* strains for use with beans (*Phaseolus vulgaris* L.) in Kenya: Tolerance of high temperature and antibiotic resistance

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Abstract

Forty one strains of *Rhizobium phaseoli* were screened for the ability to multiply at high temperatures on yeast extract-mannitol agar. Most strains were tolerant of 30°C, eight strains were tolerant of 45°C and two of 47°C although the rate of multiplication was reduced at 45–47°C. The high temperature-tolerant strains were isolated from Kenyan soils and were fast-growing. Seven of the eight strains tolerant of 45–47°C lost their infectiveness after incubation at high temperature but four strains tolerant of 40°C remained infective after incubation at that temperature.

Thirty six strains were resistant to 200 $\mu g\,ml^{-1}$ streptomycin sulphate and 29 strains to 200 $\mu g\,ml^{-1}$ spectinomycin dihydrochloride. Eight strains were resistant to both antibiotics each at 200 $\mu g\,ml^{-1}$. Two of the double-labelled antibiotic-resistant mutants lost their infectiveness on *Phaseolus vulgaris*. The response to acidity was unaltered and two of the mutants showed a decrease in temperature tolerance. The double-labelled mutants were recoverable from two Kenyan soils.

Introduction

The ideal Rhizobium inoculant strain is one which is able to survive well in soil, multiply and colonise the roots readily, compete with other rhizosphere microorganisms and initiate nitrogen fixation (Burton, 1976). Lack of knowledge of the environmental factors affecting the ecology of rhizobia introduced into soil is reflected in the frequent failure of inoculated legumes to develop active N_2-fixing symbioses.

A major environmental factor controlling the survival and establishment of rhizobia both in culture and in soil is high temperature. Growth responses by rhizobia to temperature are complicated in the legume rhizosphere due to the influence of host species and cultivars within species (Bowen and Kennedy, 1959; Day *et al.*, 1978; Gibson, 1967). High root temperatures may affect formation of root hairs, binding of rhizobia to the root hairs, formation of leghaemoglobin, nodule development and structure, nitrogen fixation and dry matter production (Munevar and Wollum, 1981). Thus plants which are dependent on symbiotically fixed nitrogen are more sensitive to elevated temperatures when compared with similar plants supplied with inorganic nitrogen. Therefore, the potential benefit of the legume-Rhizobium association may not be fully exploited when soil temperatures become limiting.

In the tropics and subtropics soil temperatures may reach 40–60°C (Alexander, 1982; Chatel and Parker, 1973; Somasegaran *et al.*, 1984), and inoculants may be exposed to ambient temperatures ranging from 20–50°C for varying lengths of time (Ayanaba, 1977). Temperatures above 48°C in the upper centimeters of a soil profile are common and prolonged periods at 38°C in at least 4 cm of the soil before canopy closure of soyabean have been reported in Northern Carolina, USA (Munevar and Wollum, 1981). Survival of *R. leguminosarum* in hot dry conditions in certain soils was observed

as early as 1927 as quoted by Parker *et al.* (1977). Similarly in Kenya nodulation of dry beans, cowpeas and pigeon peas has been observed in areas where soils have been exposed to prolonged dry periods indicating that the indigenous Rhizobium species in those soils are tolerant of high temperatures.

The lethal effects of moist heat on the survival in the soil of the strains of Rhizobium from pea, clover, lucerne and tropical legumes subjected to 40°C has been demonstrated, as well as the survival of rhizobia applied on the seed and planted in moist soil at 40°C (Bowen and Kennedy, 1959; Somasegaran *et al.*, 1984). Survival at elevated temperatures was dependent on the strain of Rhizobium, initial concentration of inoculum and period of exposure to high temperature. Although dry hot temperatures may not be deterimental to the rhizobia, problems could be experienced with the survival of rhizobia following pre-inoculation of the seed (Burton, 1976). Selecting for temperature tolerant strains of rhizobia for tropical and subtropical regions is therefore necessary before establishing inoculant production for these regions.

Antibiotic resistance is a useful marker for monitoring the performance of Rhizobium inoculants in soil (Obaton, 1973). Strains should be resistant to concentrations of antibiotics that inhibit growth of other soil bacteria on agar media and they should also retain the qualities of the parent strain (Alexander, 1982). Two potential problems with this technique are loss of symbiotic effectiveness (Schwinghamer, 1967) and loss of antibiotic resistance (Brockwell *et al.*, 1974). Resistance to antibiotics which inhibit protein synthesis for example, streptomycin and spectinomycin has little effect on symbiotic effectiveness (Pankhurst, 1977; Schwinghamer, 1967), whereas resistance to antibiotics which inhibit synthesis of cell wall and nucleic acid may affect effectiveness (Levin and Montgomery, 1974; Pankhurst, 1977; Schwinghamer, 1967). Schwinghamer and Dudman (1973) suggested using two antibiotic markers including spectinomycin which has a similar chemical structure and mode of action to streptomycin. As a further precaution marked strains should be fully characterized before use to check for alterations in important characteristics associated with spontaneous mutation for antibiotic resistance.

The aims of this study were (a) to screen *Rhizobium phaseoli* strains for tolerance to high temperature and to determine the effect of this stress on the symbiotic ability of the bacteria, (b) to identify symbiotically competent antibiotic-resistant mutants of *R. phaseoli* which would be used for ecological studies in the field.

Materials and methods

Rhizobium cultures

The *Rhizobium phaseoli* strains used in this study (Table 1) were obtained from MIRCEN, Department of Soil Science, University of Nairobi, Kenya. RCR3605 was obtained from Rothamsted Experimental Station, Harpenden, UK. The rhizobia were stored at 4°C on yeast extract mannitol (YEM) agar with the following composition ($g l^{-1}$) 0.5 K_2HPO_4, 0.2 $MgSO_4 \cdot 7H_2O$, 0.1 NaCl, 3.0 $CaCO_3$, 0.01 $FeCl_3 \cdot 6H_2O$, 10 mannitol, 0.4 yeast extract, 10 agar, distilled water.

Antibiotics

Stock solutions of streptomycin sulphate and spectinomycin dihydrochloride (Sigma Chemical Company Ltd, Poole, Dorset, UK) were prepared in distilled water, sterilised by passing through a 0.2 μm membrane filter and stored at 4°C.

Temperature tolerance

Rhizobium strains were subcultured from YEM agar slants in 50 ml YEM broth contained in 100 ml conical flasks incubated on an orbital shaker at 75 rpm at 25°C for 3 to 4 days. Each strain was then streaked on YEM agar plates in duplicate for each temperature treatment. The plates were incubated at 25, 30, 35, 40, 45 and 47°C for a maximum of 10 days and checked daily for colony development. Single colonies were picked from each plate incubated at high temperature (40, 45 and 47°C) and subcultured in YEM broth for 4 days. These isolates were transferred to YEM agar slants and stored at 4°C, then used for plant infection tests.

Table 1. Time taken (days) for colonies > 2 mm diameter to develop on YEMA for *R. phaseoli* strains at different temperatures. (means of two observations)

Parent Strain No.	Incubation temperature (°C)					
	25	30	35	40	45	47
1A	1.5	2	2	2	S	S
1C	1.5	2	2	2	S	S
2A	1.5	2	2	2	6	S
3D	1.5	2	2	2	S	S
4D	1.5	2	2	2	S	S
5E	1.5	2	2	2	S	S
7A	1.5	2	2	2	S	S
7C	1.5	2	2	3	S	S
8D	4	3	3	S	S	S
9C	1.5	2	2	2	S	S
10F	3	3	3	S	S	S
11B	4	3	3	S	S	S
13C	1.5	1	1	1	6	6
17	1.5	1	2	2	6	S
18A	1	1	1	1	6	6
19A	4	3	3	3	6	S
21C	4	S	S	S	S	S
23	4	3	3	3	6	S
29B	1	1	1	1	6	S
33A	4	3	S	S	S	S
98	4	3	3	S	S	S
107E	4	3	3	S	S	S
110	4	3	S	S	S	S
113	3.5	3	S	S	S	S
115	4	3	S	S	S	S
116	4	3	S	S	S	S
118	4	3	S	S	S	S
119	4	3	S	S	S	S
301	4	3	S	S	S	S
406	2.5	3	3	S	S	S
412	4	3	S	S	S	S
420	2.5	S	S	S	S	S
428	1.5	3	3	5	5	5
436	1.5	3	3	5	S	S
440	4	3	S	S	S	S
441	4	3	S	S	S	S
445	2	3	3	5	S	S
446	4	3	S	S	S	S
447	4	3	S	S	S	S
448	1.5	3	3	5	S	S
RCR3605	4	3	S	S	S	S

S denotes no colony development on duplicate plates within 14 days

Isolation of double-labelled antibiotic-resistant mutants

Rhizobium phaseoli strains were inoculated onto YEM agar plates containing 200 $\mu g\,ml^{-1}$ spectinomycin dihydrochloride in duplicate and incubated at 25°C for a period of 3 to 14 days. Selected single colonies were subcultured in YEM broth plus 200 $\mu g\,ml^{-1}$ spectinomycin held in 100 ml conical flask and incubated at 25°C in an orbital shaker until turbidity developed. A loopful of the culture was streaked onto YEM agar plates containing 200 $\mu g\,ml^{-1}$ streptomycin in duplicate. The plates were incubated at 25°C for a maximum of 14 days and again colony development was checked daily. Selected colonies that developed on the streptomycin plates were assumed to be resistant to both antibiotics as *spcstr* mutants. These isolates were subcultured in YEM broth containing both spectinomycin and streptomycin at 200 $\mu g\,ml^{-1}$ in 100 ml conical flasks, and when turbidity developed the isolates were transferred onto YEM agar containing both spectimomycin and streptomycin at 200 $\mu g\,ml^{-1}$ and non-antibiotic YEM agar slants and stored at 4°C.

Temperature tolerance of antibiotic resistant mutants

Antibiotic-resistant mutants and parent strains were checked for temperature tolerance as described earlier.

Acid tolerance of antibiotic resistant mutants

A modified liquid medium was used for screening antibiotic-resistant mutants of *R. phaseoli* for tolerance to low pH. The composition of the medium was (μM); 100 $CaCl_2 \cdot 6H_2O$, 500 $MgSO_4 \cdot 7H_2O$, 50 KCl, 25 FeEDTA, 10 KH_2PO_4, 10 H_3BO_3, 1 $MnSO_4 \cdot 4H_2O$, 0.5 $ZnSO_4 \cdot 7H_2O$, 0.1 $CuSO_4 \cdot 5H_2O$, 0.025 $NaMoO_4 \cdot 2H_2O$ and 0.005 $CoCl_2 \cdot 6H_2O$. After autoclaving sodium glutamate (1.8 $g\,l^{-1}$), arabinose (0.3 $g\,l^{-1}$), galactose (0.3 $g\,l^{-1}$), thiamine-HCl (100 $\mu g\,l^{-1}$) and biotin (250 $\mu g\,l^{-1}$) were added as filter-sterile solutions and pH was adjusted using HCl (SG 1.18) or filter-sterile 10% KOH. The medium was dispensed in 7 ml aliquots into plastic capped tubes. Eight *spcstr* mutants together with the eight parent strains were screened for tolerance of pH 4 and 6 in defined liquid medium as described previously (Karanja and Wood, 1988).

Recovery of the antibiotic-resistant mutants

This experiment was conducted in two soils collected from the Kenya Agricultural Research Station, Muguga and from the National Dryland Farming Research Station, Katumani, Kenya. The soils from these sites have been described as Humic Nitisol and (ferral) Chromic Luvisol respectively according to FAO/UNESCO 1974 (Siderius and Muchena, 1977). The pH values of the soils (measured in water) were 5.7 and 5.1 respectively. 1 ml of cell suspension containing 10^6 cfu ml^{-1} was added to 10 g of moist soil which was held in screw capped bottled which were loosely covered and were maintained for 5 days at 20°C. The population of each antibiotic mutant was enumerated by plating soil dilutions on YEM agar plates containing 200 $\mu g\, ml^{-1}$ of spectinomycin, streptomycin and cycloheximide (actidione) following the method of Miles and Misra (1938). The viable cell density in the inoculum was measured using YEM agar and YEM agar plus antibiotics.

Plant infection tests

Two groups of Rhizobium were tested in this experiment. Firstly the strains which were tolerant of high temperatures ranging from 40–47°C (428, 19A, 13C 18A, 115E, 23, 5E, 2A, 445, 406, 436, 29B and 9C). Secondly the double-labelled antibiotic-resistant mutants, $5E^{spcstr}$, $7C^{spcstr}$, $18A^{spcstr}$, 447^{spcstr}, 436^{spcstr}, $9C^{spcstr}$ and $3D^{spcstr}$ and $RCR3605^{spcstr}$. Seeds of *Phaseolus vulgaris* L. cv. Canadian Wonder (GLP-24) were surface sterilized by immersion in acidified 0.2% $HgCl_2$ solution for 5 minutes, rinsed 6–7 times with sterile distilled water, germinated in sterile petri dishes containing moistened filter papers and transferred into 200 × 75 mm boiling tubes containing 200 ml of sterile N-free nutrient solution. There were two seedlings per tube and the composition of the plant nutrient solution was as follows (μM): 1000 $CaCl_2 \cdot 2H_2O$, 100 KCl, 800 $MgSO_4 \cdot 7H_2O$, 10 FeEDTA, 35 H_3BO_3, 9 $MnCl_2 \cdot 4H_2O$, 0.8 $ZnCl_2$, 0.5 $NaMoO_4 \cdot 2H_2O$, 0.3 $CuSO_4 \cdot 5H_2O$, 7.2 mM KH_2PO_4 and 7.2 mM $NaHPO_4$. The tubers were inoculated with each strain at 10^3 cfu ml^{-1} in triplicate and held in blackened boxes with shoots exposed to the atmosphere in a growth room at 20–22°C and illuminated at 300 $\mu E\, m^{-2} s^{-1}$ for a 16 h day, 8 h night. After 14 days the presence or absence of nodules on the roots was recorded. Uninoculated controls were included.

Results and discussion

Temperature tolerance and infectiveness

The optimum temperature for a majority of *Rhizobium phaseoli* strains was 25–30°C except strains 21C and 420 which were sensitive to 30°C (Table 1). This group of Rhizobium like other fast-growing strains developed colonies > 2 mm in diameter on YEM agar plates within 2–5 days at the optimum temperatures. Those strains that were isolated from soils found in the hot dry regions of Kenya tolerated higher temperatures (> 30°C) than isolates from the cooler regions. A complete description of the origins of isolates 1A to 119 was given previously (Karanja and Wood, 1988). The origins of 301 to 448 are not known. Isolates 33A and 110–119 which were from soils found in the cool humid areas were sensitive to temperatures higher than 30°C while isolates 1A–11B, 98 and 107, which were from the hot dry regions where surface soils could possibly rise to > 40°C, could tolerate temperatures > 35°. Isolates 2A, 17C, 19A, 23 and 29B tolerated 45°C, and 13C and 18A, were tolerant of 47°C. Apart from strain 23 the temperature-tolerant strains were fast-growing, developing colonies > 2 mm in diameter within 48 h at optimum temperatures with a gummy or watery appearance. It is not known whether the high multiplication rate or the amount of polysaccharides produced were an advantage to the tolerant strains.

Surface soil temperatures in the tropics may be greater than 40°C (Alexander, 1982; Somasegaran *et al.*, 1984) and the fact that nodulation does occur on the roots of uninoculated bean plants sown in those soils suggests that the indigenous *R. phaseoli* populations are able to withstand high temperatures. In this study a majority of the Kenyan isolates grew normally at 35°C and over 50% could tolerate 40°C. Hence, 40°C was chosen as the value above which rhizobia were considered as temperature tolerant. Total loss in rhizobia viability was observed in chickpea, lentil and bean in-

oculants when they were exposed to ambient temperatures of 44°C (Somasegaran *et al.*, 1984) but these *R. phaseoli* isolates survived and multiplied on YEM agar plates at 45–47°C. A *Rhizobium japonicum* strain has also been reported which survived in liquid culture at 48.7°C (Munevar and Wollum, 1981).

All but one of these strains that showed tolerance to high temperatures (45–47°C) lost the ability to nodulate the host legume (Table 2). Strain 2A isolated at 45°C formed very small nodules on the roots of the host plant 14 days after inoculation. No examination of the effectiveness of this strain was made. There was no loss of infectiveness in those strains that were incubated at 40°C; they formed nodules on the host plant after 9–10 days. Non-nodulating mutants of *R. trifolii* have been found in cultures incubated at 35°C for 7 days, and the frequency of those Nod$^-$ mutants varied from 1–75% depending on the strain tested (Zurkowski and Lorkiewicz, 1978). The loss in infectiveness in those *R. phaseoli* strains that grew at 45–47°C may have been due to plasmid-curing caused by the high temperatures.

The lethal temperature for Rhizobium depends on the strain, temperature and duration of exposure. Thus a strain of 'cowpea' rhizobia which grew well at 44°C on YEM agar plates retained effectiveness and performed better than a temperature sensitive strain when cowpea roots were exposed to 38°C (Eaglesham *et al.*, 1981). A positive correlation between performance of *R. japonicum* strains as symbionts on soyabean plants at high temperatures and the response of strains in pure culture has been observed (Munevar and Wollum, 1981). This suggests that pure culture evaluation could be used as a preliminary screening procedure to identify strains which are tolerant to high temperatures for inoculant production in the tropics. Desiccation and high temperatures occur simultaneously in tropical regions and the interactions between these factors on survival of Rhizobium and symbiotic performance require investigation.

Table 2. Infectiveness on *Phaseolus vulgaris* cv. GLP-24 of *R. phaseoli* strains after incubation on YEMA plates at 40–47°C for 3–7 days (+ nodules formed and − nodules not formed within 14 days after inoculaton on six seedlings)

Parent Strain No.	Temperature (°C) at which isolates were recovered	Infectiveness
9C	40	+
406	40	+
436	40	+
445	40	+
2A	45	+
5E	45	−
19A	45	−
23	45	−
115	45	−
428	45	−
18A	47	−
13C	47	−

Properties of antibiotic-resistant mutants

When 200 μg ml^{-1} streptomycin sulfate was added to YEM agar all but 5 of the 41 *Rhizobium phaseoli* strains developed colonies (data not presented) and so spectinomycin which was more selective was chosen for initial marking of the strains. An addition of 200 μg ml^{-1} of spectinomycin resulted in a loss of 12 sensitive strains. Except for isolates 21C, 107E, 118, 119 and 301 which were tolerant to spectinomycin only, the remaining spectinomycin mutants were also resistant to 200 μg ml^{-1} streptomycin (Table 3). The double antibiotic-resistant mutants, those which grew in presence of both spectinomycin and streptomycin at a concentration of 200 μg ml^{-1}, were 3D, 7A, 7C, 9C, 29B, 447, 436 and RCR3605. Isolates 5E and 18A were tolerant to 100 μg ml^{-1} only of both spectinomycin and streptomycin. The presence of antibiotics in the medium resulted in reduction of the multiplication rate as compared to the growth rate on non-antibiotic YEM agar (Table 3).

For an antibiotic-resistant mutant to be of use in competitiveness and persistence studies in the plant rhizosphere it should retain the properties of the parent strain particularly the symbiotic ability with the homologous host plant. From Table 4, two antibiotic-resistant mutants 3D^{spcstr} and 18A^{spcstr} lost their infectiveness as no nodules developed on the roots of the seedlings inoculated with the two isolates after 14 days. The other mutants retained their infectiveness. A loss of 20% in infectiveness has been observed in spectinomycin-resistant mutants (Schwinghamer and Dudman, 1973) but little or no loss in effectiveness in streptomycin mutants (Schwinghamer, 1967). No tests on effectiveness of

Table 3. Time taken (days) for colonies > 2 mm diameter of *R phaseoli* strains to form on YEM agar plates containing 200 $\mu g\,ml^{-1}$ of spectinomycin (spc) and streptomycin (str) and on non-antibiotic YEM agar plates (means of two observations)

Parent Strain No.	Colony formation in days on YEM agar plates containing			
	spc	str	spc + str	none
1A	S	ND	ND	2
1C	S	ND	ND	2
2A	S	ND	ND	2
3D	7	4	4	2
4D	S	ND	ND	2
5E[a]	5	4	4	2
7A	7	2	2	2
7C	7	5	4	2
8D	9	4	S	3
9C	5	5	4	2
10F	S	ND	ND	3
11B	S	ND	ND	3
13C	S	ND	ND	1
17C	5	4	S	1
18A[a]	5	4	S	1
19A	S	ND	ND	4
21C	7	S	ND	4
23	5	4	ND	3
29B	5	4	4	1
33A	9	S	ND	3
98	S	ND	ND	3
107E	7	S	ND	4
110	S	ND	ND	4
113	S	ND	ND	4
115	5	S	ND	4
116	S	ND	ND	4
118	7	S	ND	4
119	12	S	ND	4
301	7	S	ND	4
406	7	S	ND	3
412	7	S	ND	4
420	9	S	ND	3
428	5	S	ND	2
436	7	5	4	2
440	8	S	ND	4
441	8	S	ND	4
445	12	S	ND	4
446	8	S	ND	4
447	8	5	5	4
448	7	S	ND	2
RCR3605	7	5	5	4

ND not determined. S no colonies formed within 14 days.

[a] mutant resistant to 100 $\mu g\,ml^{-1}$ of both spectinomycin plus streptomycin.

the double antibiotic-resistant mutants were carried out in this study. None of the Rhizobium strains (both parent and the antibiotic mutants) produced visible turbidity at pH 4.0 (Table 4) but strain 9C, 18A and the Rothamsted strain RCR3605 survived in the acid liquid medium for 14 days at 25°C (data not shown). This was also true for mutants $9C^{spcstr}$, $18A^{spcstr}$ and $RCR3605^{spcstr}$. Response to elevated temperatures by the antibiotic-resistant mutants was similar to that of the

Table 4. Infectiveness on *Phaseolus vulgaris* L. cv. GLP-24 of wild type and antibiotic-resistant mutant strains of *R. phaseoli* resistant to 200 $\mu g\,ml^{-1}$ spectinomycin and 200 $\mu g\,ml^{-1}$ of streptomycin (+ nodules formed, − nodules not formed), time taken for visible turbidity in liquid medium at pH 4 and 6 and maximum temperature for colony formation on YEM agar plates (means of two observations).

Parent strain and antibiotic resistant mutants	Infectiveness	Visible turbidity (days)		Maximum Temperature (°C)
		pH4	pH6	
3D	+	NT	3	40
$3D^{spcstr}$	−	NT	4	40
5E	+	NT	2	45
$5E^{spcstra}$	+	NT	4	45
7C	+	NT	3	35
$7C^{spcstr}$	+	NT	3	35
9C	+	NT	3	40
$9C^{spcstr}$	+	NT	3	35
18A	+	NT	2	47
$18A^{spcstra}$	−	NT	3	47
436	+	NT	3	40
436^{spcstr}	+	NT	3	35
447	+	NT	3	35
447^{spcstr}	+	NT	3	35
RCR3605	+	NT	3	35
$RCR3605^{spcstr}$	+	NT	3	35

NT denote no turbidity was observed after 14 days.
[a] the doubled mutants were only resistant to 100 $\mu g\,ml^{-1}$ spectinomycin and 100 $\mu g\,ml^{-1}$ streptomcin.

Table 5. Recovery of strains of *R. phaseoli* resistant to 200 $\mu g\,ml^{-1}$ spectinomycin and 200 $\mu g\,ml^{-1}$ streptomycin from two Kenyan soils and viable counts of the mutant strain on YEMA and YEMA plus 200 $\mu g\,ml^{-1}$ of both spectinomycin and streptomycin (means of two observations)

Strain No.	Initial broth count (cfu ml^{-1})		Recovery of the antibiotic mutants (cfu g^{-1}) from			
	YEMA	YEMA + spc + str	Katumani Soil		Muguga Soil	
			Day 0	Day 5	Day 0	Day 5
$3D^{spstr}$	3×10^5	3×10^5	5×10^3	3×10^3	5×10^3	2×10^4
$7C^{spcstr}$	5×10^6	5×10^6	1×10^3	4×10^4	3×10^3	2×10^4
$9C^{spcstr}$	5×10^6	5×10^6	3×10^3	3×10^4	4×10^3	4×10^4
436^{spcstr}	2×10^6	3×10^6	3×10^3	5×10^3	3×10^3	3×10^4
447^{spcstr}	7×10^5	8×10^5	5×10^3	4×10^4	5×10^3	5×10^2
$RCR3605^{spcstr}$	3×10^6	3×10^6	2×10^3	1×10^4	3×10^3	2×10^4

parent strains except for strains 9C and 436 whose antibiotic-resistant mutants were more temperature sensitive.

Recovery of the double antibiotic-resistant mutants from the soil

The inoculum count on YEM agar and YEM agar plus antibiotics showed no differences in populations of the mutants with respect to the type of medium used (Table 5) therefore the counts on the antibiotic medium gave an accurate estimate of the population. All six mutants were equally recoverable from the two soils. A population increase of tenfold occurred within five days of incubation but there was no difference between Muguga and Katumani soil (Table 5).

Conclusions

These and previous studies (Karanja and Wood, 1988) have indicated strains of *R. phaseoli* tolerant

of low pH, aluminium and high temperatures. Antibiotic-resistant mutants of these strains generally retain the properties of the parent strain and are readily recoverable from soils. Strains 9C and 18A which are tolerant of acidity and high temperature appear particularly useful for enhancing nodulation of *P. vulgaris* under Kenyan conditions. However, these strains need to be tested fully under field conditions before any recommendations can be made concerning their suitability for inoculant production.

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