SHORT COMMUNICATION

ANTIBIOTIC SENSITIVITIES OF THE ROOT NODULE BACTERIA*

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Variations in the susceptibility of rhizobia to antibiotic substances have been previously reported. Fogle and Allen (1948) considered lupin and soybean rhizobia to be more susceptible to various streptomycete antibiotics than other species of rhizobia. In contrast, Landerkin and Lochhead (1948) noted that strains of *Rhizobium japonicum* were less susceptible to actinomycete antibiotics than were other soil organisms tested.

Recent publications (Nilsson 1957; Kecskes and Manninger 1960; Schwinghamer and Dalmas 1961; Davis 1962) have examined the effect of purified antibiotics upon rhizobial strains. As the number and type of antibiotics tested and their concentrations have varied, no comparison of their results is possible.

The present study was undertaken to determine whether the different species of *Rhizobium* were alike in their susceptibility to antibiotics.

Media

The basal medium used throughout was a yeast extract–mannitol medium containing (in grams per litre of distilled water): baker's yeast extract, 20; mannitol, 10; agar, 20; K_2HPO_4, 0·5; $CaCl_2$, 0·2; $MgSO_4.7H_2O$, 0·1; NaCl, 0·2; $FeCl_3.6H_2O$, 0·01.

The organisms were preserved, where necessary, by lyophilization (Annear 1956), and stored in the cold.

Experimental Procedure

Nine different antibiotics, namely streptomycin, aureomycin, chloramphenicol, neomycin, terramycin, bacitracin, ledermycin, erythromycin, and sodium benzyl penicillin (penicillin G) were tested, each at three concentrations (0·1, 4·0, and 50 μg per sensitivity disk). These concentrations were decided upon following tests to determine the most significant levels. Tubes of the yeast extract medium were liquefied by autoclaving and allowed to cool to 45°C. They were inoculated, then agitated and poured. Sterile sensitivity disks were spaced around the agar plates, and the plates incubated for 3 days at 28°C. Zones of inhibition were observed by holding the plates over a viewing box in which a circular filament provided indirect lighting.

Results

Of the antibiotics tested, aureomycin, terramycin, and ledermycin seem to be most effective in inhibiting growth. Streptomycin, neomycin, and penicillin are

* Manuscript received November 22, 1962.

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inhibitory in some instances, but bacitracin, erythromycin, and chloramphenicol are mainly without effect (Table 1).

Quite appreciable species differences are apparent. Most noticeable is the high sensitivity of strains of *Beijerinckia indica* to erythromycin. Strains of the slow-growing species of *Rhizobium* appear less susceptible to antibiotics than are rhizobia of the clover–pea type.

TABLE 1

ANTIBIOTIC SENSITIVITIES OF ROOT NODULE AND ASSOCIATED BACTERIA

Number of strains inhibited by various antibiotics at three concentrations. Number of strains tested given in parentheses; n.t., not tested

Antibiotic	*Rhizobium trifolii* (17)			*Rh. leguminosarum* (12)			*Rh. phaseoli* (7)			*Rh. lupini* (10)			*Rh. japonicum* (6)		
Antibiotic concn. (μg/disk):	0·1	4	50	0·1	4	50	0·1	4	50	0·1	4	50	0·1	4	50
Streptomycin	9	16	17	6	10	12	2	4	5	—	6	7	—	—	4
Aureomycin	15	17	17	9	12	12	4	6	6	—	1	4	—	—	1
Terramycin	16	17	17	11	12	12	5	6	7	—	1	3	—	—	1
Ledermycin	16	17	17	12	12	12	4	4	6	1	1	5	—	—	2
Neomycin	1	2	12	—	—	5	—	—	2	—	—	—	—	—	—
Penicillin G	3	3	11	1	2	8	—	—	4	—	—	3	—	—	1
Bacitracin	—	1	2	—	—	1	—	1	1	—	—	—	—	—	—
Erythromycin	1	4	n.t.	—	—	n.t.	—	1	n.t.	—	—	n.t.	—	—	n.t.
Chloramphenicol	—	1	n.t.	—	—	n.t.	1	1	n.t.	—	—	n.t.	—	—	n.t.

Antibiotic	Cowpea rhizobia (11)			*Rh. meliloti* (10)			*Agrobacterium radiobacter* (7)			*A. tumefaciens* (11)			*Beijerinckia indica* (8)		
Antibiotic concn. (μg/disk):	0·1	4	50	0·1	4	50	0·1	4	50	0·1	4	50	0·1	4	50
Streptomycin	2	5	10	3	6	9	2	3	6	—	4	9	2	7	7
Aureomycin	—	1	5	7	10	10	6	7	7	9	11	11	8	8	8
Terramycin	—	—	4	10	10	10	5	6	7	9	11	11	5	8	8
Ledermycin	—	—	5	9	10	10	6	7	7	11	11	11	5	7	7
Neomycin	—	—	1	—	—	4	—	—	5	—	1	6	—	—	4
Penicillin G	—	—	5	—	1	3	—	—	2	—	—	1	—	1	5
Bacitracin	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Erythromycin	—	—	n.t.	—	—	n.t.	—	—	n.t.	—	—	n.t.	4	6	n.t.
Chloramphenicol	—	—	n.t.	—	4	n.t.	1	1	n.t.	1	1	n.t.	—	—	n.t.

In certain areas of Western Australia antibiotic production in the soil appears to be a major factor in legume establishment (Holland and Parker 1962). These results could explain why some legume species are more readily affected than others. They would also suggest that pasture species requiring slow-growing rhizobia are more likely to establish successfully in these areas. Serradella (*Ornithopus sativus* Brot.) has already shown potential (Parker 1962).

Antibiotic sensitivities of bacteria are not commonly used in the differentiation of bacteria because of the difficulty in assessing results. This may be overcome by programming digital computers to perform bacterial classification (Sneath 1957). The results obtained here suggest that antibiotic sensitivities could be of value if included in the classification of the root nodule bacteria. Other characters which could be of taxonomic value are being investigated.

Grateful acknowledgment is made to Miss M. Jones for technical assistance. The work was carried out during the tenure of the Bank of New South Wales Research Fellowship in Agriculture and was supported by a research grant from the University of Western Australia.

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