FREE-LIVING NITROGEN-FIXING BACTERIA IN NATAL SUGARCANE SOILS

I. DISTRIBUTION OF AZOTOBACTER

By J. R. ANDERSON

Summary

A survey of soil groups of the Natal sugar industry has revealed a fairly high incidence of Azotobacter. The exceptions to this finding were Table Mountain sandstone (ordinary), Dwyka and the Ecca shales. A probable explanation of low incidence in the former is the predominantly acid reaction of this soil, the majority of samples being below pH 5.5. No definite explanation for the other two soils is given.

A higher incidence of Azotobacter in soils of pH 6.0 and above, with a fairly high incidence in the range pH 5.5 to 5.9 was noted. Eighty three out of 147 samples were Azotobacter positive, of which 56.6 per cent were pH 6.0 and above, 32.5 per cent were pH 5.5 to 5.9 and 10.8 per cent below pH 5.5.

No correlation of organic matter content or agricultural practice with incidence of Azotobacter was evident, but compaction was found to limit depth distribution which in some cases was as deep as 24 inches. Liming is thought to improve the incidence of Azotobacter and is recommended for this purpose in Table Mountain sandstone (ordinary) soils.

No associations with the rhizoplan was established and no correlation of "good" or "poor" response to nitrogen applications could be obtained. The soils in these cases were of the low incidence group.

Introduction

Whereas considerable literature on the distribution and influence of atmospheric-nitrogen fixing bacteria is available for most countries, information of a similar nature is lacking for the soils of the Natal sugar industry. The need for such is now a matter of necessity in view of the observed differences in response of cane to additions of chemical nitrogen on various soil groups.

Early work indicated that Azotobacter was present in some soil groups but appeared to be absent, or in the minimum, in others. This finding is felt to be of significance, not only from the academic, but also from the practical viewpoint since it has frequently been found that whereas Recent Sands show a somewhat indifferent response to added nitrogen, Table Mountain sandstone soils generally give an excellent response. The existence and activity of Azotobacter in the former, but not in the latter soil, is considered to be a possible partial explanation for this variation in response.

The findings reported here deal with the overall distribution of Azotobacter in various soils, depth of distribution in Azotobacter positive soils, presence or absence in soils showing a response or little response to added nitrogen, associations with cane roots and a brief description of some species isolated from various soils. Quantitative aspects of nitrogen fixation are at present under investigation, together with further survey work, and will not be dealt with in this paper.

Literature Survey

Recent appraisal of the genus Azotobacter has resulted in the inclusion of *A.indicum* as a species of the new genus Beijerinckia.[4] The pH tolerance of the two genera are compared by various workers. It has been claimed for example that Azotobacter requires a pH of 6.0 and above,[15] but later findings [20, 9, 8] grant higher acid tolerance, with values of pH 4.5 being recorded. In another instance it was found[34] that 11 of 40 strains tolerated pH 5.0 and 17 tolerated pH 10.0. Beijerinckia occur[4] in the range pH 4.0 to 7.4.

Azotobacter and Beijerinckia are present in many different soil groups, the latter genus favouring lateritic or laterising soils.[4] A study of the literature [13, 18, 22, 23] reveals some confusion as to the soil groups and conditions favouring Azotobacter.

A linear ratio between Azotobacter and water soluble calcium in the soil has been found[25] but the apparent unimportance of this nutritional factor in Beijerinckia probably explains its greater distribution in lateritic and laterising soils. The essentiality of calcium for growth of Azotobacter has been stressed,[14] although the amount,[12] required does not seem to be very great. *Azotobacter agile* appears to be divorced from these calcium requirements.[28]

The presence and amount of organic matter necessary for growth and fixation of Azotobacter is yet another controversial point. The characteristics of various species are dependent on different carbon sources.[41] Few Azotobacter were reported[10] to survive incubation with various composts but on the other hand an insufficiency of organic matter was found[6] to be unsuitable for Azotobacter. An increase in Azotobacter was found except when the applied organic matter was decomposing.[27] Some associations were found[11] with cotton leaf mulches and various composts applied to maize.[10] On the other hand it has been claimed[16] that a negative relationship exists between amount of organic matter present in soil and amount of energy material available to Azotobacter. The organisms are said to utilise root secretions before utilising the nutrients in the surrounding soil.[42]

Cultivation of the soil was found to increase the Azotobacter population.[7, 22, 35, 37, 39] The presence of herbage has also been reported as stimulating these bacteria.[30]

Toxic or inhibitory factors have been widely investigated and low salt concentration,[19] excess calcium nitrate[12] and the correlation of Al+ content of soils with bacteriostatic and/or bactericidal effects[17] are a few examples of the chemical aspects. Antagonism by species of Actinomycetes[3, 33, 36] as well as between species and strains of Azotobacter,[21] specific crops[24, 31] fungi, fungus lysates and spore forming bacteria[26, 20, 40] have been established on the biological side.

Seasonal fluctuations, namely highest in Spring and Autumn[25] or in Spring and Summer[23] have been reported from northern latitudes.

Materials and Methods

Various liquid media are available for isolation of Azotobacter, the best known of which are Ashby's mannitol phosphate solution,[32] Burk's medium[1] and those of Tchan[2] and Augier.[2] These media are generally quite satisfactory for isolation purposes, but the formulations of some do not provide adequately for trace element demands and so-called unknown growth factors. The medium suggested by Augier[2] however, has taken these and other nutritional demands, into consideration and for these reasons was adopted as the medium for isolations in the work reported here. The medium was dispensed in 5 ml. aliquots in 22 mm. by 140 mm. test tubes, autoclaved at 5 lbs./10 minutes and stored in drilled, wooden framed, boxes which facilitated inoculation and incubation on a large scale.

The method of sampling the various soil groups was adapted, for convenience, to the surface three inches only, the sampler walking in a large circle in the field (chosen at random) scooping soil at various intervals and placing it in a standard cloth-type sampling bag. Wherever possible, sampling was conducted during, or just after a rainy spell. The composite samples from each field were labelled and removed to the laboratory where each bag was sub-sampled and a quantity of soil (25 grams) placed in a 250 ml. sterile water blank. This 10:1 dilution was further diluted to 1:250 by addition of 10 mls. of the 10:1 dilution to a fresh 250 ml. sterile water blank. One ml. aliquots of the two (high and low) dilutions were pipetted into the test-tubes containing the culture solution, using duplicate tubes for each dilution. Alternatively, four tubes of the 0:1 dilution only were incubated. Temperature and time of incubation was standardised at 33°C for 5 days whereafter the tubes were examined for the presence of a surface film of Azotobacter cells and the result verified microscopically. An extension of incubation period to 10 days was considered only in doubtful cases.

The survey of soils in the Natal sugar industry was conducted on the basis of percentages of various soils within seven separate regions as follows:

(1) Sezela to Amanzimtoti River.

(2) Avoca to Tongaat.

(3) Tongaat to Umvoti River.

(4) Umvoti River to Tugela River.

(5) Tugela River to Umlalazi River.

(6) Umlalazi River to Enseleni River.

(7) Ensleni River to Umfolosi River.

The proportion of various soil groups to each other was the basic consideration in sampling these regions and whereas many fields and soil groups may have been left out in one region, the soil group concerned was adequately covered in other regions. The generalised nature of the investigation which was primarily for the *incidence* of Aztobacter, should be borne in mind.

In addition to aquiring a generalised impression of soil groups within various regions, investigations into the depth distribution of Azotobacter were also conducted on fields previously sampled during the survey. The method of sampling at various depths was by means of test-pits dug at random points in a particular field with removal of soil from appropriate depths. Composite depth samples from each level in the field were sub-sampled, diluted and incubated in Augier's solution as before.

Selected soil groups which showed either good response or poor response to nitrogen applications were surface sampled as previously outlined. The purpose of this particular investigation being to compare the incidence of Azotobacter between the good response and poor response soils.

Sugarcane roots, from the top six inches of soil were gently rinsed under running tap water to remove the bulk of soil adhering to the root surface. Four additional rinsings were conducted in sterile distilled water and finally portions of root placed in 5 ml. aliquots of Augier's solution and incubated as previously. The presence or absence of growth of Azotobacter from these root portions would have established the association of Azotobacter with the rhizoplan.

Identification of species was conducted by means of single colonies obtained by streak culturing surface films from Augier's solution on Ashby's mannitol-phosphate agar. Single colonies were re-inoculated in Augier's solution and re-streaked from the resulting film onto Ashby's agar. Single colonies were then inoculated on to potato wedges, gelatin, glucose broth, nutrient broth and litmus milk. Size, shape and motility of cells, as well as Gram's stain (Hucker's modification) and flagella stain (Casare Giles) observations, recorded.

Results and Discussion

The number of Azotobacter positive and negative results obtained from a total of 187 samplings in the seven regions surveyed is given in Table 1 below.

Table 1

Total number of Azotobacter positive and negative samples from twelve soil groups of the Natal sugar industry.

Soil Type	No. of Samples	Azotobacter		% Positive
		Positive	Negative	
Beaufort series ..	5	4	1	80.0
Recent sand (red) ..	27	21	6	77.7
Schist	8	6	2	75.0
Alluvium	15	11	4	73.3
Dolerite	22	16	6	72.7
Recent sand (grey) ..	20	14	6	70.0
Granite	12	7	5	58.3
Table Mountain sandstone (Mist belt) ..	13	7	6	53.8
Middle Ecca shale ..	10	5	5	50.0
Lower Ecca shale ..	20	7	13	35.0
Dwyka	17	5	12	29.4
Table Mountain sandstone (ordinary) ..	18	2	16	11.1
TOTAL	187	105	82	56.1

Observations, at the time of sampling, on state of cultivation, approximate moisture content and age of crop could not be correlated with the proportion of positive and negative results in Table 1. Exceptionally dry soils were not included in the sampling programme, and were passed over in favour of soils under cane or known to have received recent rains. Fallow Dwyka soils were noticed to be particularly susceptible to drying out. The results in Table 1 indicate no particular preference on the part of Azotobacter for a particular soil texture since high incidences are apparent in sandy, clay, and clay loam soils.

One of the outstanding features of the survey results concerns the incidence of Azotobacter in T.M.S. (ordinary) soils. As mentioned, this soil invariably gives good response to applications of nitrogenous fertilizer and the persistently low incidence of Azotobacter may partially account for this fact. The high incidence, and indications of large (quantitative) populations, in Recent (red) sands is likewise thought to be partially responsible for the lack of similar good responses to added nitrogen as exhibited in T.M.S. (ordinary).

Consideration of nutritional properties of soils showing positive and negative results does not provide an adequate explanation for the virtual absence of Azotobacter from T.M.S. (ordinary) since as shown in Table 2 below, the organic matter status of this soil is greater than that of Recent sands. In most other chemical respects this soil is superior to some of those showing high Azotobacter incidence.

Table 2

Organic matter content and soil reaction of samples* investigated for Azotobacter.

Soil Group	O.M. % (Av.)	pH (Av.)	No. of Soils	No. of Azotobacter Positive Soils	Soils of pH 6.0 and above		Soils between pH 5.5 and 5.9		Soils below pH 5.5	
					Azotobacter		Azotobacter		Azotobacter	
					+ve	−ve	+ve	−ve	+ve	−ve
Beaufort series	3.84	5.85	5	4	2	0	2	1	0	0
Recent sand (red)	1.45	5.62	16	10	7	0	3	4	0	2
Recent sand (red) limed.. ..	1.10	8.05	4	4	4	0	0	0	0	0
Schist	4.67	5.74	8	6	3	0	1	1	2	1
Alluvium	3.07	6.28	13	10	9	1	1	1	0	1
Dolerite	3.61	6.09	14	10	6	1	3	3	1	0
Recent sand (grey)	1.67	5.57	15	9	4	0	4	0	1	6
Recent sand (grey) limed ..	1.20	7.35	3	3	3	0	0	0	0	0
Granite	3.84	5.78	12	7	1	2	4	2	2	1
Table Mountain Sandstone (Mist Belt)	5.03	5.40	9	6	0	0	4	0	2	3
Middle Ecca shale	3.40	6.00	9	4	3	0	1	2	0	3
Lower Ecca shale	6.61	6.25	13	5	3	1	2	3	0	4
Dwyka	4.28	5.85	12	3	2	2	0	4	1	3
Table Mountain Sandstone (Ordinary)	3.03	5.27	14	2	0	0	2	3	0	9
TOTAL			147	83	47	7	27	24	9	33

*Only 147 samples were available for analysis in this case.

From the above table, organic matter per cent. does not emerge as a limiting or decisive factor in the incidence of Azotobacter in any of the soils studied. In point of fact, the Recent (red) sands possess the lowest average organic matter content, yet emerge as the soil with the second highest incidence of Azotobacter. The T.M.S. (ordinary), Beaufort series, Alluvium, Dolerite, Granite and Middle Ecca soils all show an organic matter per cent of approximately the same order, yet the incidence of Azotobacter varies from virtually none in the T.M.S. (ordinary) to 80.0 per cent in the case of Beaufort series.

The average pH values of the soils sampled do not immediately arouse suspicions as to suppressing influences of values below pH 6.0 on the incidence of Azotobacter, but upon comparing the incidence of these bacteria in soils of a particular pH level, as opposed to the average value, an entirely different picture emerges. Of the 83 positive Azotobacter soils, 47 (i.e. 32.5 per cent) were from the pH group 5.5 to 5.9 representing approximately 18 per cent of all the samples in Table 2, whilst 9 positives (i.e. 10.8 per cent) were from the pH group below 5.5 representing approximately 6 per cent of the soils in the same table. Thus in 147 soil samples, 56.4 per cent were positive for Azotobacter, the majority of these (89.1 per cent) coming from soils above pH 5.5. Of this majority, 56.6 per cent were from soils of pH 6.0 and above. Most negative Azotobacter soils were found in the pH range below 5.5, with the next highest number of negative soils coming from the range pH 5.5 to 5.9, with relatively few negative soils in the group pH 6.0 and above.

It is obvious from Table 2 that the majority of T.M.S. (ordinary) soils were below pH 5.5 and that no instances of pH 6.0 and above were encountered. The next soil possessing a high number of soils below pH 5.5 was the Recent (grey) sand but in this instance there were 7 cases above pH 6.0 including limed soils, all of which were positive for Azotobacter. The form of lime applied to this and the Recent (red) sands was a filter-press containing a high proportion of $CaCo_3$. In a subsequent series of repeat samplings, all sandy soils possessing this form of filter-press were found to be very highly positive.

The presence of Actinomycetes in T.M.S. (ordinary) has been determined at various intervals and the total population established as being higher than that for both Recent sands (unlined) and dolerite soils. In view of the preference of these organisms for pH values similar to, if not higher than those preferred by Azotobacter, it is surprising that this is the case. No attempt at species identification of the Actinomycetes has been essayed but the species found to develop on Glucose-asparagin agar[38] were of an homogeneous nature, apparently confined to two or three types. It is possible that the presence of such acid-tolerant Actinomycetes are an additional antagonistic influence on the development of Azotobacter although studies in this field are required for confirmation or otherwise.

Investigations into the depth distribution in particular soil groups revealed that Azotobacter may be present at depths of 24 in. depending on soil group and physical conditions within the soil such as degree of aeration, waterlogging or compaction. The highly aerobic nature of these bacteria make adequate aeration a prerequisite for growth.

Table 3

Depth distribution of Azotobacter in some sugarcane soils.

Soil Type and Condition	Depth of Sample	Azotobacter
Table Mountain Sandstone (Mist belt). Recently tilled, damp.	Surface	+ve
	8 in.	+ve
	16 in.	+ve
	24 in.	−ve
Table Mountain Sandstone (ordinary). Under plant cane (3 months old). Between row cultivation.	Surface	−ve
	2 in.	−ve
	4 in.	−ve
	6 in.	−ve
	12 in.	−ve
	24 in.	−ve
Recent (red) sand. Limed, ploughed-out three months previous to sampling.	Surface	+ve
	2 in.	+ve
	4 in.	+ve
	6 in.	+ve
	12 in.	+ve
	24 in.	+ve
Dolerite (Black). Ploughed, harrowed. Good tilth, well aerated.	Surface	+ve
	2 in.	+ve
	4 in.	+ve
	6 in.	+ve
	12 in.	−ve
	24 in.	−ve
Dolerite (Black). Same as above; sample taken from vehicle tracks in close proximity to previous sample.	Surface	+ve
	2 in.	−ve
	4 in.	−ve
	6 in.	−ve
	12 in.	−ve
	24 in.	−ve

Cultivation, by virtue of its aerating effect on soil, is thus obviously beneficial to the depth distribution of Azotobacter, particularly in heavy clay soils. Sandy soils of the Natal coast by virtue of their open texture and excellent water retaining properties, are especially suited to the development of these organisms at depth. The sensitivity of Azotobacter to anaerobic conditions is exemplified in the above table and lends weight to the need for care in preventing compaction of mechanised fields.

Associations of Azotobacter with the rhizoplan of sugarcane were not established and with extended incubation times up to 20 days, no surface films developed in the culture medium. It is not known whether this negative association is due to toxic or inhibitory root secretions, antagonistic rhizoplan microflora or lack of a particular growth promoting, or essential nutrient. The presence of Azotobacter in soils under mature cane with extensively interwoven lateral (surface) roots does not appear to indicate a toxicity effect of sugarcane roots but likewise does not exclude the possibility of antagonistic rhizoplan micro-organisms.

An intensive sampling of soils known to give either a good or poor response to additions of nitrogen revealed an overall negative result. Five of the soils sampled were classified as giving a good response to nitrogen additions the appropriate soil groups being Dwyka (2), Ecca shale (not specified), Table Mountain Sandstone (ordinary) and Alluvium.

The remaining two soils, classified as giving a poor response to nitrogen additions, were a Dwyka and an Ecca shale (unspecified).

Whereas most of the Dwyka, Ecca shale and Table Mountain Sandstone (ordinary) soils have been shown (table 2) to yield negative Azotobacter results, the Alluvium soils generally show positive Azotobacter reactions. It was noticed however that this latter soil, being situated in low aspect near the Tugela River, was extremely wet although not quite waterlogged. The so-called poor response soils sampled, being of the Ecca shale and Dwyka groups are known to have a low incidence of Azotobacter, and therefore the negative results obtained were not altogether unexpected. Probably the reason for poor response to nitrogen additions is coupled with some other nutritional or physiological factor.

Cultural characteristics and morphological observations of several isolates from various soils have indicated the presence of several different forms of Azotobacter in Natal sugarcane soils. Four typical isolates are presented in Table 4 below.

Table 4

Cultural and morphological characteristics of four strains or variants of Azotobacter spp. isolated from Natal sugarcane.

Origin of Culture	Test Applied	Characteristics
Recent (red) sand (Limed).	Glucose broth	Surface film fairly thick, creamy white at first later slightly brown. (28° c/5 days).
Culture No. 1.	Nutrient broth	No growth at 28°C or 33°C.
	Litmus milk	Cleared in 8 days at 28°C.
	Gelatin* stab	No liquefaction.
	Potato wedges	Dense creamy-white growth. Slimy tinged with brown. (28°C for 5 days).
	Mannitol phosphate agar ..	Translucent, slimy, oval-shaped colonies. 5 mm. in diameter after 28°C. for 5 days.
	Augier's solution	Fairly strong, thick film. White to grey-white, later grey, heavily wrinkled at margin.
	Gram stain	Negative.
	Size, shape, etc.	Oval to rod-shaped, 6-8μ x 5-6μ, motile by peritrichous flagella.
Recent (red) sand (Not limed) pH. 6.0 Culture No. 2	Glucose broth	Surface film thin, yellowish-brown becoming predominantly brown after 5 days at 28°C.
	Nutrient broth	No growth at 28°C or 33°C.
	Litmus milk	Cleared in 10 days at 28°C.
	Gelatin stab	—
	Potato wedges	Slimy yellow white growth, not profuse, after 5 days at 28°C.
	Mannitol phosphate agar ..	White, slimy, flecked with grey. Granular. Colonies approx. 5 mm. in diameter after 5 days at 28°C.
	Augier's solution	Fairly thick, creamy-brown at first, later dark brown, but white at periphery.
	Gram stain	Negative.
	Size, shape, etc.	Oval, 4-7μ x approx. 5μ, frequently in pairs. Motile by peritrichous flagella.
Table Mountain sandstone pH 6.0. Culture No. 3.	Glucose broth	Very slight, delicate, film. 28°C 5 for days).
	Nutrient broth	No growth at 28°C and 33°C.
	Litmus milk	Partially cleared, inconclusive after 14 days, at 28°C.
	Gelatin stab	No liquefaction.
	Potato wedges	Individual, small yellow-brown colonies. Moist, not slimy. 28°C for 5 days.
	Mannitol phosphate agar ..	Greyish-white colonies, approximately 3 mm. in diameter. Granular.
	Augier's solution	Thin, delicate, brown film; slightly wrinkled.
	Gram stain	Negative.
	Size, shape, etc.	Small oval cells 3-4μ x 2-3μ single and in pairs. Non-motile.
Table Mountain sandstone (Mist belt) pH 6.0. Culture No. 4.	Glucose broth	Thick, creamy-white pellicle; tendency for slime-threads.
	Nutrient broth	No growth at 28°C and 33°C.
	Litmus milk	Cleared after 10 days at 28°C.
	Gelatin stab	—
	Potato wedges	Dense, slimy yellow-brown to brown growth after 5 days at 28°C.
	Mannitol phosphate	Creamy-white, slimy, granular colonies approx. 5 mm. in diameter after 5 days at 28°C.
	Augier's solution	Creamy-white, thick pellicle. Slime threads.
	Gram stain	Negative.
	Size, shape, etc.	Large, almost spherical cells 6-8μ x 5-6μ single and in pairs. Motile by peritrichous flagella.

The cultural and morphological characteristics of the above four cultures are not identical in all respects to the type-species described.[5] Consequently they may only be likened to typical species in certain respects and designated as variants or strains of type-species. Thus, cultures 1 and 4 are similar in some respects to *A.agile* Beijerinck but vary in cellular size and colony habit in agar media. Culture number 2 was a closer fit to the type-species mentioned above especially in cultural characteristics. Culture 3 was of a similar pattern to the type-species *A.chroococcum* Beijerinckia.

Whereas identification and classification are important in providing a basis upon which to gauge differences in nitrogen fixation ability, it is not regarded as being of great importance for the current project of establishing the presence or absence of Azotobacter. The brief morphological and cultural partitioning outlined above will thus serve only to differentiate strain ability in fixation in the next stage of this investigation.

Conclusions

The soils of the Natal sugar belt not only indicate a general incidence of Azotobacter, but also a definite variation in species. The majority of soil groups showed a fairly high incidence of these bacteria, the exceptions being T.M.S. (ordinary), Dwyka and Ecca shales. The former soil group has a predominantly low pH value, generally being below pH 5.5, and this factor is thought to account adequately for the low proportion of positive Azotobacter results obtained.

From the results obtained it is clear that Azotobacter prefer soil pH values of an alkaline nature, preferably above pH 6.0. In the range pH 5.5 to 5.9 Azotobacter are present in many instances but not to the same degree as at more neutral values, whilst values below pH 5.5 are barely tolerated the exceptions probably being acid tolerant strains.

Whereas antagonistic principles such as those imparted by Actinomycetes, may have a bearing on the T.M.S. (ordinary) soil, the availability of organic matter can be discounted since soils with lower values of organic matter have shown a persistently higher incidence of these bacteria. It is concluded that organic matter is not a limiting factor in Natal sugarcane soils.

Various forms of agricultural practice did not correlate with incidence of Azotobacter but compaction of soils was found to be a limiting factor in depth distribution of these bacteria and liming of soils seemed to provide an added stimulus to their existence. Liming of soils, whilst being undesirable in many other respects, is considered to be beneficial in creating more suitable pH conditions for development of these bacteria and may well be considered a prerequisite for the establishment of Azotobacter in T.M.S. (ordinary) soils.

The existence of Azotobacter in the rhizoplan has not been established for sugarcane, the exact reason for which is not clear although toxicity of sugarcane roots does not seem applicable in view of their existence in the rhizosphere.

The investigation of certain "good" and "poor" response soils, as gauged from nitrogen applications, gave an overall negative result which was not unexpected in even the "poor" response soils since the soils concerned where of the Dwyka, Ecca shale and T.M.S. (ordinary) type, the one exception being an Alluvium, a soil group which is generally Azotobacter positive. In this particular case the soil was not only classified as a "good response to nitrogen" soil but showed a slight tendency towards waterlogging.

References

[1]Allen, O. N., 1957. Experiment in Soil Bacteriology. *Burgess Pub. Co., Minnesota.*

[2]Augier, J., 1956. A propos de la numeralton des Azotobacter en milieu liquide. *Ann. Inst. Pasteur 91 (5) Nov. 1956 p. 759-764.*

[3]Babak, N. M., 1958. The sensitivity of Azotobacter to the antagonistic action of Actinomycetes and to some antibiotics. Abst.: *Soils and Fertilisers, 22, 1:43.*

[4]Becking, J. H., 1961. Studies on nitrogen-fixing bacteria of the genus Beijerinckia. I Geographical and ecological distribution in soils. *Plant and Soil XIV: 1:49-81.*

[5]Breed, R. S., Murray, E. G. D. and Hitchens, A. P., 1948. Bergey's manual of determinative bacteriology. *Bailliere, Tindall and Cox., London. 6th Edition.*

[6]Bernard, V. V. and Voronkova, E. A., 1960. The multiplication of Azotobacter and the fixation of nitrogen in the presence of organic manures and other sources of organic matter. Abst.: *Soils and Fertilisers*, 23, 3-193.

[7]Bilodub, H., 1960. Investigations on Azotobacter in the experimental plots of the Laskowice Olawskie experimental station. Abst.: *Soils and Fertilisers, 24, 3:207.*

[8]Blinkov, G. N. and Novoselova, A. N., 1959. The Azotobacter of Podzolic soils of Siberia. Abst.: *Soils and Fertilisers, 23, 4:271.*

[9]Bychkovskaya, A. L. and Shklyar, M. Z., 1959. An acid tolerant variety of Azotobacter. Abst.: *Soils and Fertilisers, 23, 3:192.*

[10]Chizhevskii, M. G. and Dikusar, M. M., 1959. The effect of different forms of natural organic matter on the development of Azotobacter and the productivity of its nitrogen fixation. Abst.: *Soils and Fertilisers 23, 3:192.*

[11]Darzneik, Yu. O., 1960. The effect of the cotton plant on the development of Azotobacter in soils. Abst.: *Soils and Fertilisers, 24, 3:208.*

[12]Ebert, K., 1959. The effect of amide, ammonium and nitrate nitrogen on the growth and nitrogen fixation of Azotobacter. Abst.: *Soils and Fertilisers, 23, 2:113.*

[13]Elivan, S. H. and Mahmoud, S. A. Z., 1960. Note on the bacterial flora of the Egyptian desert in Summer. Abst.: *Soils and Fertilisers, 24, 1:35.*

[14]Federov, N. V. and Kalininskaya, T. A., 1958. Physiological characteristics of strains of typical Azotobacter isolated from limed sod-podzotic soils. Abst.: *Soils and Fertilisers, 21, 6:381.*

[15]Gainey, P. L., 1918. Soil reaction and the presence of Azotobacter. *Science N.S. 48. 139.*

[16]Garbosky, A. J., 1957. Distribution of Azotobacter in depth in soils of Tucuman. Abst.: *Soils and Fertilisers, 21, 4:241.*

[17]Gromÿko, E. P., 1960. Causes of the toxicity of podzotic soil to Azotobacter. Abst.: *Soils and Fertilisers, 23, 3:193.*

[18]Gupta, S. R. S., 1956. A new factor in the study of nitrogen fixation in soil. *Indian Jour. Mycol. Res. 2: 29-26. (Soils and Fertilisers, 22, 1:42.)*

[19]Iswaran, V. and Sen, A., 1958. Effect of salinity on nitrogen fixation by Azotobacter species in some Indian soils. *Journ. Indian Soc. Soil Sci. 6: 109-113.*

[20]Jensen, H. L., 1955. *Azotobacter macrocytogenes* n. sp. a nitrogen-fixing bacteria resistant to acid reactions. *Acta Agr. Scand., 5: 278-294.*

[21]Kirakosyan, A. V. and Karimyan, R. S., 1958. Intra- and inter-specific relations in Azotobacter. Abst.: *Soils and Fertilisers, 22, 4:284.*

[22]Kirsanina, E. F. and Volkova, V. A., 1960. Some data on the distribution of Azotobacter in soils of the mountainous Altai autonomous region. Abst.: *Soils and Fertilisers, 23, 6:410.*

[23]Kolker, I. I. and Dakhnova, E. N., 1960. Distribution of Azotobacter in soils of the Crimea. Abst.: *Soils and Fertilisers, 24, 1:39.*

[24]Konobeeva, G. M., 1960. Resistance of some species of soil micro-organisms to the action of Anabasine. Abst.: *Soils and Fertilisers, 23, 4:272.*

[25]Kukharkov, A. M., 1956. The effect of some physico-chemical and biological conditions on the distribution of Azotobacter in soils. Abst.: *Soils and Fertilisers, 22, 2:117.*

[26]Mal'tseva, N. M., 1957. The relations between Azotobacter and Bicillus mycoides. Abst.: *Soils and Fertilisers, 22, 4:284.*

[27]Morales, J., 1960. A new biological study of the genus Azotobacter. Abst.: *Soils and Fertilisers, 24, 3:207.*

[28]Norris, J. R. and Jensen, H. L., 1957. Calcium requirements of Azotobacter. *Nature, Lond., 180 1493-1494.*

[29]Novogrudskii, D. M. and Karaguishieva, D., 1957. New data on the biology of Azotobacter in soils. Abst.: *Soils and Fertilisers, 22, 1:43.*

[30]Rybkina, N. A., 1960. Distribution of anaerobic nitrogen fixing bacteria in the fields of a rotation. Abst.: *Soils and Fertilisers, 24, 1:38.*

[31]Sadovskya, R. O., 1959. The effect of the root system of the grapevine on the development of Azotobacter. Abst.: *Soils and Fertilisers, 23, 4:271.*

[32]Salle, A. J., 1954. Laboratory manual on Fundamental prinples of Bacteriology. *McGraw-Hill Book Co.*

[33]Segi, I., 1958. Interaction between *Azotobacter chroococcum* and soil Actinomycetes. Abst.: *Soils and Fertilisers, 24, 2:123.*

[34]Sen, A. and Iswaran, V. 1959. Variation of characteristics and nitrogen fixing capacities of Azotobacter in some Indian soils. *Soil Sci. 87, 46-49.*

[35]Shamis, D. L. and Galimova, R. A., 1958. The microflora of irrigated serozems cultivated in different ways. (*Soils and Fertilisers, 22, 6:447.*

[36]Szegi, J., 1959. The effect of soil Actinomycetes on the development of Azotobacter chroococcum under natural conditions. Abst.: *Soils and Fertilisers, 22, 5:373.*

[37]Tanatin, B. Ya., 1956. The effect of cultivating virgin soils on the growth of Azotobacter. Abst.: *Soils and Fertilisers, 22, 4:284.*

[38]Waksman, S. A., 1950. The Actinomycetes. *Chronica Botanica. Co.*

[39]Yuzhina, Z. I., 1958. Survival of Azotobacter in cultivated and virgin soils of the Kola peninsula. Abst.: *Soils and Fertilisers, 22, 2:117.*

[40] , 1958. The relation between the toxicity to Azotobacter of the soils of the Kola peninsula and the number of microbes antagonistic to Azotobacter. Abst.: *Soils and Fertilisers, 22, 2:43.*

[41]Zinov'eva, Hk. G., 1956. The relationship between specific strain of Azotobacter v. various sources of carbon nutrition. Abst.: *Soils and Fertilisers, 21, 1:36.*

[42] , 1958. The effect of the root secretions and root extracts of some agricultural plants on Azotobacter. Abst.: *Soils and Fertilisers, 22, 3:192.*

Dr. Dick, in the chair, stated that it was sometimes difficult for the layman to realise how much work goes into the fundamental research attempts at solving the difficult problems of why we obtained unexpected results from field trials. Mr. Anderson had already given a paper on one aspect of the nitrogen problem and this paper described another aspect.

He asked if Mr. Anderson could give an estimate of the amount of nitrogen fixed in the soil by the action of micro-organisms.

Mr. Anderson replied that in the literature there was a great difference of opinion on this matter, due to the difficulty of accurately determining the amount of fixation. Some microbiologists estimated the fixation to be equivalent to the nitrogen supplied by 350 lbs. of ammonium sulphate per acre. Some chemists maintained that no nitrogen was fixed by azotobacter in the soil but only by these bacteria in culture.

Mr. du Toit said the question of response or no response to nitrogen was a difficult one, and he was therefore pleased that the author had now tackled it from the microbiological aspect in his two papers presented to Congress this year. A possible reason why no response to nitrogen was sometimes found, was that it was made available from the atmosphere or from the breakdown of organic matter.

The author seemed to show that in the Grey Recent Sands there was a large number of azotobacter whereas in Table Mountain sandstone soils there was an insufficient amount of the micro-organism. This fitted in fairly well with the nitrogen responses in these two soil types, but unfortunately when the author confined himself to field experiments, results were rather contradictory. This showed that much more work would have to be done on the subject, but this first step by the author was extremely welcome.