Plant and Soil XI, no. 3 September 1959

NITROGEN-FIXING BACTERIA OF THE GENUS *BEIJERINCKIA* IN SOUTH AFRICAN SOILS

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INTRODUCTION

In 1939 Starkey and De [23] described a new *Azotobacter* species, *Azotobacter indicum* isolated from an acid rice soil in India, which is capable of fixing atmospheric nitrogen under acid conditions [21 22].

In 1950 Derx [2 3] found this microbe to occur abundantly in some Bogor (W. Java) soils and proposed the new generic name of *Beijerinckia* for it in view of differences in morphology and cultural characteristics that distinguish the latter from the other *Azotobacter* species.

In recent reviews [9 14 26 27] on nitrogen-fixing bacteria the setting up of a new genus *Beijerinckia* for *Azotobacter indicum* seems to be accepted.

Unlike the almost ubiquitous occurrence of *Azotobacter* which has been encountered in all soils with suitable pH independent of their geographical localization, the distribution of *Beijerinckia* is restricted to tropical regions. There exists no definite explanation for this phenomenon.

In addition to the localities mentioned above, *Beijerinckia* has been reported from Malaya (Altson [1]), French West Africa (Kauffmann and Toussaint [11 12]), Madagascar (Dommergues [6]), Australia north of the 20°S. latitude (Tchan [28]), and South America (Döbereiner and De Castro [5], Kluyver and Becking [13]). Meiklejohn [15] reported the isolation of a green fluorescent pigment-producing *Beijerinckia* strain from an acid Tanganyika soil, but later she identified this micro-organism as belonging to *Azotobacter vinelandii* Lipman [16].

Unpublished results obtained by the author indicate the occurrence of *Beijerinckia* in lateritic soils or latosols from the Hawaii Islands, New Guinea (Papua), China (Kwantung province, Hongkong), Ethiopia, East Africa (Tanganyika, Uganda, Kenya) and in some regions of South America (Trinidad Island, Dutch Guiana, Brazil, and Bolivia).

Again all these localities for *Beijerinckia* are tropical, although some of them are at rather high altitudes *i.e.* at 7,000 to 9,000 feet in the Ethiopian and East African highlands.

There is only one record of the occurrence of *Beijerinckia* outside the tropics. In a very recent publication Suto [24 25] describes the isolation of this bacterium from an acid volcanic ash soil at Sendai (latitude 38° N.) in Japan.

The present paper deals with the unsuspected occurrence of *Beijerinckia* in South African soils. South Africa lies outside the tropical bounderies between latitudes 23° and 34° S. This fact is of considerable importance since Tchan [28] has stated explicitly that *Beijerinckia* does not occur in the Australian continent at latitudes more south than 20° S.

MATERIALS

The soils were sampled in the field in small, sterile containers at their natural moisture content. The samples were all surface soils. They were not a random sample. Most of them were collected in forest plantations and along roadsides. Soil pH was measured by a glass electrode in the soil paste obtained by stirring some soil with a small amount of distilled water.

The time between soil sampling and microbiological examination was about 3–6 weeks. It is assumed that this delay has been of no influence on the results as *Beijerinckia* appears to be very persistent in soils in which it naturally occurs. Tropical latosols stored for five years in an air-dry condition at room temperature still reveal living *Beijerinckia* germs on examination.

A geographical map (Fig. 1) gives the distribution of the soil samples over the Union of South Africa. In the list of soil samples (Table 1) some details concerning the various samples are given such as locality, latitude, annual rainfall, altitude, occurrence of frost, soil type, soil pH, and cover. Most of these data are derived from the publications on South African soils and forest trees of Van der Merwe [17] and Poynton [19]. Geological information was obtained from the geological map (scale 1 : 1,000,000) of the Department of Mines, Geological Survey 1955, Pretoria.

METHOD OF EXAMINATION

The predominant characteristic of the bacteria of the genus *Beijerinckia* is their acid tolerance. Growth is obtained between pH = 3.0 and pH = 10.0. Their development is most vigorous at a pH = 4.0 to 5.0, which is usually about the reaction of their natural surroundings. However, growth of *Beijerinckia* in general is much slower than that of *Azotobacter*.

For the reasons mentioned above and in order to eliminate *Azotobacter*, an acid enrichment medium was employed. This medium differs only from the usual enrichment medium of *Azotobacter* by its acidity of pH = 5.0.

The composition of the medium used is quite simple:

Distilled water	1000 ml	KH_2PO_4	1.0 g
Glucose	20.0 g	$MgSO_4.7H_2O$	0.5 g

The phosphate buffer gives a pH of about 5.0. The supply of trace elements in the medium was obviously provided by the soil used as inoculum. Supplementary trace-element addition yielded no better results especially as fungus growth was enhanced.

The enrichment medium was poured in very thin layers (2 to 3 mm) in petri dishes (9 cm diameter) to ensure adequate aeration and to reduce butyric-acid fermentation. Petri dishes as well as enrichment media were sterilized before use in order to eliminate *Beijerinckia* infections from other sources than the soil used as inoculum.

For inoculation approximately 0.1 to 0.5 g of soil per petri dish was used. Every soil was tested with 10 to 15 replicates.

The enrichment cultures were examined microscopically for the presence of *Beijerinckia* cells which owing to their peculiar morphological characteristics can easily be recognized. *Beijerinckia* cells are motile or non-motile gram-negative rods (approx. 1.2 to 4.8–7.2 μ) showing a large lipoid globule stainable with Sudan III at each end of the cells. (See Plate 1A). Some strains produce large capsules with a distinct wall layer enveloping a large number of cells (see Plate 1B).

If necessary, the microscopic examination of the enrichment cultures was extended over a longer period of 3 to 4 weeks. A positive result on the basis of microscopic examination was always

checked by streaking an appropriate dilution of the enrichment medium on plates containing 2% agar, 2% glucose, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.01% $FeCl_3.6H_2O$, 0.002% $Na_2MoO_4.2H_2O$ (pH = 5.0). Calcium was not added to the medium as *Beijerinckia* needs no calcium for development. On this medium *Beijerinckia* forms highly raised, often plicated, glistening colonies of a very tenacious and elastic slime. The copious production of slime is a distinct feature of the genus (see Plate 2).

As a rule the presence of *Beijerinckia* cells in the enrichment medium can already be established with certainty by judging the appearance of the medium. In a somewhat advanced stage of development the medium changes into a viscous slimy mass which can be drawn out by a loop in long threads. The yeast, *Lipomyces* species, often present in these nitrogen-poor enrichment cultures, never produces such a tenacious slime.

RESULTS

Table 1 shows that most of the South African soils tested are distinctly acid. Only five of the forty samples collected showed pH values above pH = 6.0, *viz* a calcareous littoral soil (pH = 7.7) from Port Durnford, an alluvial (Olifant's river C.P.) desert soil from the Little Karroo, a savannah soil (pH = 6.7) from Pretoriuskop (Krüger National Park), a dressed (limed?) citrus soil (pH = 6.6) from Nelspruit, and a garden soil (pH = 6.1) from the Tokai Arboretum near Muizenberg, Cape Peninsula.

Twelve of the thirty-five more acid soils (pH $\leqslant$ 6.0) and two

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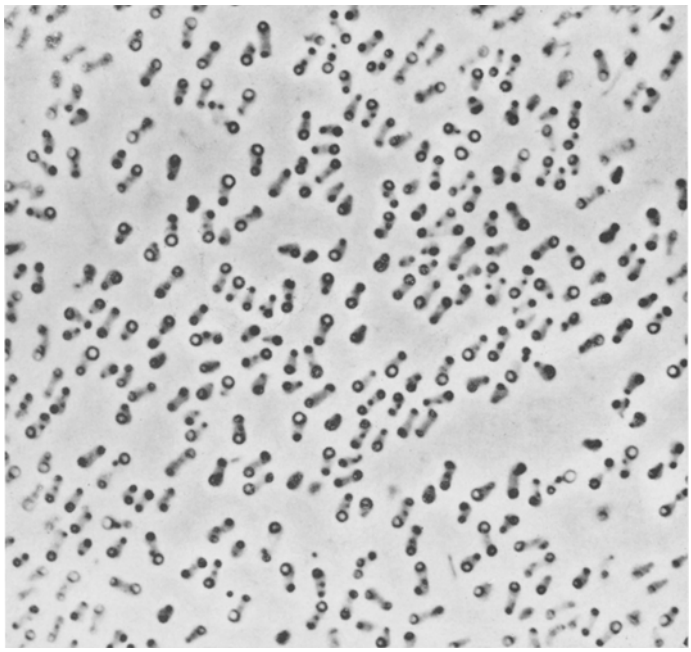
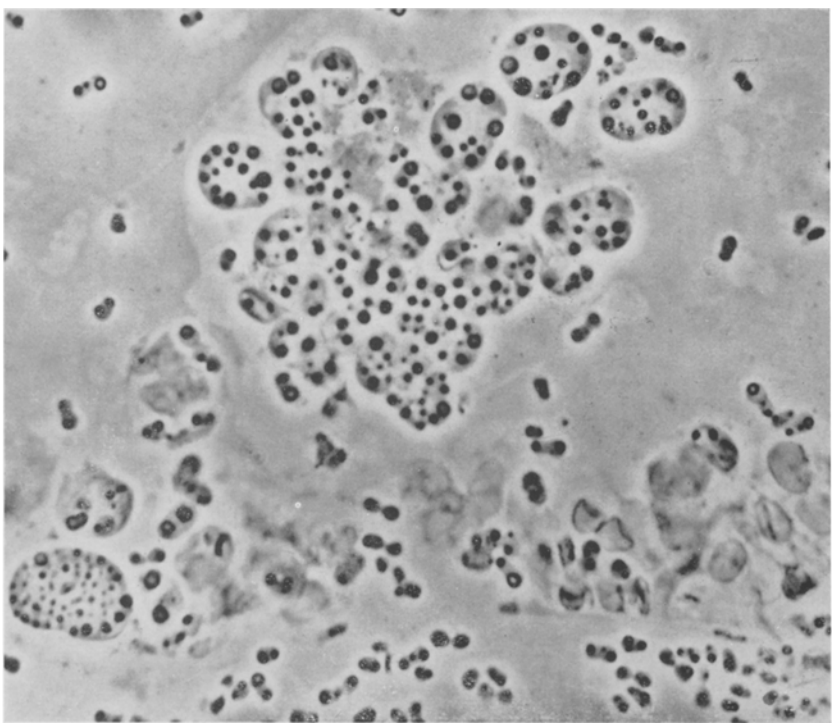
Plate 1: Both pictures are phase-contrast photographs of living cells grown on nitrogen-free glucose agar (pH = 5.0) at 30° C. (Magnification 2000 ×).

A. Cells of a *Beijerinckia* strain isolated from a South African "Mistbelt" soil. The photograph shows typical *Beijerincka* cells characterized by a large, highly refractive fat globule at each end of the cell.

B. *Beijerinckia* strain isolated from South African (Tweefontein plantation, Sabie) soil of pH = 4.5.
This strain shows the property of producing large capsules with a distinct wall layer enclosing many cells.
Similar strains were isolated from some other South African soils (*e.g.* Amatikulu soil) and from other regions (China, Java, Brazil).

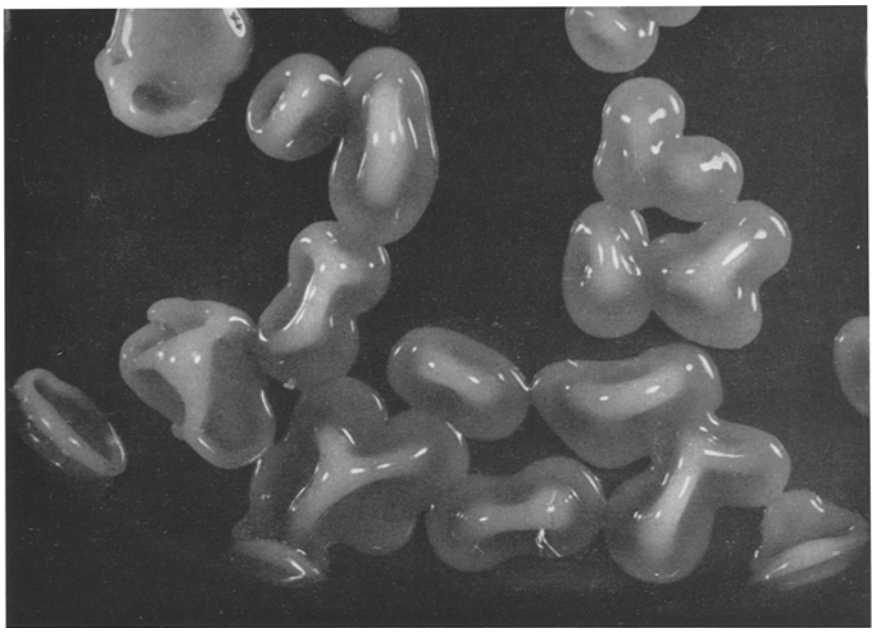


A



B

Plate 1



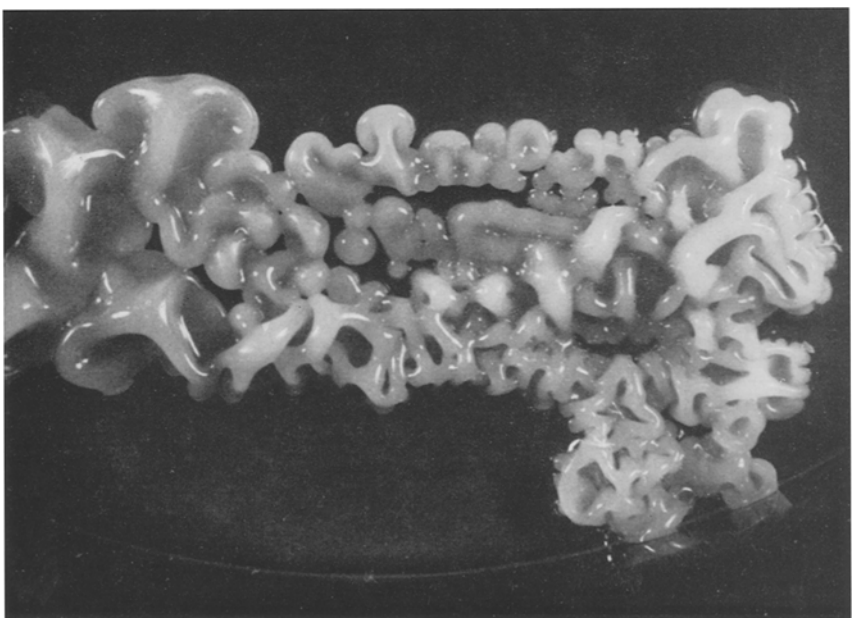


Plate 2

of the five soils with pH values higher than pH = 6.0 showed *Beijerinckia* development. As evident from Table 1 *Beijerinckia* was absent in the very acid soils (pH < 4.3) and the alkaline soils (pH > 7.0).

As indicated in the geographical map (Fig. 1) eleven of the twenty soils collected around or north of latitude 29° S. yielded *Beijerinckia,* but only three of an equal number of soils collected south of this latitude. This confirms to a certain extent the tropical distribution of the genus *Beijerinckia.*

Beijerinckia was found to be present abundantly in the northern part of Transvaal in the soils (pH = 5.8 to 6.1) of the De Hoek plantations, north of Tzaneen in the Drakensberg Mountains, in the soils (pH = 5.3) of Wilgenboom plantation, north of Acornhoek, and in the soils around Sabie at Tweefontein plantation, Witklip plantation and the S.A.F.I. estate (Hendriksdal) with pH values of 4.5, 5.4 and 5.8, respectively.

It occurred with *Azotobacter* in the savannah soil (pH = 6.7) from Pretoriuskop in the Krüger National Park. Moreover, *Beijerinckia* was present in considerable numbers in the acid soil (pH = = 4.5) of the Usutu pine plantations near Mbabane in Swaziland.

In Natal the acid sands (pH = 4.8) of Kwambonambi, St. Lucia Bay and the acid coastal soils near Eshowe, *viz* those near Port Durnford (pH = 4.5) and Amatikulu (pH = 5.9), contained some *Beijerinckia* germs.

Beijerinckia was found to be distributed far more sporadically in soil samples collected in the Cape Province. Only in three cases a positive result was obtained, *i.e.* in a forest soil (pH = 4.4) of the Forest Sanctuary near Stutterheim, in a well-developed *Pinus taeda* forest soil (pH = 5.4) with root penetration to 2 meters (very good site) at Blueliliesbush, and in a garden soil (pH = 6.1) of the Tokai Arboretum near Muizenberg on the Cape Peninsula. In the latter soil *Beijerinckia* occurred together with *Azotobacter.*

Soil cover seems of little influence on the distribution of *Beije-*

Plate 2: Typical colony types of *Beijerinckia* strains.

Most strains produce these highly raised, often plicated, glistening colonies of a very tenacious and elastic slime.

Colony development upon nitrogen-free glucose agar plates (pH = 5.0) incubated 3 weeks at 30° C. (Magnification 2 ×).

rinckia as the latter occurs in soils under indigenous grass cover (North Transvaal), savannah vegetation (Low Veld), sugar-cane cultivations (Amatikulu), exotic forest plantations (Transvaal, Swaziland, Natal), natural forest (Stutterheim Forest Sanctuary) or garden soil (Tokai Arboretum).

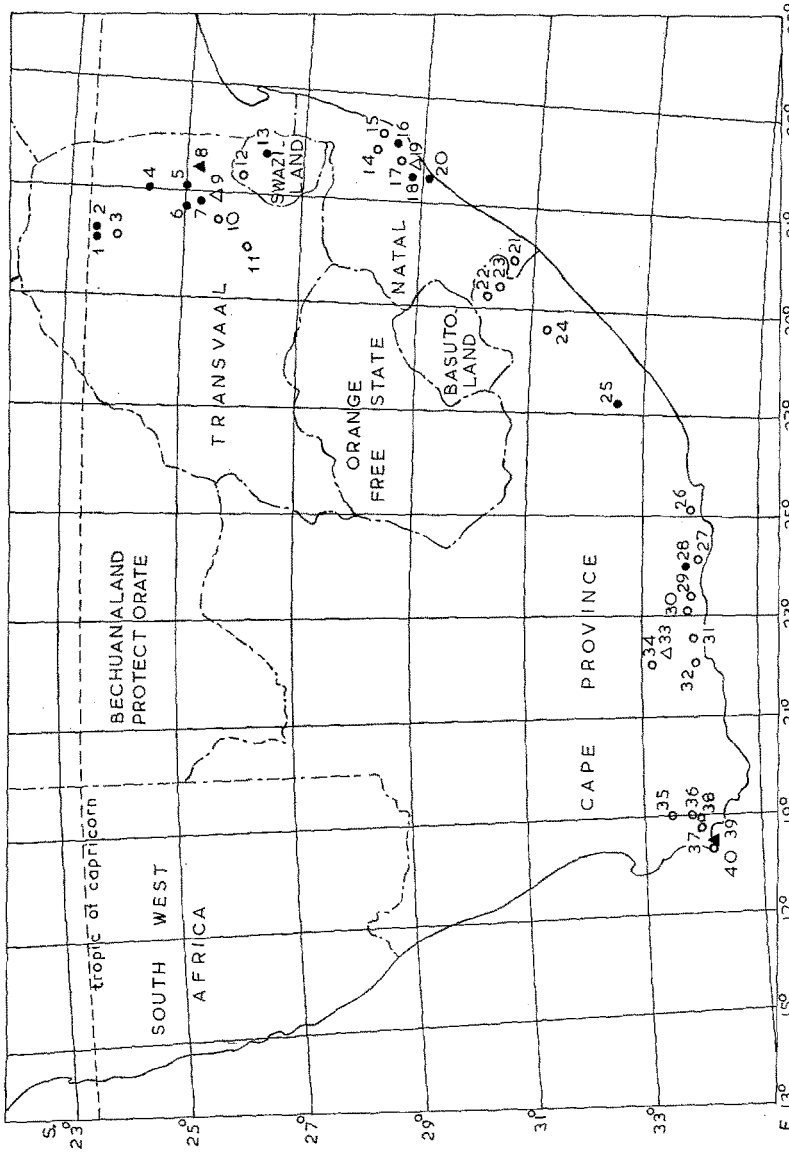


Fig. 1. Geographical distribution of the soil samples collected in the Union of South Africa. Notation: negative samples (○), soils containing *Beijerinckia* (●), *Azotobacter* (△) and both species (▲). The numbers correspond to the soil sample numbers presented in Table 1.

Climate – apart of its influence on soil type – also shows little influence. In northern Transvaal *Beijerinckia* occurs predominantly in the high-rainfall (40–66″) area in the Drakensberg Mountains, sometimes at rather high altitudes (3,000–6,000′) where occasional frosts occur. But this micro-organism is also present in the semi-arid savannah climate of the Low Veld with extremely hot day temperatures and in soils of the Tokai Arboretum with a moderate climate.

In contrast to the factors mentioned, soil type appears to have very great influence on the geographical and ecological distribution of *Beijerinckia*. This will be discussed more in detail in the next section.

DISCUSSION

The ecological and geographical conditions governing the remarkable tropical distribution of the genus *Beijerinckia* are not clearly understood.

In view of the fact that these micro-organisms have been usually reported from more or less acid soils, one may surmise that a low pH, by suppressing the competition of many other soil bacteria, might be a factor determining the presence of *Beijerinckia*. The negative outcome of tests with so many and diverse acid soils of temperate regions shows the untenability of this idea.

There is also no reason for the belief that the low temperature in the temperate zones may be responsible for the absence of *Beijerinckia* in these soils. Growth experiments with *Beijerinckia* strains at several temperatures showed no marked differences between temperature optima and minima of these bacteria and those of *Azotobacter* strains. In fact, the reverse was found to be true of what would have been expected from a tropical micro-organism as no *Beijerinckia* strain was found to produce growth at 37° C while most *Azotobacter* strains grew profusely at this temperature. Some *Azotobacter* strains still produced growth at 40° C. These experiments will be described in a subsequent paper.

Moreover, *Beijerinckia* is fairly cold and frost resistant. Cells of these micro-organisms suspended in tap water or nitrogen-free mineral medium and stored for twenty months at + 4° C or — 4° C in a refrigerator retained some viability as shown by growth when

TABLE 1

Sample No.	Locality	Lat. °S.	Annual rainfall, inches	Altitude Ft.	Sample No.
	TRANSVAAL				
1	De Hoek plantations, 7.5 miles N. of Mokeetsi, N. of Tzaneen	23.5	66	4000	1
2	„ „ „ 7.5 „ „ „ „ „ „ „ „ „ , Bruynzeel's Sawmill	23.5	66	4000	2
3	Westfalia estate, between Duivelskloof and Tzaneen	24.0	40	2800	3
4	Wilgenboom plantation, 12 miles NW. of Acornhoek	24.5	50	3000	4
5	Tweefontein plantation, road between Graskop and Sabie	25.0	48	4100	5
6	Witklip plantation, SE. of Sabie	25.0	45	3800	6
7	South African Forest Investment estate, Hendriksdal, 7 miles S. of Sabie	25.0	45	4400	7
8	Krüger National Park, Pretoriuskop	25.0	22	1060	8
9	Citrus and Subtropical Horticulture Research Station, Nelspruit	25.5	30	2350	9
10	Berlin plantation, 20 miles SW. of Nelspruit	25.5	42	4400	10
11	Jessievale plantation, 25 miles N. of Ermelo	26.0	36	5800	11
	SWAZILAND				
12	Piggspeak plantations, 10 miles N. of Piggspeak	26.0	47	4500	12
13	Usutu plantation, 30 miles NE. of Mbabane	26.0	45	4500	13
	NATAL				
14	Dududuku plantation, 10 miles NW. of St. Lucia	28.0	38	200	14
15	Vergoeding plantation, 8 miles NW. of St. Lucia	28.0	38	200	15
16	Kwambonambi, 14 miles NE. of Empangeni	28.5	50	100	16
17	5 miles N. of Empangeni	28.5	45	200	17
18	Port Durnford, 20 miles E. of Eshowe	29.0	60	300	18
19	„ „ 20 „ „ „ „	29.0	60	50	19
20	Amatikulu, 20 miles SE. of Eshowe	29.0	36	100	20
21	Weza plantations, 30 miles E. of Kokstad	30.5	45	4500	21
	CAPE PROVINCE				
22	Houtbosfontein plantation, 20 miles N. of Kokstad	30.5	45	5000	22
23	„ „ , 15 miles N. of Kokstad	30.5	45	3000	23
24	Arboretum Nqadu, 15 miles NW. of Umtata	31.5	39	2900	24
25	Forest Sanctuary, 20 miles W. of Stutterheim	32.5	42	2500	25
26	Loerie plantations, 30 miles SW. of Uitenhage	34.0	31	2400	26
27	Witelsbos, 60 miles W. of Humansdorp	34.0	45	700	27
28	Blueliliesbush, 80 miles W. of Humansdorp	34.0	40	700	28
29	Kransbos, 10 miles NE. of Knysna	34.0	31	1200	29
30	Concordia plantations, 10 miles N. of Knysna	34.0	38	800	30
31	Saasfeld, 15 miles E. of George	34.0	34	600	31
32	Kleinplaat, 20 miles W. of George	34.0	22	2000	32
33	Little Karroo, Olifant's river, 10 miles N. of Oudtshoorn	33.5	10	1100	33
34	Swartberg Pass, 10 miles S. of Prince Albert	33.5	18	3000	34
35	Kluitjeskraal, 1 mile W. of Wolseley	33.5	26	900	35
36	Franschhoek, Helshoogte, 5 miles NE. of Stellenbosch	34.0	29	1000	36
37	Jonkershoek, 10 miles SE. of Stellenbosch	34.0	42	900	37
38	„ 10 „ „ „ „	34.0	42	900	38
39	Tokai Arboretum, Muizenberg	34.0	38	200	39
40	Hout Bay, 10 miles S. of Cape Town	34.0	25	100	40

plated on nitrogen-free nutrient agar. Such extreme conditions are not likely to occur in temperate zones. Ecological evidence also points to a considerable cold tolerance of *Beijerinckia* as it was found to occur at rather high altitudes in the Red Earths of the

TABLE 1 (continued)

Sample No.	Frost	Soil type	Soil pH	Soil cover	B*	A**	Sample No.
1	Light	Red lateritic loam	5.8	*Eucalyptus microcorys* cultivation	+	—	1
2	,,	,, ,, ,,	6.0	Indigenous grassland	+	—	2
3	,,	,, ,, ,,	5.0	*Eucalyptus saligna* cultivation	—	—	3
4	,,	,, ,, ,,	5.3	*E. cloeziana* cultivation	+	—	4
5	Moderate	,, ,, ,,	4.5	*E. saligna* cultivation, 9 years old	+	—	5
6	Light	,, ,, ,,	5.4	*Pinus patula* cultivation	+	—	6
7	,,	,, ,, ,,	5.8	*P. patula* cultivation, 35 years old	+	—	7
8	None	Reddish-brown sandy soil	6.7	Savannah vegetation	+	+	8
9	,,	,, ,, ,,	6.6	*Citrus* cultivation	—	+	9
10	Moderate	Yellow-brown sandy soil	4.2	*Pinus patula* cultivation, old plantation	—	—	10
11	Severe	Red lateritic loam	4.6	*P. patula* cultivation, young plantation	—	—	11
12	Mild	Red lateritic earth	5.2	*Pinus patula* cultivation	—	—	12
13	,,	,, ,, ,,	4.5	*P. patula* cultivation, 6 years old	+	—	13
14	None	Light brown littoral sand	4.4	*Pinus caribaea* cultivation, 8 years old	—	—	14
15	,,	,, ,, ,, ,,	4.5	*Eucalyptus paniculata* cult., 3–4 years old	—	—	15
16	,,	Brown fine sand	4.8	*Pinus elliottii* cultivation	+	—	16
17	,,	Humus-rich forest soil	5.6	*Casuarina equisetifolia* cultivation	—	—	17
18	,,	Reddish-brown fine sand	4.5	*Eucalyptus maculata* cult., 30 years old	+	—	18
19	,,	Dark littoral sand	7.7	*Casuarina equisetifolia* reafforestation	—	+	19
20	,,	Yellow-brown ferruginous earth	5.9	Sugar-cane cultivation	+	—	20
21	Moderate	Yellow-brown lateritic loam	4.5	*Pinus patula* cultivation, 31 years old	+	—	21
22	,,	Red (eluvial) dolorite soil, slopes	5.7	Natural pasture	—	—	22
23	,,	Dark (illuvial) soil, valleys	5.4	Uncultivated	—	—	23
24	,,	Reddish ferruginous earth	4.3	Arboretum	—	—	24
25	Light	Red lateritic loam	4.4	Bare slope	+	—	25
26	,,	Gray fine loam	5.1	*Pinus pinaster* cultivation	—	—	26
27	,,	,, ,, ,,	5.1	*P. pinaster* and *P. radiata* cult., poor site	—	—	27
28	,,	,, ,, ,,	5.4	*P. taeda* cult., 41 years old, very good site, root development to 2 m	+	—	28
29	,,	,, ,, ,,	4.4	*P. taeda* cultivation	—	—	29
30	None	Dark sand (old dunes)	3.6	,, ,, ,,	—	—	30
31	Light	Grey ferruginous sand	5.9	Natural forest (groenbos)	—	—	31
32	,,	Dark sandy soil	3.6	Forest felling area	—	—	32
33	Severe	Heavy loam (riverside)	7.5	Cultivated soil	—	+	33
34	,,	Brown sand	4.7	Reafforestation of eroded land	—	—	34
35	Light	Iron-coated coarse sand	4.9	*Pinus taeda* cultivation	—	—	35
36	,,	Yellow-gray sandy soil	5.1	,, ,, ,,	—	—	36
37	,,	Dark litter-rich sandy soil	5.5	*Pinus radiata* cultivation	—	—	37
38	,,	Reddish-gray fine sand	5.1	Natural forest (fijnbos)	—	—	38
39	None	Garden soil	6.1	Arboretum	+	+	39
40	,,	Reddish coarse granitic sand	5.1	Indigenous vegetation	—	—	40

*B = presence of *Beijerinckia*. **A = presence of *Azotobacter*.

Ethiopian Mountain Plateau (Addis Ababa and Entoto hills at 8,300 and 9,300′, respectively), in the latosols of the East African Highlands (Kenya, 6,800′) and of the Drakensberg Mountains (4,000′) in South Africa, and in some mountain forest soils (6,700′

to 10,000') in Java. Several of these localities suffer occasional frosts.

We must therefore conclude that the occurrence of *Beijerinckia* is determined by some complex of ecological factors which is only realized in the tropics.

Several hypotheses have been developed to explain the tropical distribution of *Beijerinckia*.

Derx [4] attributes the tropical distribution to a possible association with some non nodule-forming tropical legumes of the genus *Caesalpiniaceae* (e.g. *Cassia tora*) and suggests that *Beijerinckia* is a facultative symbiont which in contrast to *Rhizobium* has not lost the power to fix atmospheric nitrogen outside the plant.

Ruinen [20] reports the abundant occurrence of *Beijerinckia* on leaves ("phyllosphere") of tropical plants. She is inclined to think that there exists in tropical regions a mutual benificial relationship between tropical plants and *Beijerinckia*. The plant would provide the bacterium through cuticular excretion with sugar and nutrient salts, whilst the bacterium would fix atmospheric nitrogen which might become available to the plant.

Kluyver and Becking [13] have drawn attention to the fact that in nearly all cases in which *Beijerinckia* has been isolated from soil, a tropical soil of the eluvial type, *i.e.* a lateritic soil (*e.g.* laterite, tropical Red Earth or latosol) is concerned. *Beijerinckia* is observed to be absent in tropical illuvial soils (*e.g.* calcareous Margalitic soils, Black Cotton soils, Regur or Mbuga soils) and in tropical alkaline alluvial (river or marine deposits) clay soils.

They suggest that the leached lateritic soils are a favourable habitat for *Beijerinckia*. If we accept the view that the various *Beijerinckia* species are especially adapted to lateritic soils, we have to a certain extent an explanation of their restriction to the tropics as this type of rock weathering and clay mineral synthesis is only clearly displayed in the tropics.

From the geographical map (Fig. 1) it is evident that most *Beijerinckia* positive localities are situated between longitude 29° and 32° E. and latitude 23° and 29° S. This region comprises the Drakensberg Mountains forming a wall-like barrier from south to north extending form the SE. point of the Basuto Highlands, along the western boundery of Natal and the eastern side of Swaziland (Mbabane) into Transvaal past Pilgrims Rest, Tzaneen to SE. of Louis Trichardt in Northern Transvaal.

The escarpment of the Drakenberg Mountain rises abruptly from the Low Veld forming a transitional belt between the Low Veld and the High Veld north of Basutoland and the Low Veld and the Middle Veld in the

north. These mountain barriers, towering from 2,000 to 5,000 feet above the Low Veld, intercept the moisture-laden winds from the Indian Ocean, causing an increased precipitation on its eastern slope. This area – generally known as the "Mistbelt" – experiences the highest rainfall and the most humid atmosphere of the whole of South Africa.

Van der Merwe [17] (p. 182), in the publication "Soil groups and subgroups of South Africa", remarks: "Coincident with the "Mistbelt" there occur soil zones unique in South Africa. Although these zones differ from each other morphologically and chemically, they are classified as (a) Laterites, (b) Lateritic Red Earths and (c) Lateritic Yellow Earths". He classifies the soils near Sabie and Graskop (soil samples No's 5, 6, 7) as belonging to laterites, those near Tzaneen (soil samples No's 1, 2) and Mbabane (soil sample No. 13) as lateritic Red Earths, and those of Jessievale (soil sample No. 11) as lateritic Yellow Earth.

According to the geological map of the Geological Survey 1955, these lateritic soils of the "Mistbelt" are mainly derived from acid igneous rock, Old Granite of archaeozoic origin.

The remarkable coincidence given above between the distribution of *Beijerinckia* and the presence of laterites and lateritic Earths in South Africa strengthens our suggestion that *Beijerinckia* is mainly associated with lateritic soil types. Moreover it provides the first evidence of an earlier prediction of ours [13] that this micro-organism may occur also in lateritic soils outside the tropics.

The other soils observed to be positive for *Beijerinckia* are more or less ferruginous acid forest soils, near Stutterheim and Blue-liliesbush. The presence of *Beijerinckia* in a garden soil of the Tokai Arboretum on the Cape Peninsula indicates its occurrence in South Africa as far south as Cape Town lat. 34° S.

The abundant distribution of *Beijerinckia* in the latosols of the "Mistbelt" may be of some value in the nitrogen economy of these soil types. The pH values of these soils are mostly fairly low so that *Azotobacter* is absent.

As is well known, laterization leads to an accumulation of iron and aluminium in the upper soil layers and to a leaching away of silica, potassium, sodium and magnesium and more particularly calcium. The soils are moreover mostly phosphorus deficient owing to phosphate fixation by iron-aluminium complexes. Judged by the high electrical resistance, these soils contain very little soluble salts. The soils have a characteristic low base-exchange capacity due to the dominant presence of the clay mineral kaolinite.

It is tempting to relate the extreme poverty in calcium of the

natural surroundings of *Beijerinckia* with Jensen's [7 8] observation that these bacteria, in contrast to *Azotobacter*, require no calcium for their development. This observation has been confirmed by the present author. According to a recent publication by Norris and Jensen [18], however, calcium is not essential for the growth of *Azotobacter agile* either, although they found it to have a more or less stimulatory effect.

In these lateritic soils the accumulation of sesquioxides of iron and aluminium combined with a high acidity leads to a rather high content of mobile iron and aluminium and a low concentration of phosphate in the soil environment. The occurrence of *Beijerinckia* under these particular chemical soil conditions corresponds with our observations that *Beijerinckia* requires far more iron and less phosphate for optimal development than *Azotobacter*. Moreover, Katznelson [10] has shown that *Beijerinckia* (=*Azotobacter indicum*) is considerably less susceptible to the toxic effect of free aluminium than *Azotobacter* strains. A more detailed account on the mineral nutrition of *Beijerinckia* will be published in a separate paper.

SUMMARY

In contrast to the ubiquitous distribution of the nitrogen-fixing bacteria of the genus *Azotobacter* which can be encountered in all soils with suitable pH, the nitrogen-fixing bacteria of the genus *Beijerinckia* seem to be restricted to the tropics.

All attempts to isolate *Beijerinckia* from north-european soils have so far failed. The conditions underlying this peculiar phenomenon are not clearly understood. The selective action of low soil pH, low temperature, frost resistance, etc. cannot be responsible for the absence of *Beijerinckia* from temperate zones.

In an earlier paper [13] it was emphasized that the presence of *Beijerinckia* is almost invariably connected with the occurrence of laterites, tropical Red Earths or latosols. As these soil types are almost restricted to the tropics, this may be the reason why the genus *Beijerinckia* is mainly confined to tropical regions.

It was also suggested in that paper that *Beijerinckia* might also occur in lateritic soils – fossil or developed under particular climatic conditions – outside the tropics.

The present paper gives the first evidence of the occurrence of *Beijerinckia* in lateritic soils outside the tropics in South Africa. There *Beijerinckia* occurs abundantly in the lateritic soils of the "Mistbelt", but it occurs also – although more sporadically – in soils as far south as Cape Town (lat. 34° S.).

Hence, non-symbiotic nitrogen fixation may occur in some of these soils which are too acid to contain *Azotobacter*.

ACKNOWLEDGEMENTS

The soil samples were collected by Prof. Dr. J. H. Becking of the Department of Forestry, Agricultural University of Wageningen, The Netherlands, during a study tour in the Union of South Africa from the 5thNovember 1957 till 5th January 1958.

The author also wishes to express his indebtedness to the Department of Forestry at Pretoria for checking the geophysical data mentioned in Table 1.

Received October 15, 1958

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