Studies on the Nodule Bacteria. VIII.

Influence of Ash Content of the Nodules on the Growth of Nodule Bacteria with a Special Reference to the Titanium Salts.

By

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In a preceding paper[1], it was reported that the nodules of leguminous plants stimulated the growth of nodule bacteria best of all the parts used. In this paper, the influence of ash contents in the nodules was investigated with a special reference to the titanium salts, and the results are presented.

It was reported by KONISHI and his co-worker[2] that the nodules are rich with titanium as their results of spectrum analysis, and that the addition of titanium increased the formation of nodules and it was suggested that titanium may have some intimate relation with the physiological activities of nodule bacteria.

Experimental.

I. *Cultures Used :*

The cultures were the same as used in the previous investigation, namely Nodule bacterial strains A, B and C of Genge and one of each bean and clover.

II. *Chemical Analysis of the Nodules and other Parts of Bean Plant :*

The samples were burnt to ashes and analysed for various inorganic constituents, as noted in table 1, in the soluble portion, in hot hydrochloric acid. Titanium was determined by HILDEBRAND'S[4),5)] method which is carried out as follows: the HCl solution is evaparated to dryness and taken up with $KHSO_4$ solution to which H_2O_2 is added and the quantity of titanium is determined colorimetrically. The results of analysis are shown in Table 1.

(See Table 1 on next page.)

Table 1 indicates that the ash content was largest in leaves and stems which amounted to 8.016 percent and the least in the nodules, 3.013 percent on the dry matter basis. The insoluble matter in hot HCl was rather high in the roots and

Table 1.

Inorganic Constituents in various Parts of Bean Plants.

Constituents.	Dry matter.				Ash.			
	Seeds.	Stem and leaves.	Roots.	Nodules.	Seeds.	Stem and leaves.	Roots.	Nodules.
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Ash.	4.285	8.016	4.947	3.013	100.000	100.000	100.000	100.000
Insol. matter in HCl.	0.857	0.865	0.617	0.202	1.330	10.791	12.472	6.704
SO_3	0.011	0.188	0.101	0.037	0.252	2.340	2.038	1.221
Fe_2O_3	0.014	0.023	0.053	0.031	0.320	0.287	1.063	1.019
CaO	0.230	0.898	0.458	0.335	5.356	11.199	9.266	11.112
Na_2O	0.418	0.799	0.632	0.406	9.757	9.971	12.775	13.468
K_2O	1.276	1.299	0.934	0.668	29.767	16.201	18.874	22.184
MgO	0.443	0.959	0.418	0.411	10.336	11.966	8.450	13.641
P_2O_5	1.300	2.340	1.044	0.671	30.338	29.189	21.100	22.273
TiO_2	0.000	0.000	trace.	trace.	0.000	0.000	trace.	trace.

leaves-stems while very low in the seeds. The sulfate salts were low in all parts but comparatively high in the leaves and stems. The iron content was also rather small except in the roots and nodules which contained comparatively large amount. The calcium content was highest in the seeds. Na_2O was found in the order of nodules, roots, leaves and stems and seeds while the distribution of K_2O was different, the order being the seeds, nodules, roots, leaves and stems; MgO was highest in the nodules and lowest in the roots; P_2O_5 was highest in the seeds and least in the roots; TiO_2 was found only in trace in the roots and nodules. As noted above, a considerable amount of P_2O_5, K_2O, Na_2O, MgO and CaO were found while the others were very small quantity. The presence of MgO in the nodules is noteworthy although it was found in other parts as well, so that it may not have special significance on the nodule bacteria. However it is worthy to note that even a trace of TiO_2 was found only in the roots and nodules. Consequently further quantitative determination of TiO_2 was carried out by extracting it with $KHSO_4$ directly from the ash instead of the hot HCl solution since it is difficultly dissolved in hot HCl. The results are given in Table 2.

Table 2.

Titanium Contents in various Parts of Bean Plants. (P. P. M.)

	Seeds.	Stem and leaves.	Roots.	Nodules.
Dry matter.	0.00000	0.43458	1.73059	1.54448
Ash.	0.00000	5.52502	34.45304	52.31001

As shown above, a small quantity of TiO_2 was found in all parts except the seeds, and greatest in the nodules followed by the roots.

1.) *Influence of ash constituents of the bean nodules on the growth of nodule bacteria.*

In place of the yeast extract, the ash corresponding to 1 percent of the original nodule was added to the mannit agar medium, and the growth of nodule bacteria was examined by the naked eyes and also by the weight of bacterial cells. The results are given in Table 3.

Table 3.

Influence of Ash Constituents of Bean Nodules on the Growth of Genge, Bean and Clover Nodule Bacteria.

Nodule bacteria.	Substance added.	Rate of growth by days.				Weight of bacterial cell after 7 days. (mg.)
		2	4	7	Sum of +.	
Genge Strain A.	Control.	+	+	+	3	2.1
	Yeast.	+	|||	|||	9	5.4
	1% Nodule.	+	|||	||||	11	14.8
	Ash A.	+	+	+	3	1.4
	Ash B.	+	+	+	3	2.8
Genge Strain B.	Control.	+	+	+	3	6.9
	Yeast.	+	|||	|||	8	11.3
	1% Nodule.	+	|||	||||	11	17.4
	Ash A.	+	+	+	3	3.4
	Ash B.	+	+	+	3	4.6
Genge Strain C.	Control.	+	+	+	3	3.2
	Yeast.	+	|||	|||	9	11.7
	1% Nodule.	+	|||	||||	11	13.4
	Ash A.	+	+	+	3	5.2
	Ash B.	+	+	+	3	3.0
Bean.	Control.	+	++	++	5	5.8
	Yeast.	+	||||	||||	10	12.1
	1% Nodule.	+	|||	||||	11	18.4
	Ash A.	+	+	+	3	5.0
	Ash B.	+	+	+	3	4.5
Clover.	Control.	+	++	++	5	2.6
	Yeast.	++	|||	|||	11	9.4
	1% Nodule.	++	||||	||||	13	14.8
	Ash A.	+	++	++	5	3.7
	Ash B.	+	++	+++	6	5.0

Notes: The number of + indicates the rate of growth.

Table 3 indicates that the addition of nodules stimulated the growth very markedly which was followed by the yeast extracts while the ash did not show any benefical effect and almost equal to the control.

2.) Influence of titanium salts on the growth of nodule bacteria.

The same experimental procedure was used as in the preceding experiment except various concentrations of titanic acid [$Ti(OH)_4$], titanium sulfate and potas-

Table 4.

Influence of Titanium Salts on the Growth of Genge Nodule Bacteria, Strain A.

Ti salts.	Conc. (%)	Without yeast extracts. Rate of growth by days. 2	4	7	14	Sum of +.	Morphology on 14th day. Form.	Size. (μ)
Control.		+	++	++	++	7	Short rod.	0.3×0.6–0.4×1.0
Titanic acid.	5.000	–	–	–	–	0	—	—
	2.000	–	–	–	–	0	—	—
	1.000	–	–	+	+	2	Short rod.	0.3×0.6–0.4×1.0
	0.500	–	+	+	+	3	Rod.	0.3×0.6–0.4×1.2
	0.100	+	+	+	+	4	Short rod.	0.3×0.5–0.4×1.0
	0.050	+	+	+	+	4	Rod.	0.3×0.5–0.4×1.2
	0.010	+	+	+	++	5	Short rod.	0.3×0.5–0.4×1.0
	0.005	+	++	++	++	7	„	0.3×0.5–0.4×1.0
	0.001	+	++	++	++	7	„	0.3×0.5–0.4×0.8
Titanium sulfate.	0.500	. . .	. . .	. . .	. . .	. . .	. . . . .	. . . . .
	0.100	+	+	+	+	4	Short rod.	0.3×0.5–0.4×0.8
	0.050	+	+	+	+	4	„	0.3×0.6–0.4×1.0
	0.010	+	+	++	++	6	„	0.3×0.5–0.4×0.8
	0.005	+	++	++	++	7	Rod.	0.3×0.6–0.4×1.2
	0.001	+	++	++	++	7	„	0.3×0.6–0.4×0.8
Potassium acid titanate.	5.000	–	–	–	–	0	—	—
	2.000	–	–	–	–	0	—	—
	1.000	–	–	–	–	0	—	—
	0.500	–	–	–	–	0	—	—
	0.100	–	+	+	+	3	Coccic.	0.3–0.4
	0.050	+	+	++	++	6	Rod.	0.3×0.5–0.4×1.2
	0.010	+	+	++	++	6	Short rod.	0.3×0.5–0.4×0.8
	0.005	+	+	++	++	6	„	„
	0.001	+	+	+	+	4	Coccic-short rod.	0.3×0.4–0.4×0.5

sium acid titanate were added to the mannit agar medium, and the growth was examined by the naked eyes and the morphological examination was carried out microscopically staining with Carbol fuchsin.

The data in Table 4 indicate that while the yeast extracts have marked influence on the growth, the titanium salts have very slight influence. As the concentration of titanate increases, the growth became worse and 0.1% was the limit for the growth while very small quantity caused stimulation. Morphologi-

Table 4. (Continued.)

Ti salts.	Conc. (%)	With yeast extracts. Rate of growth by days. 2	4	7	14	Sum of +	Morphology on 14th day. Form.	Size. (μ)
Control.		+	++++	++++	++++	10	Short rod.	0.2×0.4 – 0.3×0.8
Titanic acid.	5.000	–	–	–	–	0	—	—
	2.000	–	–	–	–	0	—	—
	1.000	–	–	–	–	0	—	—
	0.500	–	+	+	+	3	Coccic-short rod.	0.3×0.4 – 0.4×0.8
	0.100	++	++	++	++++	9	Rod.	0.3×0.5 – 0.4×1.2
	0.050	++	++++	++++	++++	11	Short rod.	0.3×0.5 – 0.5×1.0
	0.010	+	++++	++++	++++	10	"	"
	0.005	+	++++	++++	++++	10	Rod.	0.3×0.6 – 0.5×1.3
	0.001	++	++	++	++++	9	Short rod-coccic.	0.3×0.4 – 0.4×0.8
Titanium sulfate.	0.500	–	+	+	+	3	Rod.	0.2×0.5 – 0.6×1.2
	0.100	+	++++	++++	++++	10	Short rod.	0.3×0.4 – 0.4×0.8
	0.050	+	++++	++++	++++	10	Rod.	0.3×0.5 – 0.5×1.5
	0.010	++	++++	++++	+++++	12	Short rod.	0.2×0.4 – 0.4×0.8
	0.005	++	+++++	+++++	+++++	14	Rod.	0.2×0.5 – 0.4×1.2
	0.001	++	++++	++++	+++++	12	"	0.3×0.5 – 0.7×1.5
Potassium acid titanate.	5.000	–	–	–	–	0	—	—
	2.000	–	–	–	–	0	—	—
	1.000	–	–	–	–	0	—	—
	0.500	–	–	–	–	0	—	—
	0.100	–	+	+	++	4	Short rod-coccic.	0.3×0.4 – 0.6×0.8
	0.050	–	++	++	++	6	Short rod.	0.3×0.5 – 0.5×1.0
	0.010	++	++++	++++	+++++	12	Rod.	0.3×0.5 – 0.6×1.2
	0.005	++	+++++	+++++	+++++	14	Short rod.	0.3×0.5 – 0.6×1.0
	0.001	++	+++++	++++++	++++++	16	"	0.2×0.5 – 0.5×0.8

Notes: The number of + indicates the rate of growth.

cally no great variation was noted except some bacteroids were noted where the 0.005% titanic acid and 0.001% titanium sulfate were added.

Same experiment as the preceding one was carried out with Strain B of Genge nodule bacteria, and the results are reported in Table 5.

The results indicate that titanic acid stimulates the growth and the optimum concentration was 0.05% in the presence of yeast extract and when no

Table 5.

Influence of Titanium Salts on the Growth of Genge Nodule Bacteria, Strain B.

Ti salts.	Conc. (%)	Without yeast extracts.						
		Rate of growth by days.					Morphology on 14th day.	
		2	4	7	14	Sum of +.	Form.	Size. (μ)
Control.		+	++	++	++	7	Short rod.	0.3×0.6–0.4×1.0
Titanic acid.	5.000	−	−	−	−	0	—	—
	2.000	−	−	−	−	0	—	—
	1.000	−	−	+	+	2	Short rod.	0.3×0.5–0.4×1.0
	0.500	−	+	+	+	3	„	„
	0.100	+	+	+	+	4	„	„
	0.050	+	++	++	++	7	„	0.3×0.6–0.4×0.8
	0.010	+	+++	+++	+++	10	„	0.3×0.5–0.4×1.0
	0.005	+	+++	+++	+++	10	„	0.3×0.5–0.4×0.8
	0.001	+	+++	+++	+++	10	„	0.3×0.5–0.4×0.8
Titanium sulfate.	0.500	. . .	. . .	. . .	. . .	. . .	. . . . .	. . . . .
	0.100	−	+	++	++	5	Short rod.	0.3×0.5–0.4×1.0
	0.050	+	++	++	++	7	„	0.3×0.5–0.4×0.8
	0.010	+	++	+++	+++	9	Rod.	0.3×0.6–0.4×1.2
	0.005	+	+++	+++	+++	10	Short rod.	0.3×0.5–0.4×0.8
	0.001	+	++	++	++	7	„	0.2×0.5–0.4×1.0
Potassium acid titanate.	5.000	−	−	−	−	0	—	—
	2.000	−	−	−	−	0	—	—
	1.000	−	−	−	−	0	—	—
	0.500	−	−	−	−	0	—	—
	0.100	−	−	−	−	0	—	—
	0.050	−	−	−	−	0	—	—
	0.010	+	+	++	++	6	Short rod.	0.3×0.5–0.4×0.8
	0.005	+	++	++	++	7	„	„
	0.001	+	++	++	++	7	„	0.3×0.6–0.4×1.0

yeast extract was present, even in 1.0% concentration some growth was observed. The optimum concentration of titanium sulfate was 0.005% while that of potassium acid titanium was 0.001% and the limit for the growth was 0.1 and 0.01% in the presence and absence of yeast extract respectively. Morphologically only slight change was observed except some bacteroids were found in 0.005% titanium sulfate.

Table 5. (Continued.)

Ti salts.	Conc. (%)	With yeast extracts.						
		Rate of growth by days.					Morphology on 14th day.	
		2	4	7	14	Sum of +.	Form.	Size. (μ)
Control.		++	++++	++++	++++	14	Rod.	0.3×0.5–0.4×1.2
Titanic acid.	5.000	–	–	–	–	0	—	—
	2.000	–	–	–	–	0	—	—
	1.000	–	–	–	–	0	—	—
	0.500	–	+	+	+	3	Rod.	0.3×0.4–0.4×1.5
	0.100	++	++++	++++	++++	13	Short rod.	0.3×0.5–0.4×0.8
	0.050	++	+++++	+++++	+++++	17	Rod-oval.	0.3×0.5–0.6×1.2
	0.010	++	++++	++++	++++	14	Short rod-coccic.	0.3×0.4–0.4×0.7
	0.005	++	++++	++++	++++	14	Short rod.	0.3×0.5–0.4×1.0
	0.001	+++	++++	++++	++++	15	Short rod-coccic.	0.3×0.5–0.5×0.8
Titanium sulfate.	0.500	–	+	+	++	4	„	0.3×0.4–0.5×1.0
	0.100	+	+++	+++	+++	10	Short rod.	0.2×0.5–0.4×1.0
	0.050	+	+++	+++	+++	10	Rod.	0.2×0.5–0.3×1.2
	0.010	++	+++	++++	++++	13	Short rod.	0.3×0.5–0.4×1.0
	0.005	++	+++++	+++++	+++++	17	Rod.	0.3×0.5–0.6×1.5
	0.001	++	++++	++++	++++	14	Short rod.	0.2×0.5–0.4×1.0
Potassium acid titanate.	5.000	–	–	–	–	0	—	—
	2.000	–	–	–	–	0	—	—
	1.000	–	–	–	–	0	—	—
	0.500	–	–	–	–	0	—	—
	0.100	+	+	++	++	6	Rod.	0.3×0.5–0.6×1.5
	0.050	+	+++	+++	+++	10	Short rod.	0.3×0.5–0.5×0.8
	0.010	++	++++	++++	+++++	15	„	0.2×0.4–0.3×1.0
	0.005	++	++++	+++++	+++++	16	Coccic.	0.3×0.4–0.4×0.7
	0.001	++	++++	+++++	+++++	16	Short rod.	0.2×0.5–0.5×0.7

Notes: The number of + indicates the rate of growth.

Further the similar experiment was carried out with Strain C of Genge nodule bacteria, and the results are presented in Table 6.

As noted in Table 6, titanic acid has no stimulation where the yeast extract was absent while in its presence 0.1% stimulated the growth fairly well, and 1.0 and 0.5% were the limit for the growth in the absence and presence of yeast

Table 6.

Influence of Titanium Salts on the Growth of Genge Nodule Bacteria, Strain C.

Ti salts.	Conc. (%)	Without yeast extracts. Rate of growth by days. 2	4	7	14	Sum of +.	Morphology on 14th day. Form.	Size. (μ)
Control.		+	++	++	++	7	Short rod.	0.3×0.6–0.4×1.0
Titanic acid.	5.000	–	–	–	–	0	—	—
	2.000	–	–	–	–	0	—	—
	1.000	–	–	+	+	2	Short rod.	0.3×0.5–0.4×1.0
	0.500	–	+	+	+	3	Rod.	0.3×0.5–0.4×1.2
	0.100	–	++	++	++	6	Short rod.	0.3×0.5–0.4×0.8
	0.050	+	++	++	++	7	„	0.3×0.5–0.4×0.8
	0.010	+	++	++	++	7	„	„
	0.005	+	++	++	++	7	Coccic-short rod.	0.3×0.4–0.4×0.8
	0.001	+	++	++	++	7	Short rod.	0.3×0.5–0.4×0.8
Titanium sulfate.	0 500	. . .	. . .	. . .	. . .	. . .	. . . . .	. . . . .
	0.100	–	–	+	+	2	Short rod.	0.3×0.5–0.4×1.0
	0.050	+	++	++	++	7	„	0.3×0.5–0.4×0.8
	0.010	+	++	++	++	7	„	„
	0.005	+	++	++	++	7	„	„
	0.001	+	++	++	++	7	„	0.3×0.5–0.4×0.8
Potassium acid titanate.	5.000	–	–	–	–	0	—	—
	2.000	–	–	–	–	0	—	—
	1.000	–	–	–	–	0	—	—
	0.500	–	–	–	–	0	—	—
	0.100	–	–	–	–	0	—	—
	0.050	–	–	–	+	1	Coccic.	0.3–0.4
	0.010	+	++	++	++	7	Short rod-coccic.	0.3×0.4–0.4×0.8
	0.005	+	++	++	++	7	Short rod.	0.3×0.5–0.4×0.8
	0.001	+	++	++	++	7	„	0.3×0.5–0.4×1.0

extract. Titanium sulfate has no stimulation at all as well as potassium acid titanium. No marked morphological change was observed.

The similar experiment was carried out as the foregoings, with the bean nodule bacteria, and the results are given in Table 7.

Table 6. (Continued.)

Ti salts.	Conc. (%)	With yeast extracts. Rate of growth by days. 2	4	7	14	Sum of +.	Morphology on 14th day. Form.	Size. (μ)
Control.		++	++	+++	+++	11	Short rod-rod.	0.3×0.4 – 0.4×0.8
Titanic acid.	5.000	–	–	–	–	0	—	—
	2.000	–	–	–	–	0	—	—
	1.000	–	–	–	–	0	—	—
	0.500	–	+	+	+	3	Short rod.	0.3×0.5 – 0.5×1.0
	0.100	++	+++	+++	+++	13	"	0.3×0.5 – 0.4×0.8
	0.050	++	++	+++	+++	11	Rod.	0.3×0.5 – 0.4×1.2
	0.010	++	++	+++	+++	11	Short rod.	0.3×0.5 – 0.4×0.8
	0.005	++	+++	+++	+++	12	"	0.2×0.5 – 0.5×1.0
	0.001	++	++	++	+++	9	"	0.3×0.5 – 0.5×0.8
Titanium sulfate.	0.500	–	+	+	+	3	Short rod-coccic.	0.2×0.4 – 0.4×1.0
	0.100	+	+	++	+++	7	Coccic-short rod.	0.3×0.5 – 0.5×0.9
	0.050	+	++	+++	+++	9	Short rod.	0.2×0.5 – 0.3×0.8
	0.010	+	++	+++	+++	9	Rod.	0.3×0.5 – 0.5×1.2
	0.005	++	++	+++	+++	10	"	0.2×0.5 – 0.4×1.2
	0.001	+	++	++	+++	8	"	0.2×0.5 – 0.5×1.5
Potassium acid titanate.	5.000	–	–	–	–	0	—	—
	2.000	–	–	–	–	0	—	—
	1.000	–	–	–	–	0	—	—
	0.500	–	–	–	–	0	—	—
	0.100	+	+	++	++	6	Short rod-coccic.	0.3×0.4 – 0.5×0.7
	0.050	–	+	++	+++	6	Short rod.	0.3×0.5 – 0.4×1.0
	0.010	++	++	+++	+++	10	"	0.3×0.5 – 0.5×1.0
	0.005	++	+++	+++	+++	11	"	0.2×0.5 – 0.4×1.0
	0.001	++	++	+++	+++	10	Rod.	0.2×0.5 – 0.5×1.2

Notes: The number of + indicates the rate of growth.

As shown below the growth of bean nodule bacteria was stimulated by 0.01% concentration of titanic acid with the yeast extract and no action without it; the

Table 7.

Influence of Titanium Salts on the Growth of Bean Nodule Bacteria.

Ti salts.	Conc. (%)	Without yeast extracts.						
		Rate of growth by days.					Morphology on 14th day.	
		2	4	7	14	Sum of +.	Form.	Size. (μ)
Control.		+	++	++	++	7	Rod.	0.3×0.5–0.4×1.3
Titanic acid.	5.000	−	−	−	−	0	—	—
	2.000	−	−	+	+	2	Rod.	0.3×0.5–0.4×1.5
	1.000	−	+	+	+	3	„	0.3×0.7–0.6×1.8
	0.500	−	+	+	+	3	„	0.3×0.5–0.5×1.2
	0.100	+	++	+++	+++	9	Short rod.	0.3×0.8–0.5×1.0
	0.050	−	+++	+++	+++	9	„	0.3×0.5–0.4×0.8
	0.010	−	+++	+++	+++	9	„	0.3×0.5–0.4×1.0
	0.005	+	++	++	++	7	„	„
	0.001	+	++	++	++	7	„	0.3×0.5–0.5×1.0
Titanium sulfate.	0.500	. . .	. . .	. . .	. . .	. . .	. . . . .	. . . . .
	0.100	+	+	+	+	4	Short rod.	0.3×0.5–0.4×0.8
	0.050	−	++	++	++	6	„	„
	0.010	−	++	++	++	6	„	0.3×0.5–0.4×1.0
	0.005	+	+++	+++	+++	10	„	0.3×0.5–0.5×1.0
	0.001	+	++	++	++	7	„	0.3×0.8–0.4×1.0
Potassium acid titanate.	5.000	−	−	−	−	0	—	—
	2.000	−	−	−	−	0	—	—
	1.000	−	−	−	−	0	—	—
	0.500	−	−	−	−	0	—	—
	0.100	−	−	−	−	0	—	—
	0.050	−	−	+	+	2	Short rod.	0.3×0.5–0.4×0.8
	0.010	−	+	+	+	3	„	0.3×0.5–0.4×1.0
	0.005	+	++	++	+++	8	Rod.	0.3×0.5–0.4×1.2
	0.001	+	++	++	+++	8	Short rod.	0.3×0.5–0.5×0.8

limit was 2.0 and 1.0% without and with the yeast extract respectively. The optimum concentration of titanium sulfate was 0.001 and 0.005% with and without

Table 7. (Continued.)

Ti salts.	Conc. (%)	With yeast extracts. Rate of growth by days. 2	4	7	14	Sum of +.	Morphology on 14th day. Form.	Size. (μ)
Control.		++	++	++	++	8	Short rod.	0.2×0.5 – 0.3×0.7
Titanic acid.	5.000	–	–	–	–	0	—	—
	2.000	–	–	–	–	0	—	—
	1.000	–	–	+	+	2	Short rod.	0.2×0.5 – 0.4×0.8
	0.500	–	++	++	++	6	„	0.2×0.4 – 0.3×1.0
	0.100	++	++	+++	+++	10	„	0.2×0.5 – 0.4×0.8
	0.050	+	++	++++	++++	11	„	0.3×0.5 – 0.5×1.0
	0.010	++	++++	++++	++++	14	„	0.2×0.5 – 0.5×1.0
	0.005	++	+++	+++	+++	11	„	0.2×0.5 – 0.5×0.8
	0.001	++	+++	+++	+++	11	„	0.2×0.4 – 0.3×1.0
Titanium sulfate.	0.500	–	+	+	+	3	Rod.	0.1×0.5 – 0.2×1.2
	0.100	+	++	++	+++	8	Short rod.	0.2×0.4 – 0.3×0.7
	0.050	+	+++	+++	+++	10	„	0.2×0.5 – 0.3×0.8
	0.010	+	+++	+++	+++	10	„	0.2×0.5 – 0.3×1.0
	0.005	+	+++	+++	+++	10	„	0.2×0.5 – 0.5×1.0
	0.001	++	++++	++++	++++	14	Rod.	0.2×0.5 – 0.5×1.5
Potassium acid titanate.	5.000	–	–	–	–	0	—	—
	2.000	–	–	–	–	0	—	—
	1.000	–	–	–	–	0	—	—
	0.500	–	–	–	–	0	—	—
	0.100	+	+	+	++	5	Short rod.	0.2×0.5 – 0.4×1.0
	0.050	+	++	++	++	7	„	0.2×0.5 – 0.3×1.0
	0.010	+	+++	+++	+++	10	Short rod-coccic.	0.3×0.5 – 0.5×1.0
	0.005	++	+++	+++	+++	11	„	0.2×0.5 – 0.5×1.0
	0.001	++	+++	+++	+++	11	Rod.	0.2×0.5 – 0.4×1.0

Notes: The number of + indicates the rate of growth.

the yeast extract respectively. Some bacteroids were observed only in 0.001% titanium sulfate with the yeast extract.

Table 8.

Influence of Titanium Salts on the Growth of Clover Nodule Bacteria.

Ti salts.	Conc. (%)	Without yeast extracts.						
		Rate of growth by days.					Morphology on 14th day.	
		2	4	7	14	Sum of +.	Form.	Size. (μ)
Control.		+	++	+++	+++	9	Rod.	0.3×0.8–0.6×2.0
Titanic acid.	5.000	−	−	−	−	0	—	—
	2.000	−	+	+	+	3	Rod.	0.3×0.5–0.4×2.0
	1.000	−	+	++	++	5	„	0.3×0.8–0.8×3.0
	0.500	+	++	+++	+++	9	„	0.3×0.8–0.7×4.0
	0.100	+	++	+++	+++	9	„	0.3×0.8–0.7×2.5
	0.050	+	+++	+++	+++	10	„	0.4×0.8–0.7×2.0
	0.010	+	++	+++	+++	9	„	0.3×0.7–0.6×2.0
	0.005	+	+++	+++	+++	10	„	0.3×1.0–0.6×2.0
	0.001	+	+++	+++	+++	10	„	0.4×1.0–0.8×3.0
Titanium sulfate.	0.500	. . .	. . .	. . .	. . .	. . .	. . . . .	. . . . .
	0.100	−	+	++	++	5	Rod.	0.5×0.7–0.7×2.5
	0.050	+	++	++	++	7	„	0.3×0.8–0.6×2.0
	0.010	+	+++	+++	+++	12	„	0.3×0.5–0.6×1.8
	0.005	+	++	+++	+++	9	„	0.3×0.8–0.6×2.0
	0.001	+	+++	+++	+++	10	„	0.3×0.8–0.4×2.5
Potassium acid titanate.	5.000	−	−	−	−	0	—	—
	2.000	−	−	−	−	0	—	—
	1.000	−	−	−	−	0	—	—
	0.500	−	−	−	−	0	—	—
	0.100	−	−	−	−	0	—	—
	0.050	−	−	−	−	0	—	—
	0.010	−	+	+++	+++	7	Rod.	0.3×0.8–0.6×2.0
	0.005	+	+++	+++	+++	10	„	„
	0.001	+	+++	+++	+++	10	„	0.3×0.8–0.5×2.0

Again the similar experiment was carried out with clover nodule bacteria and the results are noted in Table 8.

Table 8. (Continued.)

Ti salts.	Conc. (%)	With yeast extracts. Rate of growth by days. 2	4	7	14	Sum of +.	Morphology on 14th day. Form.	Size. (μ)
Control.		++	++	++	++	17	Rod.	0.3×0.6 – 0.5×1.2
Titanic acid.	5.000	–	–	–	–	0	—	—
	2.000	–	–	–	–	0	—	—
	1.000	+	+	+	+	4	Rod.	0.3×0.4 – 0.5×2.0
	0.500	+	++	++	++	10	„	0.3×0.5 – 0.5×2.0
	0.100	++	++	++	++	19	Oval.	0.3×0.5 – 0.7×1.0
	0.050	++	++	++	++	17	„	0.5×0.8 – 0.7×1.5
	0.010	++	++	++	++	18	Rod.	0.3×0.5 – 0.6×1.5
	0.005	++	++	++	++	18	„	0.2×0.5 – 0.6×2.0
	0.001	++	++	++	++	19	„	0.2×0.5 – 0.4×1.5
Titanium sulfate.	0.500	+	++	++	++	10	„	0.3×0.5 – 0.6×1.5
	0.001	++	++	++	++	15	Oval.	0.3×0.5 – 0.7×1.0
	0.050	++	++	++	++	14	„	0.4×0.5 – 0.8×1.2
	0.010	++	++	++	++	17	„	0.2×0.5 – 0.7×1.2
	0.005	++	++	++	++	20	Oval-rod.	0.3×0.6 – 0.6×2.0
	0.001	++	++	++	++	20	Rod.	0.4×0.6 – 0.7×2.5
Potassium acid titanate.	5.000	–	–	–	–	0	—	—
	2.000	–	–	–	–	0	—	—
	1.000	–	–	–	–	0	—	—
	0.500	–	–	–	–	0	—	—
	0.100	+	++	++	++	9	Rod.	0.3×0.5 – 0.4×1.5
	0.050	++	++	++	++	18	„	0.3×0.5 – 0.5×1.2
	0.010	++	++	++	++	22	„	0.2×0.7 – 0.7×2.0
	0.005	++	++	++	++	18	Oval.	0.4×0.5 – 0.7×2.0
	0.001	++	++	++	++	18	Rod-oval.	0.4×0.8 - 1.0×1.5

Notes: The number of + indicates the rate of growth.

The stimulation effect of titanic acid was noted only in 0.1% with the yeast extract while none without the yeast extract, and the limits for growth were 2.0 and 1.0% without and with the yeast extracts respectively; the optimum concentration of titanium sulfate was 0.001 and 0.005% with and without the yeast extract respectively, and no lethal limit was found clearly. The optimum concentration of potassium acid titanium was 0.01 and 0.001% with and without the yeast extract respectively; the lethal limit was 0.1 and 0.01% respectively with and without the yeast extract. Morphologically some bacteroids were found in some cases especially some oval cells were observed.

Summary.

In a preceding paper, it was reported that the nodules of leguminous plants stimulated the growth of nodule bacteria best of all the parts used. In this paper, the influence of ash contents in the nodules was investigated with a special reference to the titanium salts, and the following results were obtained:

1.) The ash content of the nodules is comparatively small relative to the other portions of leguminous plants, and it was 3.013 percent while the leaves and stems contained 8.016 percent.

2.) Comparatively large amount of Na_2O, MgO and TiO_2 were found in the ash from the nodules which amounted to 13.463 percent; 13.614 percent and 52.3 p.p.m. respectively.

3.) The seeds contained somewhat a large amount of K_2O, P_2O_5 while more of SO_3 and CaO were found in the leaves and stems; and in the roots, the insoluble matter in hydrochloric acid and Fe_2O_3 were prevalent.

4.) An addition of ash equivalent to 1 percent nodules to the culture medium did not effect the growth of nodule bacteria at all so that the stimulation of growth of bacteria obtained by the addition of nodules seems due chiefly to the organic constituents of the nodule rather than the inorganic components.

5.) The influence of titanic acid, titanium sulfate and potassium titanate on the growth of nodule bacteria was tried and found that the titanium as a whole has slightly beneficial effect. As the concentration of titanium increased the growth of bacteria became worse, and in case of titanic acid and potassium titanate, the growth was prohibited altogether at certain concentration which varies somewhat by different strain of bacteria as well as by the constituents in the medium. The critical concentration of titanic acid was somewhat 1 percent, and 0.1 percent for potassium titanate, and the optimum concentration was about 0.05 percent for the former and 0.001—0.005 percent for the latter.

6.) No special influence of titanic compounds on the morphological change of nodule bacteria was noted and a few bacteroids were found.

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