

Studies on Thermophilic Bacteria with special Reference to Cellulose Decomposition.

I. Distribution of the Bacteria in Surface Soil, on the Rice-straw and Unhulled Rice by Different Seasons.

By

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For the preparation of artificial compost as well as from the scientific interest, it is important to know as to the distribution of thermophilic bacteria with special reference to the cellulose decomposition. As the sources, the surface soil, rice-straw and unhulled rice were investigated in different seasons. The term, thermophilic is used in this paper to designate those bacteria which are capable of resisting any higher temperature than that of ordinarily considered regardless of the optimum temperature. The actinomycetes are included in this investigation also.

Experimental.

I. Collection of samples :

The samples were taken from four different plots in the experimental field at this Institute as follows ; Plot A.—the plot where no fertilizer has been applied since 1930 and only the rice, variety of 'Ehime sinriki' has been planted without the winter crop ; Plot B.—the rice, called 'Kūshin' and the barley have been planted ; Plot C.—a rush, *Inuncus effusus* was raised previous to the rice crop, 'Ehime sinriki' and was located near a farm-house ; and Plot D.—the glutinous rice was raised, followed by the barley.

The samples were collected at four different times : first in November, right after the harvest, the straw and unhulled rice were examined, and the second time in February, the examinations were made on the straw stored indoors and out-of-doors, and also the surface soils were investigated. The weather records were taken and a calm day was chosen to collect the samples under the usual precautions. Thus the third in May and the fourth in August, the tests were made.

II. Determination of Bacterial Number :

The number of bacteria was determined by the plating method, using the albumin agar, incubated at 28° and 37°C. In case of the soil and unhulled rice, 10 g., and straw, 1 g. were taken for examination. For sake of convenience, those grew at 37°C. are called thermophilic. The results of the first examination are given in Table I.

Table I.
Number of Bacteria on Surface Soil, Rice-straw and Unhulled Rice.

Samples.	Surface soil.			Rice-straw.			Unhulled rice.		
	37°C.	28°C.	I.T.R.*	37°C.	28°C.	I.T.R.*	37°C.	28°C.	I.T.R.*
A	7,174	11,462	62.6	113,477	168,655	67.3	1,599	2,951	54.2
B	4,440	9,059	49.0	115,346	143,072	80.6	3,185	3,992	79.8
C	20,260	20,839	97.2	177,094	242,585	73.0	4,012	12,366	32.4
D	14,757	19,393	76.1	108,163	187,998	57.5	5,540	7,049	78.6
Average.	11,658	15,188	71.2	128,520	185,578	69.6	3,584	6,589	61.3

Note : Number of bacteria given in the table, is by thousands per 1 g. dry soil.

* I. T. R. = Index of thermophilic bacteria.

As Table I indicates, the number of bacteria vary greatly and the rice-straw was the greatest, followed by the soil and unhulled rice in the order. The average ratio between those grown at 37° and 20°C. shows that the soil was highest and followed by the rice-straw and unhulled rice in the order. The results of second quantitative determination of bacteria which was made in February are given in Table II.

Table II.
Number of Bacteria on Surface Soil, Rice-straw, stored Indoors and Out-of-doors.

Samples.	Surface soil.			Indoor straws.			Out-of-doors straws.		
	37°C.	28°C.	I.T.R.*	37°C.	28°C.	I.T.R.*	37°C.	28°C.	I.T.R.*
A	9,921	19,295	51.4	216,273	295,565	73.2	138,872	411,695	33.7
B	11,378	15,839	71.8	136,689	150,892	90.6	215,033	628,148	34.3
C	9,574	16,502	58.0	238,137	583,130	40.8	168,977	375,664	45.0
D	15,785	23,677	66.7	105,511	333,721	31.6	49,154	249,785	19.7
Average.	11,664	18,828	62.0	174,152	340,811	59.1	143,009	415,823	32.2

Note : Number of bacteria given in the table, is by thousands per 1 g. dry soil.

* I. T. R. = Index of thermophilic bacteria.

As noted in Table II, the determinations were carried out on the surface soil and the rice-straw in and out-of-doors. In all cases, the number of bacteria was found greater than the first determination with a few exceptions, and more bacteria on the straw than that in the soil were found; among the straws, those stored out-of-doors contained more bacteria especially those grew at 28°C. The thermophilic ratio was smallest on the straw kept out-of-doors.

The third determination was made on the later part of May, in the rainy season. The results are given in Table III.

Table III.
Number of Bacteria on Surface Soil, Rice-straw, stored
Indoors and Out-of-doors.

Samples.	Surface soil.			Indoor straws.			Out-of-doors straws.		
	37°C.	28°C.	I.T.R.*	37°C.	28°C.	I.T.R.*	37°C.	28°C.	I.T.R.*
A	30,943	44,849	69.0	124,486	200,896	62.0	452,356	988,482	45.8
B	70,608	72,850	96.9	56,865	74,018	76.8	439,372	838,754	52.4
C	19,502	17,031	114.5	95,103	182,470	52.1	682,274	1,314,185	51.9
D	37,468	33,404	112.2	68,555	95,932	71.5	323,034	797,128	40.5
Average.	39,630	42,033	98.2	86,252	138,329	65.6	474,259	984,637	47.5

Note: Number of bacteria given in the table, is by thousands per 1g. dry soil.

* I. T. R. = Index of thermophilic bacteria.

As Table III indicates, the straws kept out-of-doors contained the largest number of bacteria and the others were irregular. It is noteworthy that the thermophilic ratio became over 100 in case of samples C and D of the soil samples while it was nearly 50 per cent for the straws kept out-of-doors. The fourth determination was carried out in the later part of August, of which the results are given in Table IV.

Table IV.
Number of Bacteria on Surface Soil, Rice-straw, stored
Indoors and Out-of-doors.

Samples.	Surface soil.			Indoor straws.			Out-of-doors straws.		
	37°C.	28°C.	I.T.R.*	37°C.	28°C.	I.T.R.*	37°C.	28°C.	I.T.R.*
A	47,383	8,767	61.4	9,221	14,523	63.5	208,142	213,517	97.5
B	13,463	16,228	83.0	6,891	16,538	41.7	218,870	220,371	99.3
C	15,969	15,669	101.9	27,243	63,491	42.9	558,033	1,026,895	54.3
D	23,278	28,901	80.5	55,803	72,689	52.0	774,284	992,313	78.0
Average.	14,273	17,391	81.7	24,789	41,810	50.0	439,832	613,274	82.3

Note: Number of bacteria given in the table, is by thousands per 1g. dry soil.

* I. T. R. = Index of thermophilic bacteria.

The above data show that the bacterial number was greatest on the straws kept out-of-doors, and smallest in the soil. In general, the results resemble to those of the preceding determination. In examining the results obtained thus far in conjunction with the season, for each sample, the following summary may be made, as shown in Tables V, VI, VII and Graph I.

Table V.
Bacterial Number in Soil by Season.

Samples.	November.			February.			May.			August.		
	37°C.	28°C.	* I.T.R.	37°C.	28°C.	* I.T.R.	37°C.	28°C.	* I.T.R.	37°C.	28°C.	* I.T.R.
A	7,174	11,462	62.6	9,921	19,295	51.4	30,943	44,849	69.0	5,383	8,767	61.4
B	4,440	9,059	49.0	11,378	15,839	71.8	70,605	72,850	96.9	13,463	16,228	83.0
C	20,260	20,839	97.2	9,574	16,502	58.0	19,502	17,031	114.5	15,969	15,669	101.9
D	14,757	19,393	76.1	15,785	23,677	66.7	37,468	33,404	112.2	23,278	28,901	80.5
Average.	11,658	15,188	71.2	11,664	18,828	62.0	39,630	42,033	98.2	14,523	17,391	81.7

Note: Number of bacteria given in the table, is by thousands per 1 g. dry soil.

* I. T. R. = Index of thermophilic bacteria.

As Table V indicates, between the first and second determinations, no appreciable difference was found although on an average it was slightly greater in case of the second. The number increased very rapidly in the third and decreased again in the fourth. The results thus obtained are in accord with those reports, such as WAKSMAN¹⁾ who noted that the number of bacteria are greater in the summer time than in the winter and greatest in July and August. Again REMY²⁾ reported that the number of bacteria increased with the rise of temperature in the spring. The index of thermophilic bacteria was greatest in May.

Rice-straw stored Indoors :

The rice-straw stored indoors was examined for the change of bacterial number during the storage in the same manner as in the previous experiments and the results are noted in Table VI.

(See Table VI on next page.)

According to the data shown in Table VI, the second determination which was made in the later part of February gave the largest number of bacteria and the smallest in the fourth or in August. CONN³⁾ reported that the number of bacteria in the frozen soils is rather high in the cold seasons which agrees with the results in this case.

The number of thermophilic bacteria was about the same in all cases but the average was highest in the first and followed by the third, second and fourth.

Table VI.
Bacterial Number on Rice-straw kept Indoors.

Samples.	November.			February.			May.			August.		
	37°C.	28°C.	I.T.R.*	37°C.	28°C.	I.T.R.*	37°C.	28°C.	I.T.R.*	37°C.	28°C.	I.T.R.*
A	113,477	168,655	67.3	216,273	295,565	73.2	134,486	200,896	62.0	9,221	14,523	63.5
B	115,346	143,072	80.6	136,689	150,829	90.6	56,865	74,018	76.8	6,890	16,538	41.7
C	177,094	242,585	73.0	238,137	583,130	40.8	95,102	182,470	52.1	2,724	6,349	42.9
D	108,163	187,998	57.5	105,511	333,721	31.6	68,555	95,932	71.5	2,549	1,056	52.0
Average.	128,520	185,578	69.6	174,153	340,811	59.1	86,252	38,329	65.6	4,846	9,617	50.0

Note: Number of bacteria given in the table, is by thousands per 1 g. dry soil.

* I. T. R. = Index of thermophilic bacteria.

Rice-straw stored Out-of-doors :

The rice-straw stored out-of-doors was subjected to the same investigation as the previous case and the results are presented in Table VII.

Table VII.
Bacterial Number on Rice-straw kept Out-of-doors.

Samples.	November.			February.			May.			August.		
	37°C.	28°C.	I.T.R.*	37°C.	28°C.	I.T.R.*	37°C.	28°C.	I.T.R.*	37°C.	28°C.	I.T.R.*
A	113,477	168,655	67.3	138,872	411,695	33.7	452,356	988,482	45.8	208,142	213,517	97.5
B	115,346	143,072	80.6	215,033	626,148	34.3	439,372	838,754	52.4	218,870	220,371	99.3
C	177,094	242,585	73.0	168,977	375,664	45.0	682,274	1,314,185	51.9	558,033	1,026,895	54.3
D	108,163	187,998	57.5	49,154	249,785	19.7	323,034	797,128	40.5	774,284	992,313	78.0
Average.	128,520	185,578	69.6	143,009	415,823	33.2	474,259	984,637	47.7	439,832	613,274	82.3

Note: Number of bacteria given in the table, is given by thousands per 1 g. dry soil.

* I. T. R. = Index of thermophilic bacteria.

As Table VII indicates the number of bacteria was greatest in the third determination, followed by the fourth, and was smallest in the first.

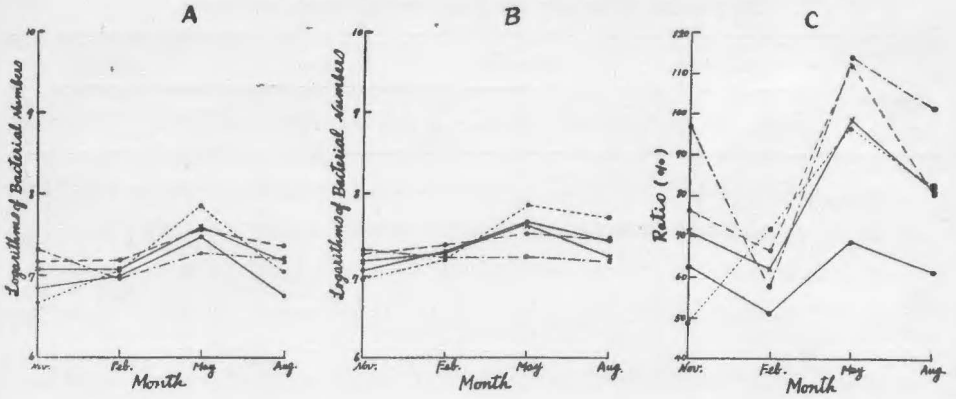
The results as a whole, resemble to those of the surface soil.

According to the average, the index of thermophilic bacteria was 82.3% in the fourth test which was the largest, and 33.2% the smallest in the second.

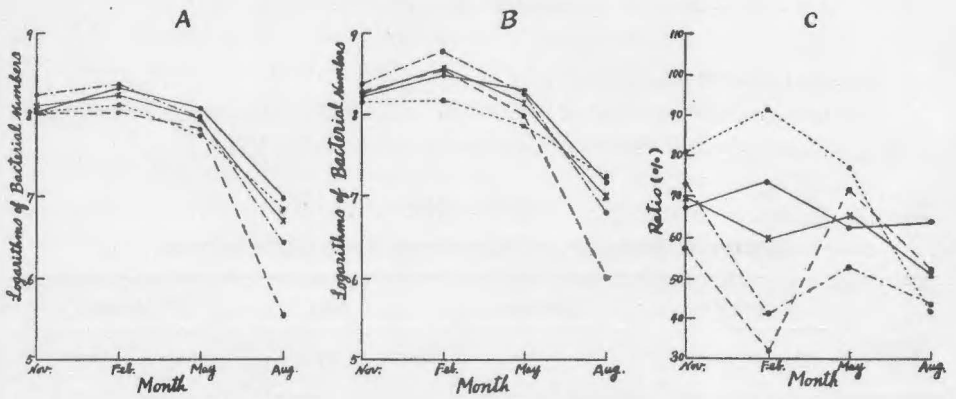
The seasonal changes of bacterial number in the surface soil, the rice-straw stored indoors and out-of-doors, are presented graphically in Graph I.

Graph I.
Seasonal Change of Bacterial Number.

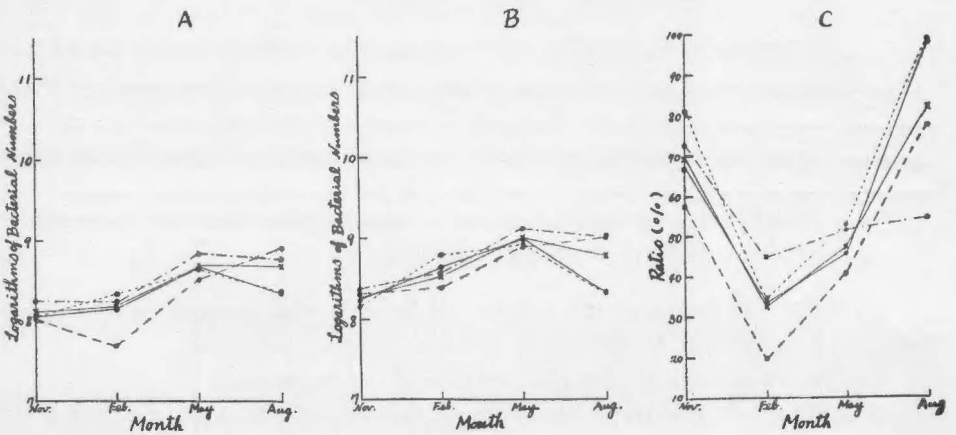
Surface Soil.



Rice-straw kept Indoors.



Rice-straw kept Out-of-doors.



Notes: A = Cultured at 37°C.; B = At 28°C.; C = Index of thermophilic bacteria.
 —x— Sample A; Sample B; ----- Sample C; ----- Sample D;
 — Average of samples.

From the foregoing results, the following statements may be made :

The number of bacteria on the straw decrease when the straw stored indoors while the increase takes place for that stored out-of-doors.

The index of thermophilic bacteria is influenced very little by the seasonal change in case of indoors but it increases with the rise of temperature in the summer in case of out-of-doors. However the reverse tendency was noted in the winter.

In case of the surface soil, the variation is almost half way between the straw stored indoors and out-of-doors.

III. Grouping of Bacteria Found on the Rice-straw according to the Method Used by the Society of American Bacteriologists :

The bacteria which were found in the foregoing experiments were grouped by the following method :

By the rate of growth under the optimum conditions on the glucose broth and glucose broth agar, the bacteria are grouped in the following four groups :

- Group 1. Those grow in the glucose broth within 24 hours at 37°C.
- Group 2. Same as above except those grow within 48 hours at 25°C.
- Group 3. Weak growth in the glucose broth but grow well on the glucose agar within 24 hours at 37°C.
- Group 4. Those grow on the glucose agar within 48 hours at 25°C. and without belonging to any other group.

From the plate culture made from the samples C and D, incubated at 37°C., the organisms were isolated as many as possible, and grouped them according to the above method. At the sametime the actinomycetes were identified microscopically so that their number was determined.

Then the index of growth was determined by dividing the number of bacterial growth by the total number of bacteria isolated.

The results of the first examination are given in Table VIII.

(See Table VIII on next page.)

Table VIII indicates that Group I predominates and none of Group IV was found in C and D of the surface soil. Many actinomycetes were found in D and reached 26.1 percent of the total number. In case of the unhulled rice, all four groups were found in their order for C ; but for D, Group II was highest which was followed by Group I, and Group III was lowest, and no actinomycetes was found in neither C nor D. For the straw, C was similar to that of the unhulled rice while for D, Group II was highest followed by Group I and none of Group III was found.

The results of the second examination are noted in Table IX :

(See Table IX on next page.)

In the surface soil C, the bacterial number was in the following order Group I, III and II while none of Group IV was found ; in D, Group I, II and III were found in their order and also none of Group IV was found ; the number of actinomycetes was similar in both C and D being about 13 percent.

Table VIII.
Groups of Bacteria isolated from Surface Soil, Husks
and Rice-straws.

Samples.	Groups.				Un- certain.	Actino- mycetes.	Growth.	% growth.	No. of stocks isolated.		
	I.	II.	III.	IV.							
Surface soil.	C {	Bacteria.	6	4	2	0	3	3	18	52.9	34
		%	33.3	22.2	11.1	0	16.7	16.7	100.0		
	D {	Bacteria.	14	7	9	0	4	12	46	93.9	49
		%	30.4	15.2	19.6	0	8.7	26.1	100.0		
Husks.	C {	Bacteria.	6	5	3	1	3	0	18	94.7	19
		%	33.3	27.8	16.7	5.6	16.7	0	100.0		
	D {	Bacteria.	6	4	10	5	9	0	34	79.1	43
		%	17.6	11.8	29.4	14.7	26.5	0	100.0		
Rice-straws.	C {	Bacteria.	5	4	3	1	12	0	25	61.0	41
		%	20.0	16.0	12.0	4.0	48.0	0	100.0		
	D {	Bacteria.	3	6	0	1	12	0	22	100.0	22
		%	13.6	27.3	0	4.5	54.5	0	100.0		

Table IX.
Groups of Bacteria isolated from Surface Soil, Indoor
and Out-of-doors Straws.

Samples.	Groups.				Un- certain.	Actino- mycetes.	Growth.	% growth.	No. of stocks isolated.		
	I.	II.	III.	IV.							
Surface soil.	C {	Bacteria.	6	2	3	0	3	2	16	76.2	21
		%	37.5	12.5	18.8	0	18.8	12.5	100.0		
	D {	Bacteria.	16	4	3	0	11	5	37	77.1	48
		%	43.2	10.8	8.1	0	29.7	13.5	100.0		
Indoor straws.	C {	Bacteria.	15	5	0	2	10	3	35	47.3	74
		%	42.9	14.3	0	5.7	28.6	8.6	100.0		
	D {	Bacteria.	11	7	1	3	11	2	35	85.4	41
		%	31.4	20.0	2.9	8.6	31.4	5.7	100.0		
Out-of-doors straws.	C {	Bacteria.	14	4	1	2	12	2	35	70.0	50
		%	40.0	11.4	2.9	5.7	34.3	5.7	100.0		
	D {	Bacteria.	18	5	0	0	2	1	16	64.0	25
		%	50.0	31.3	0	0	12.5	6.3	100.0		

On the straw stored indoors, C and D, Group I, II, IV and III were the order except no Group III was present on C; the actinomycetes was about 5–10 percent on C and D. On the straw kept out-of-doors, Group I was highest in number and followed by Group II while Group III was lowest; but none of Group III and IV were present on D; the actinomycetes was present in small number.

The results of third examination are shown in Table X :

Table X.
 Groups of Bacteria isolated from Surface Soil, Indoor
 and Out-of-doors Straws.

Samples.		Groups.				Un- certain.	Actino- mycetes.	Growth.	% growth.	No. of stocks isolated.	
		I.	II.	III.	IV.						
Surface soil.	C {	Bacteria.	7	1	2	3	5	2	20	95.2	21
		%	35.0	5.0	10.0	15.0	25.0	10.0	100.0		
	D {	Bacteria.	8	2	0	0	9	3	22	75.8	29
		%	36.4	9.1	0	0	41.0	13.6	100.0		
Indoor straws.	C {	Bacteria.	11	0	1	2	5	2	21	84.0	25
		%	52.4	0	4.8	9.5	23.8	9.5	100.0		
	D {	Bacteria.	22	5	0	8	7	1	43	87.8	49
		%	51.2	11.6	0	18.6	16.3	2.3	100.0		
Out-of-doors straws.	C {	Bacteria.	10	1	0	4	15	2	32	68.1	47
		%	31.3	3.1	0	12.5	46.9	6.3	100.0		
	D {	Bacteria.	8	3	0	2	9	0	22	91.7	24
		%	36.4	13.6	0	9.1	40.9	0	100.0		

As Table X indicates, Group I was highest in the surface soil as in the previous experiment but others were different by C or D; not more than 10 percent of actinomycete was present.

The straw stored indoors, on C and D, Group I was high on both, occupying more than 50 percent and followed by Group IV and in order, and none of Group III was present on D; only a few actinomycetes were present. In case of the straw kept out-of-doors, on C, Group I, IV, II and III were present in the order and on D, Group I, II, IV and III were the order which dose not agree with that of C but in both cases Group I was highest; a few actinomycetes were present on C but none on D.

The results of the fourth test are given in Table XI as follows :

(See Table XI on next page.)

Table XI indicates that in case of the surface soil, the similar results were obtained between C and D, and Group I was the highest followed by Group II, III and IV in the order; 10–30 percent of actinomycetes were present.

Table XI.
Groups of Bacteria isolated from Surface Soil, Indoor
and Out-of-doors Straws.

Samples.		Groups.				Un- certain.	Actino- mycetes.	Growth.	% growth.	No. of stocks isolated.
		I.	II.	III.	IV.					
Surface soil.	C { Bacteria.	8	8	6	3	5	12	42	84.0	50
	{ %	19.0	19.0	14.3	7.1	11.9	28.6	100.0		
	D { Bacteria.	24	11	5	1	14	6	61	87.1	70
	{ %	39.3	18.0	8.2	1.6	23.0	9.8	100.0		
Indoor straws.	C { Bacteria.	3	1	3	0	2	10	19	100.0	19
	{ %	15.8	5.3	15.8	0	10.5	53.6	100.0		
	D { Bacteria.	6	7	5	1	2	4	25	92.6	27
	{ %	24.0	28.0	20.0	4.0	8.0	16.0	100.0		
Out-of-doors straws.	C { Bacteria.	3	3	12	8	9	4	39	95.1	41
	{ %	7.7	7.7	30.8	20.5	23.1	10.3	100.0		
	D { Bacteria.	14	1	8	2	12	10	47	97.9	48
	{ %	29.8	2.1	17.0	4.3	25.5	21.3	100.0		

Table XII.
Summary Table of Groups of Bacteria isolated.

Samples.		Groups.				Un- certain.	Actino- mycetes.	Growth.	% growth.	No. of stocks isolated.
		I.	II.	III.	IV.					
Surface soil.	C { Bacteria.	27	15	13	6	16	19	96	76.2	126
	{ %	28.1	15.6	13.5	6.3	16.7	19.8	100.0		
	D { Bacteria.	62	24	17	1	38	26	168	85.7	196
	{ %	36.9	14.3	10.1	0.6	22.6	15.5	100.0		
Indoor straws.	C { Bacteria.	34	10	7	5	29	15	100	62.9	159
	{ %	34.0	10.0	7.0	5.0	29.0	15.0	100.0		
	D { Bacteria.	42	25	6	13	32	7	125	89.9	139
	{ %	33.6	20.0	4.8	10.4	25.6	5.6	100.0		
Out-of-doors straws.	C { Bacteria.	32	12	16	15	48	8	131	73.2	179
	{ %	24.4	9.2	12.2	11.5	36.6	6.1	100.0		
	D { Bacteria.	33	15	8	5	35	11	107	89.9	119
	{ %	30.8	14.0	7.5	4.7	32.7	10.3	100.0		

The indoor straws showed some difference between C and D but Group I was the highest amounting to more than 30 percent which was followed by Group II. On an average 10 percent of actinomycetes were present.

The out-of-doors straws contained the largest number of Group I in both C and D and not much difference was found among other groups, contained about 10—20 percent of actinomycetes.

The foregoing results are summarized in Table XII :

In all the cases, Group I was found in majority for both the surface soil and the straw while Group IV was the least in the surface soil which was found in large number on the straw under both treatment. The actinomycetes were found abundantly in the surface soil and also on the indoor straw by the end of August, and a similar tendency was noted in case of the out-of-doors straw.

As to the seasonal changes, the number of organisms increased with a rise of temperature in all the cases, and all the groups of organisms were found. The order of groups was somewhat changed toward the warmer season.

IV. Test on the Thermal Resistance :

Those bacteria grew on glucose broth agar at 37°C. were taken and subjected to various temperature from 55° to 105°C., as noted in Table XIII, in an oil bath ; and the number of surviving bacteria was divided by those number grown at 37°C. and noted as the surviving index.

Table XIII.
Thermal Resistance of Bacteria found in Surface Soil,
Husks and Straws.

Samples.		Temperature of oil bath in °C.							
		37	55	65	75	85	95	105	
Surface soil.	C	{Bacteria.	18	17	16	16	12	12	6
		{Survived %.	100.0	94.9	88.9	88.9	66.7	66.7	33.3
	D	{Bacteria.	46	41	40	39	38	37	32
		{Survived %.	100.0	89.1	87.0	84.8	82.6	80.4	69.6
Husks.	C	{Bacteria.	18	16	15	15	11	9	3
		{Survived %.	100.0	88.9	83.3	83.3	61.1	50.0	16.7
	D	{Bacteria.	33	30	30	30	20	16	11
		{Survived %.	100.0	90.9	90.9	90.9	60.6	48.5	33.3
Rice-straws.	C	{Bacteria.	25	19	19	19	15	12	10
		{Survived %.	100.0	76.0	76.0	76.0	60.0	48.0	40.0
	D	{Bacteria.	22	17	17	17	14	12	5
		{Survived %.	100.0	77.3	77.3	77.3	63.6	54.5	22.7

Table XIV.
 Thermal Resistance of Bacteria found in Surface Soil,
 Indoor and Out-of-doors Straws.

Samples.		Temperature of oil bath in °C.							
		37	55	65	75	85	95	105	
Surface soil.	C	Bacteria.	16	15	15	15	15	14	13
		Survived %.	100.0	93.8	93.8	93.8	93.8	87.5	81.3
	D	Bacteria.	38	30	29	28	28	28	23
		Survived %.	100.0	78.9	76.3	73.7	73.7	73.7	60.5
Indoor straws.	C	Bacteria.	34	30	30	30	30	27	12
		Survived %.	100.0	88.2	88.2	88.2	88.2	79.4	35.3
	D	Bacteria.	33	28	27	27	26	21	5
		Survived %.	100.0	84.8	81.8	81.8	78.8	63.6	15.2
Out-of-doors straws.	C	Bacteria.	35	25	25	25	24	23	10
		Survived %.	100.0	71.4	71.4	71.4	68.6	65.7	28.6
	D	Bacteria.	16	16	61	16	16	15	11
		Survived %.	100.0	100.0	100.0	100.0	100.0	93.8	68.8

Table XV.
 Thermal Resistance of Bacteria found in Surface Soil,
 Indoor and Out-of-doors Straws.

Samples.		Temperature of oil bath in °C.							
		37	55	65	75	85	95	105	
Surface soil.	C	Bacteria.	20	15	14	13	12	12	8
		Survived %.	100.0	75.0	70.0	65.0	60.0	60.0	40.0
	D	Bacteria.	22	18	18	18	17	17	10
		Survived %.	100.0	81.8	81.8	81.8	77.3	77.3	45.5
Indoor straws.	C	Bacteria.	20	17	17	16	16	16	13
		Survived %.	100.0	35.0	85.0	80.0	80.0	80.0	65.0
	D	Bacteria.	43	41	40	40	39	38	34
		Survived %.	100.0	95.3	93.0	93.0	90.7	88.4	79.1
Out-of-doors straws.	C	Bacteria.	30	14	14	14	13	12	11
		Survived %.	100.0	46.7	46.7	46.7	43.3	40.0	36.7
	D	Bacteria.	22	19	19	16	16	16	13
		Survived %.	100.0	86.4	86.4	72.7	72.7	72.7	59.1

Table XVI.
Thermal Resistance of Bacteria found in Surface Soil,
Indoor and Out-of-doors Straws.

Samples.		Temperature of oil bath in °C.							
		37	55	65	75	85	95	105	
Surface soil.	C	Bacteria.	42	37	37	37	37	35	25
		Survived %.	100.0	88.1	88.1	88.1	88.1	83.3	59.5
	D	Bacteria.	61	53	53	53	52	50	36
		Survived %.	100.0	86.9	86.9	86.9	85.2	82.0	59.0
Indoor straws.	C	Bacteria.	19	18	17	17	17	17	16
		Survived %.	100.0	94.7	89.5	89.5	89.5	89.5	84.2
	D	Bacteria.	25	23	23	23	23	23	19
		Survived %.	100.0	92.0	92.0	92.0	92.0	92.0	76.0
Out-of-doors straws.	C	Bacteria.	39	39	39	39	39	39	30
		Survived %.	100.0	100.0	100.0	100.0	100.0	100.0	76.9
	D	Bacteria.	47	43	43	43	43	41	32
		Survived %.	100.0	91.5	91.5	91.5	91.5	87.2	68.1

As shown in Table XIII, those bacteria which came from the surface soil showed the strongest resistance and those from the husk were the weakest. As the temperature rises to 95°—105°C. the death rate increased rapidly. At 55°C., the surface soil and the husk showed a similar surviving index which was about 90 percent while it was below 80 percent in case of the straw.

The same test was carried out at three more different times as indicated previously and the results are presented in Table XIV, XV and XVI respectively :

Table XIV indicates that the bacteria from the surface soils were strongest and no difference was noted between the straw under different treatments. According to Table XV, the straw stored indoors contained most resistant bacteria while those of the out-of-doors straw as well as the surface soils were less resistant.

The results of the fourth test are shown in Table XVI which indicates that the straw kept indoors carried the strongest bacteria followed by the out-of-doors straw and the surface soils.

The foregoing results are summarized in Table XVII :

(See Table XVII on next page.)

The data indicate that in case of the surface soils, more resistant bacteria were found in the cold season or at the second test ; for the indoor straw, they increased with the rise of temperature or in the summer time ; it was not clearly shown in case of the out-of-doors straw. However it was apparent that the resistance of bacteria was different by the host and the seasonal change respectively.

Table XVII.
Summary Table of Thermal Resistant Tests.

Samples.		Temperature of oil bath in °C.							
		37	55	65	75	85	95	105	
Surface soil.	C {	Bacteria.	96	84	82	81	76	73	52
	Survived %	100.0	87.8	85.2	84.0	77.2	74.4	53.5	
	D {	Bacteria.	167	142	140	138	135	132	101
	Survived %	100.0	84.2	83.0	81.8	79.7	78.4	58.7	
Husks.	C {	Bacteria.	18	16	15	15	11	9	3
	Survived %	100.0	88.9	83.3	83.3	61.1	50.0	16.7	
	D {	Bacteria.	33	30	30	30	20	16	11
	Survived %	100.0	90.9	90.9	90.9	60.6	48.5	33.3	
Indoor straws.	C {	Bacteria.	98	84	83	82	78	72	51
	Survived %	100.0	86.0	84.7	83.4	79.4	74.2	56.1	
	D {	Bacteria.	123	109	107	107	102	94	63
	Survived %	100.0	87.4	86.0	86.0	81.3	74.6	48.3	
Out-of-doors straws.	C {	Bacteria.	129	97	97	97	91	86	61
	Survived %	100.0	73.5	73.5	73.5	68.0	63.4	45.5	
	D {	Bacteria.	107	95	95	92	89	84	61
	Survived %	100.0	88.8	88.8	85.4	82.0	77.1	54.7	

V. Test for the Presence of Thermophilic Cellulose Decomposing Bacteria :

In connection with this experiment, the senior author isolated *Bacillus thermofibrincolus* n. sp.³⁾ and reported as to their morphological and physiological characteristics and in regard to their application in preparation of the artificial compost⁴⁾. In this test, it was intended to find out their numerical change and their distribution.

The test was carried out by using VILJOEN'S⁵⁾ Cellulose peptone solution medium and in one case 1 g. of each surface soil and husk, and 0.5 g. straw respectively was used as the inoculant, and in the other, an elective method was employed by taking 5 cc. of 10 percent suspension of the surface soil and husk, and 1 percent of straw respectively and inoculated into the medium which was incubated at 65°C., and noted the destruction of filter paper by which the presence or absence of the cellulose decomposing bacteria was determined. In the former, three tubes in a set were used and five in the latter. To make the results clearly understood, the number of filter paper destroyed was divided by the total number and noted as the index of growth.

This test was applied at four different times as noted previously, and the results are presented in Table XVIII, XIX, XX, XXI and XXII together with the summary table.

Table XVIII.
Surviving Indexes of Bacteria at 55° and 150°C.

Tests.	Surface soil.				Indoor straws.				Out-of-doors straws.			
	C		D		C		D		C		D	
	55°C.	105°C.	55°C.	105°C.	55°C.	105°C.	55°C.	105°C.	55°C.	105°C.	55°C.	105°C.
1.	94.4	33.3	89.1	69.6	78.0	40.0	77.3	22.7	76.0	40.0	77.3	22.7
2.	93.8	81.3	78.9	60.5	88.2	35.3	84.8	15.2	71.4	28.6	100.0	68.8
3.	75.0	40.0	81.8	45.5	85.0	65.0	95.3	79.1	46.7	36.7	86.4	59.1
4.	88.1	59.5	86.9	59.0	94.7	84.2	92.0	76.0	100.0	76.9	91.5	68.1

Table XIX.
Number of Cellulose Decomposing Bacteria found in Surface Soil, Husks and Straws.

Samples.	A		B		C		D		Average.
	Original.	Solution.	Original.	Solution.	Original.	Solution.	Original.	Solution.	
Surface soil. . .	100.0	60.0	100.0	80.0	100.0	80.0	100.0	80.0	87.5
Husks.	0	0	0	0	0	0	0	0	0
Rice-straws. . .	33.3	0	66.7	0	66.7	0	66.7	0	29.2

The above table indicates that the greatest number of cellulose decomposing bacteria was found in the soil, followed by the straw and none on the husk.

The results of the second test are given in Table XX.

Table XX.
Number of Cellulose Decomposing Bacteria found in Surface Soil, Indoor and Out-of-doors Straws.

Samples.	A		B		C		D		Average.
	Original.	Solution.	Original.	Solution.	Original.	Solution.	Original.	Solution.	
Surface soil. . . .	100.0	40.0	100.0	40.0	66.7	80.0	100.0	100.0	78.3
Indoor straws. . .	66.7	0	66.7	0	33.3	0	33.3	0	25.0
Out-of-doors straws.	66.7	0	66.7	0	66.7	0	66.7	0	33.4

Table XX shows that the growth index was highest in the soils, an average of 78.3 percent, the out-of-doors straw, 33.4 and 25.0 percent for the indoor straw.

Table XXI gives the results of the third test.

Table XXI.

**Number of Cellulose Decomposing Bacteria found in Surface Soil,
Indoor and Out-of-doors Straws.**

Samples.	A		B		C		D		Average.
	Original.	Solution.	Original.	Solution.	Original.	Solution.	Original.	Solution.	
Surface soil. . . .	100.0	80.0	100.0	100.0	100.0	80.0	100.0	40.0	87.5
Indoor straws. . .	66.7	0	33.3	0	33.3	0	100.0	0	29.2
Out-of-doors straws.	33.3	0	33.3	0	33.3	0	66.7	0	20.8

As shown above, the growth index was highest in the soils, followed by the indoor straw which is contrary to the results obtained in the previous test.

The results of the fourth test are given in Table XXII.

Table XXII.

**Number of Cellulose Decomposing Bacteria found in Surface Soil,
Indoor and Out-of-doors Straws.**

Samples.	A		B		C		D		Average.
	Original.	Solution.	Original.	Solution.	Original.	Solution.	Original.	Solution.	
Surface soil. . . .	100.0	60.0	100.0	100.0	100.0	100.0	100.0	100.0	95.0
Indoor straws. . .	66.7	0	100.0	0	66.7	0	66.7	0	37.5
Out-of-doors straws.	66.7	0	66.7	0	66.7	0	33.3	0	29.2

The above results agree exactly to those obtained in the third test.

The average of growth indexes obtained in the foregoing tests, by the seasonal change, are presented in the following table :

Table XXIII.

**Number of Cellulose Decomposing Bacteria found in Surface Soil,
Indoor and Out-of-doors Straws.**

Samples.	No. of experiments.				Average.
	1.	2.	3.	4.	
Surface soil.	87.5	78.3	87.5	95.0	87.1
Indoor straws.	29.2	25.0	29.2	37.5	30.6
Out-of-doors straws. .	29.2	33.4	20.8	29.2	28.2

The above table indicates that the growth index was higher in the warmer season in all cases except the out-of-doors straw which was highest in the winter.

As a whole, the growth index was highest for the soils, followed by the out-of-doors straw but sometimes the indoor straw were higher than those of the out-of-doors by the season.

The out-of-doors straw showed the increase of index with the rise of temperature while the indoor straw as highest at the start and decreased later.

Although the microscopical examination as to the morphology of these bacteria was made and observed that a majority of them were the rod shaped organisms and some were filamentous and vibrio, which will be reported later in detail.

Summary and Conclusions.

In this investigation, the distribution and the seasonal change of thermophilic bacteria in the surface soil, on the rice-straws and husks together with their cellulose decomposition, and the results are summarized as follows :

1.) At the harvest in November, the total number of bacteria was greatest on the straws followed by the soils and husks. Not much difference was found among them as to the number of thermophilic bacteria but the soils contained most followed by the straws and husks.

2.) Between the straws kept indoors and out-of-doors, the bacterial number decreased gradually in case of indoors while it increased out-of-doors. The index of thermophilic bacteria varied little by the seasonal change in case of indoors while the out-of-doors straw were influenced greatly and it was greater in the hot weather.

3.) As to the four groups of bacteria investigated after the classification of the S. A. B., the first group was present abundantly in all the cases while the others were less.

4.) A large number of actinomycetes was found in the soils and more so in the summer while none was found on the straws and husks.

5.) As to the thermal resistance of bacteria found, no great difference was noted between the soils and straws, and in both cases the number decreased by fifty percent at 105°C., while sixty to eighty percent decrease was found in case of the husks.

6.) The number of thermophilic bacteria which are capable of decomposing the cellulose, was greatest in the soils followed by the straws, kept indoors or out-of-doors depending on the season. The growth indexes for the soils, the straws of indoors and out-of-doors were 87.1, 30.6 and 28.2 respectively.

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