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CHEMICAL FRACTIONS OF ORGANIC NITROGEN IN ACID HYDROLYSATES GIVEN FROM MICROBIAL CELLS AND THEIR CELL WALL SUBSTANCES AND CHARACTERIZATION OF DECOMPOSABLE SOIL ORGANIC NITROGEN DUE TO DRYING

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1) The major part of organic nitrogen fractions in acid hydrolysate which is mineralized through the decomposition process of cells of *B. subtilis* in sand for 2 weeks was the form of amino acid N, but the form of amino sugar N was hardly mineralized. Their cytoplasmic substances were mineralized quickly, but their cell wall substances comparatively slowly. When the latter, however, was pretreated with ultrasonic or oven-drying treatment, their mineralization was accelerated. The mineralization rate of the form of amino sugar N was remarkably high.

2) The major part of the decomposable organic nitrogen fractions due to the ultrasonicating or oven-drying effects in acid hydrolysates given from microbial cells and their cell wall substances was the forms of amino acid N and amino sugar N. The accelerating effect of oven-drying pretreatment on the mineralization of the form of amino sugar N was larger than that of ultrasonicating pretreatment under this experimental conditions.

3) The amino acid composition of the amino acid N fraction in acid hydrolysates given from cells of *B. subtilis* and their cell wall substances which become decomposable due to the ultrasonicating or oven-drying effects almost equalled that of the mucopeptides in cell wall substances, and the quantity of mineralization was proportional to the contents of individual amino acids in cell wall substances. Namely, they were in the following order: Ala, Glu>Asp, Gly, Lys, Val>Ileu, Leu, Ser, Thr, Arg.

4) These results might complementarily prove that microbial cells, especially their cell wall substances, contribute considerably to a source of the decomposable soil organic matter, which was described in the previous paper (*7*).

HAYASHI and HARADA (*3*) reported that the major part of organic nitrogen in cultivated soil which become decomposable due to drying originated from the fractions of amino acid N and amino sugar N in acid hydrolysate.

KAI and AHMAD *et al.* (*1, 4*) examined the factors affecting immobilization and release of nitrogen in soil and the chemical characteristics of soil organic nitrogen by using ^{15}N. They showed that the major nitrogen compound mineralized from soil

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organic nitrogen compounds which become decomposable due to air-drying was the amino acid N fraction in acid hydrolysate. When the soil oven-dried, however, the amino sugar N fraction was also mineralized considerably. From the results obtained, they assumed that the principal decomposable organic nitrogen compounds might belong to the peptide compounds existing in microbial cell walls.

Furthermore, in the previous paper (*5*), we reported that the major amino acids newly synthesized through the decomposition process of rye-grass in soil might be almost similar to those existing in microbial cell walls; therefore microbial cell walls might contribute considerably to soil organic matter newly synthesized. However, an examination on the chemical fractions of the decomposable organic nitrogen of microbial cells and their cell wall substances has never been done so far.

So, in this paper, the following examinations were carried out on the chemical fractions of organic nitrogen in acid hydrolysates given from microbial cells and their cell wall substances and on the characterization of the decomposable organic nitrogen.

There are some problems to show clearly the contribution of each organic nitrogen fraction to mineralized nitrogen according to the difference of each fraction between the control at the beginning and the treatment after 2 weeks of incubation. It would be, however, likely to characterize the decomposable soil organic nitrogen due to drying on the basis of the differences of the amino acid nitrogen and amino sugar nitrogen fractions between the control and the treatments.

EXPERIMENT 1 ORGANIC NITROGEN FRACTIONS IN ACID HYDROLYSATES GIVEN FROM MICROBIAL CELLS AND THEIR CELL WALL SUBSTANCES AND CHARACTERIZATION OF THE DECOMPOSABLE ORGANIC NITROGEN

Materials and methods

1) *Bacillus subtilis.* The preparations of the cells of *B. subtilis* and their cell wall substances were made as described in the previous paper (*7*). Ten g of sea sand (diameter: 0.50–0.25 mm) was weighed into a 200 ml Erlenmeyer flask and fresh cells were added to it (: Control), after which one part was oven-dried at 80°C for 2 hr (: Oven-dried). Besides, cells ultrasonicated (19.5 kHz, Kaijo Denki Co.) for 20 min were added to the sand (: Ultrasonicated). Then, mineral nutrition and inoculum (*6*) were added to each sand and with distilled water its moisture content was adjusted to 60 per cent of the maximum water holding capacity, covered with polyethylene film, and incubated at 30°C for 2 weeks.

Furthermore, in the case of cell wall substances prepared by ultrasonic treatment, the incubation procedure was done the same as mentioned above, and the sample at the beginning was shown as "Control," the fresh sample after 2 weeks of incubation was shown as "Ultrasonicated" and the oven-dried sample after 2 weeks of incubation was shown as "Ultrasonicated+Oven-dried."

2) *Saccharomyces cerevisiae.* The preparation of cell wall substances from *S.*

Table 1. Organic nitrogen fractions in acid hydrolysate given from cells of *B. subtilis*.

Treatment	Organic N added	Mineral N	Organic N	Non-hydrolyzable N	Hydrolyzable N			
					Ammonium N	Amino sugar N	Amino acid N	Unidentified N
				(mg N per 100 g sand)				
(1) Control:	161.3			3.3	19.0	8.6	82.7	47.7
at the	[100.0]			[2.0]	[11.8]	[5.3]	[51.3]	[29.5]
beginning	(100.0)					(100.0)	(100.0)	(100.0)
(2) Control: after 2 weeks of incubation	161.3	64.9	96.4 〈100.0〉	3.9 〈4.0〉	44.9 〈46.6〉	8.5 〈8.8〉	18.3 〈19.0〉	20.8 〈21.6〉
(3) Ultrasonicated: after 2 weeks of incubation	161.3	70.1	91.2	3.8	30.0	4.5	14.0	38.9
(4) Oven-dried: after 2 weeks of incubation	161.3	69.8	91.5	3.5	30.4	2.4	12.3	42.9
Amounts of mineralized N								
Control			64.9	−0.6	−25.9	0.1	64.4	26.9
(1)-(2)			(40.2)			(1.2)	(77.9)	(56.4)
Ultrasonicated			70.1	−0.5	−11.0	4.1	68.7	8.8
(1)-(3)			(43.5)			(47.7)	(83.1)	(18.4)
Oven-dried			69.8	−0.2	−11.4	6.2	70.4	4.8
(1)-(4)			(43.3)			(72.1)	(85.1)	(10.1)

[ ]: Figures show per cent of organic N added.
〈 〉: Figures show per cent of organic N after 2 weeks of incubation.
( ): Figures show per cent of each organic N fraction.

cerevisiae was made as described in the previous paper (*7*). Ten g of artificial soil (8 g of sea sand+2 g of montmorillonite clay prepared from paddy soil taken from the Agricultural Experiment Station of Nagano Prefecture) was weighed into a 200 ml Erlenmeyer flask and the cell wall substances were added to it. The experimental condition was the same as described in paragraph 1).

3) Determination of the mineral nitrogen and the various forms of organic nitrogen in acid hydrolysate. After 2 weeks of incubation, the sample was brought out from the incubation chamber and analyzed. The determination method of the mineral nitrogen in sand or soil was the same as described in the previous paper (*7*). The various forms of organic nitrogen in acid hydrolysate were determined after Bremner's method (*2*).

Results and discussion

Organic nitrogen fractions in acid hydrolysate given from fresh cells of *B. subtilis* are shown in Table 1. In the case of fresh cells, as indicated about 5.3 per cent and 51.3 per cent of organic nitrogen added were contained in the forms of amino sugar N and amino acid N, respectively (see (1) Control at the beginning). After 2 weeks of incubation, the forms of amino acid N and unidentified N were decomposed, especially the form of amino acid N, but the form of amino sugar N was hardly decomposed at all (see (2) Control after 2 weeks of incubation). This result shows that mineralization of the form of amino sugar N was very slow, but that of the form of amino acid N was very fast and 78 per cent was mineralized. Considering that the mineralization of the nitrogen compounds of cytoplasmic substances in fresh cells was very fast, but that of their cell wall substances was very slow as described in the previous paper (*7*), it is asserted that the major part of amino acid N originated from cytoplasmic substances in fresh cells mineralized quickly and that originated from their cell wall substances mineralized quickly and that originated from their cell wall substances mineralized slowly. That is, it can be assumed that residual matter in sand after 2 weeks of incubation was mostly cell wall substances.

Next, as seen in the analytical values of the sample oven-dried previously just after the addition of fresh cells (see (4) Oven-dried), the forms of amino sugar N and amino acid N decreased remarkably. This result indicates that about 70 per cent of the form of amino sugar N given from the fresh cells decomposed due to the effect of oven-drying (: hereinafter reffered to as the oven-drying effect). The form of amino acid N also became decomposable due to oven-drying, and as compared with "Control" after 2 weeks of incubation the mineralization rate increased about 7 per cent, *i. e.*, about 85 per cent of the form of amino acid N given from the fresh cells were mineralized. From the result obtained, it is suggested that mineralization of the amino sugar and peptide compounds contained in cell wall substances in soil are comparatively slow as compared with those in cytoplasmic substances, but when once oven-dried, they turn decomposable.

Furthermore, after 2 weeks of incubation, the forms of amino sugar N and amino acid N of the samples added with the cells of ultrasonic pretreatment decreased remark-

Table 2. Organic nitrogen fractions in acid hydrolysate given from cell wall substances of *B. subtilis*.

Treatment	Organic N added	Mineral N	Organic N	Non-hydrolyzable N	Hydrolyzable N			
					Ammonium N	Amino sugar N	Amino acid N	Unidentified N
	(mg N per 100 g sand)							
(1) Control:	65.3			2.6	11.2	8.5	30.0	13.0
at the	[100.0]			[4.0]	[17.2]	[13.0]	[45.9]	[19.9]
beginning	(100.0)			(100.0)	(100.0)	(100.0)	(100.0)	(100.0)
(2) Ultrasonicated: after 2 weeks of incubation	65.3	24.6	40.7	2.7	19.2	2.8	9.2	7.5
(3) Ultrasonicated +Oven-dried: after 2 weeks of incubation	65.3	28.7	36.6	2.4	10.4	1.5	9.4	12.9
Amounts of mineralized N								
Ultrasonicated			24.6	−0.1	−8.0	5.7	20.8	5.5
(1)−(2)			(37.7)			(67.1)	(69.3)	(42.3)
Ultrasonicated +Oven-dried			28.7	0.2	0.8	7.0	20.6	0.1
(1)−(2)			(44.0)	(7.7)	(7.1)	(82.4)	(68.7)	(0.8)

[ ]: Figures show per cent of organic N added.
( ): Figures show per cent of each organic N fraction.

ably (see (3) Ultrasonicated). This result indicated the same tendency as in "Oven-dried." Besides, as compared with "Oven-dried," "Ultrasonicated" was slightly low in the decrease of the forms of amino sugar N and amino acid N, *i. e.*, about 48 per cent of the form of amino sugar N and about 83 per cent of the form of amino acid N were mineralized in fresh cells. These results indicate that the effect of ultrasonicating on the mineralization of organic matter in soil (: hereinafter refferred to as the ultrasonicating effect) is similar to the oven-drying effect and both effects accelerate decomposition of the amino sugar and peptide compounds in cell wall substances. Further, it is suggested that the oven-drying effect much more accelerate mineralization of the amino sugar compounds in cell wall substances than the ultrasonicating effect.

The organic nitrogen fractions in acid hydrolysates given from cell wall substances of *B. subtilis* and *S. cerevisiae* are shown in Table 2 and Table 3, respectively. As seen in Table 2, 13 per cent and about 46 per cent of the organic nitrogen added were contained in the forms of amino sugar N and amino acid N in acid hydrolysate given from cell wall substances of *B. subtilis,* respectively (see (1) Control). In "Ultrasonicated" after 2 weeks of incubation (see (2) Ultrasonicated), about 67 per cent of the form of amino sugar N and about 69 per cent of the form of amino acid N were mineralized. Further, in "Ultrasonicated+Oven-dried" after 2 weeks of incubation (see (3) Ultrasonicated+Oven-dried), about 82 per cent of the form of amino sugar N and about 69 per cent of the form of amino acid N were mineralized. That is, the oven-drying treatment accelerated the mineralization rate of the amino sugar compounds by 15 per cent as compared with the ultrasonic treatment, but did not accelerate decomposition of the amino acid compounds. These results indicate that both the oven-drying and ultrasonicating effects have a similar effect on mineralization of the amino acid compounds in cell wall substances under this experimental conditions, but the former is larger on mineralization of the amino sugar compounds than the latter, as described above.

As seen in Table 3, the results given for cell wall substances of *S. cerevisiae* showed the same tendency as those for cell wall substances of *B. subtilis*. Namely the mineralization of cell wall substances of *S. cerevisiae* prepared by the ultrasonic treatment was accelerated (see (2) Ultrasonicated); furthermore when their cell wall substances were oven-dried, their mineralization was accelerated still more (see (3) Ultrasonicated +Oven-dried).

Besides, as to the accelerating effect on the mineralization of amino sugar compounds in cell wall substances due to both pretreatments, the difference in the mineralization rate between *B. subtilis* and *S. cerevisiae* was indicated. It may be considered that this difference originated from the differences in composition and structure of the amino sugar compounds in both microbial cell wall substances.

Table 3. Organic nitrogen fractions in acid hydrolysate given from cell wall substances of *S. cerevisiae*.

Treatment	Organic N added	Mineral N	Organic N	Non-hydrolyzable N	Hydrolyzable N			
					Ammonium N	Amino sugar N	Amino acid N	Unidentified N
				(mg N per 100g soil)				
(1) Control: at the beginning	175.6 [100.0] (100.0)			5.4 [3.1] (100.0)	16.2 [9.2]	13.3 [7.6] (100.0)	70.0 [39.9] (100.0)	70.7 [40.2] (100.0)
(2) Ultrasonicated: after 2 weeks of incubation	175.6	74.4	101.2	9.2	23.4	12.8	41.5	14.3
(3) Ultrasonicated +Oven-dried: after 2 weeks of incubation	175.6	98.8	76.8	5.2	19.3	4.4	40.3	7.6
Amounts of mineralized N								
Ultrasonicated (1)-(2)			74.4 (42.4)	−3.8	−7.2	0.5 (3.8)	28.5 (40.7)	56.4 (79.8)
Ultrasonicated +Oven-dried (1)-(3)			98.8 (56.3)	0.2 (3.7)	−3.1	8.9 (66.9)	29.7 (42.4)	63.1 (89.3)

[ ]: Figures show per cent of organic N added.
( ): Figures show per cent of each organic N fraction.

EXPERIMENT 2 AMINO ACID COMPOSITION IN AMINO ACID NITROGEN FRACTIONS OF ACID HYDROLYSATES GIVEN FROM CELLS OF *B. SUBTILIS* AND THEIR CELL WALL SUBSTANCES, AND CHARACTERIZATION OF THE DECOMPOSABLE AMINO ACID COMPOUNDS

Materials and methods

Amino acid composition of amino acid N fractions of acid hydrolysates given from cells of *B. subtilis* and their cell wall substances in Experiment 1 was analyzed. Individual amino acids were determined by using the automatic amino acid analyzer (Japan Electric Co., J. L. C-5AH Type) after the isolation of the amino acid N fraction by ion-exchange chromatography.

Results and discussion

Change of amino acid composition occurring through the decomposition process in cells of *B. subtilis* is shown in Fig. 1. Because the cytoplasmic substances of cells are almost decomposed for 2 weeks as described in the previous paper (*7*), it may be reasonable to consider that the amino acids in "Control" after 2 weeks of incubation originate from peptide compounds in cell wall substances remaining in the sand as residual matter. Amino acid composition showed the following order: Ala, Glu, Asp, Gly>

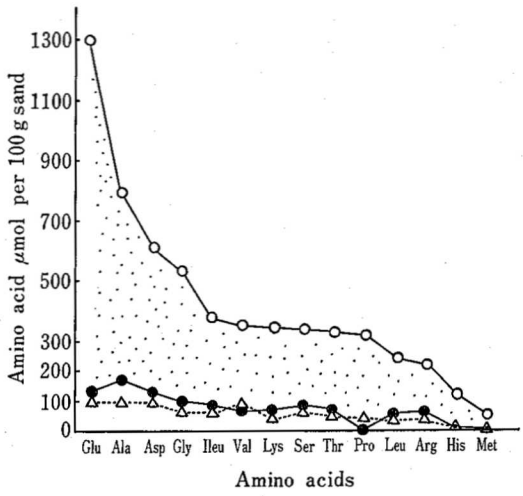

Fig. 1. Change of amino acid composition occurring through the decomposition process in cells of *B. subtilis*.
○, control at the beginning: ●, control after 2 weeks of incubation; △, oven-dried after 2 weeks of incubation.

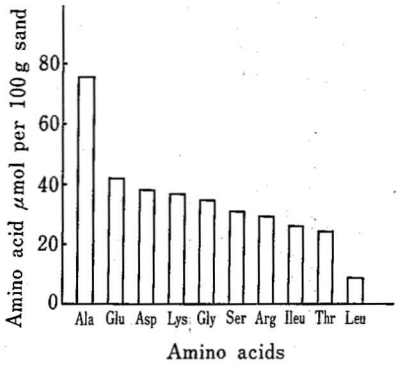

Fig. 2. Amino acids in cells of *B. subtilis* which become decomposable due to oven-drying.

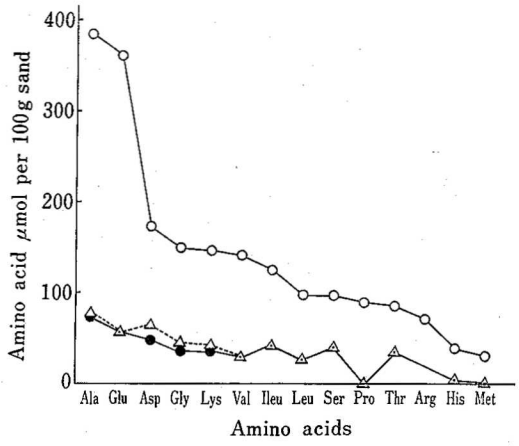

Fig. 3. Change of amino acid composition occurring through the decomposition process in cell wall substances of *B. subtilis*.
○, control at the beginning; ●, ultrasonicated after 2 weeks of incubation; △, ultrasonicated+oven-dried after 2 weeks of incubation.

Ileu, Ser, Val, Thr, Lys>Arg, Leu (see Fig. 1). The composition of major amino acids is similar to those in cell wall substances of *B. subtilis* as described later (see Fig. 3).

Furthermore, it may be considered that the dotted part in Fig. 1 indicates the amounts of amino acids in cytoplasmic substances which were mineralized for the 2-week incubation.

Composition and amounts of decomposable amino acids in cells of *B. subtilis* which are computed from the difference between the amounts of amino acids in "Control" after 2 weeks of incubation and those of amino acids in "Oven-dried" after 2 weeks of incubation are shown in Fig. 2. Decomposable major amino acid composition almost equalled that of the peptide compounds in cell wall substances (see Fig. 3) and decomposable amounts of individual amino acids were almost proportional to their amounts in cells. This suggests that some peptide compounds in cell wall substances become decomposable due to the oven-drying effect.

Change of the amino acid composition occurring through the decomposition process of cell wall substances of *B. subtilis* is shown in Fig. 3. The amino acid composition in peptide compounds of cell wall substances showed the following order: Ala, Glu> Asp, Gly, Lys, Val>Ileu, Leu, Ser. It is assured that a great deal of Ala and Glu in the amino acid composition was owing to mucopeptide existing in cell wall substances because *B. subtilis* belongs to the gram positive bacteria. As described in Experiment 1, in the case of cell wall substances of *B. subtilis* which were prepared by ultrasonic treatment, mineralization of amino sugar N in the sample previously oven-dried was accelerated, but was not accelerated that of amino acid N in them.

These results led to the conclusion that the oven-drying effect is quite similar to the ultrasonicating effect on the mineralization of the form of amino acid N in the cell wall substances of *B. subtilis*. As seen in Fig. 3, amino acid composition in "Ultrasonicated" after 2 weeks of incubation was entirely analogous to that in "Ultrasonicated +Oven-dried" after 2 weeks of incubation. And on the whole their amino acid composition was similar to that of peptide compound in "Control" at the beginning, *i. e.*, cell wall substances of *B. subtilis*. This clearly shows that the amino acid composition becoming decomposable due to both pretreatments is similar to that of the peptide compounds in cell wall substances.

The results described in Experiment 1 and Experiment 2 might complementarily prove that microbial cells, especially their cell wall substances, contribute considerably as a source of organic matter becoming decomposable due to soil drying, as described in the previous paper (*1, 4, 7*).

REFERENCES

1) AHMAD, Z., YAHIRO, Y., KAI, H., and HARADA, T., Factors affecting Immobilization and Release of Nitrogen in Soil and Chemical Characteristics of the Nitrogen Newly Immobilized, IV. *Soil Sci. Plant Nutr.* (*Tokyo*), **19**, 287-298 (1973)

2) BREMNER, J.M., Method of Soil Analysis, Agronomy, No. 9, Part 2, American Society of Agronomy. Inc., Madison, 1965. p. 1238

3) HAYASHI, R. and HARADA, T., Characterization of the Organic Nitrogen Becoming Decomposable through the Effect of Drying of a Soil, *Soil Sci. Plant Nutr.* (*Tokyo*), **15**, 226-234 (1969)

4) KAI, H., AHMAD, Z., and HARADA, T., Factors Affecting Immobilization and Release of Nitrogen in Soil and Chemical Characteristics of the Nitrogen Newly Immobilized, III. *Soil Sci. Plant Nutr.* (*Tokyo*), **19**, 275-286 (1973)

5) MARUMOTO, T., FURUKAWA, K., YOSHIDA, T., KAI, H., YAMADA, Y., and HARADA, T., Contribution of Microbial Cells and Their Cell Walls to an Accumulation of the Soil Organic Matter Becoming Decomposable Due to Drying a Soil (Part 1), *J. Sci. Soil Manure, Japan*, **45**, 23-28 (1974)

6) MARUMOTO, T., KAI, H., YOSHIDA, T., and HARADA, T., Relationship between an Accumulation of Soil Organic Matter Becoming Decomposable Due to Drying of Soil and Microbial Cells, *Soil Sci. Plant Nutr.* (*Tokyo*), **23**, 1-8 (1977)

7) MARUMOTO, T., KAI, H., YOSHIDA, T. and HARADA, T., Drying Effect on Mineralization of Microbial Cells and Their Cell Walls in Soil and Contribution of Microbial Cell Walls as a Source of Decomposable Soil Organic Matter, *Soil Sci. Plant Nutr.* (*Tokyo*), **23**, 9-19 (1977)