B-6-(3)-③: Quantitative estimation of cellulolytic enzyme system in soil and its relation to global climate warming

Contact person:Tomoyuki Hakamada, Associate Division Director for Research, Division of Environmental Planning,National Institute of Agro-environmental Sciences. Kan-nondai 3-1-1, Tsukuba, Ibaraki, 305 Japan

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Global production of cellulose by plant is calculated to be 10^{11} ton/year and most of the cellulose is supplied to soil as plant residue. Cellulase is a key enzyme in decomposition of plant residue because it operates at first step in the process that cellulose-C is mineralized to carbon dioxide and released to atmosphere. This study is aimed to predict the impact of global climate warming on cellulolytic activity in soil. For this purpose, we investigate the cellulase activity in soils sampled from various agricultural sites in the north latitude 25° to 45° in Japan. Especially, we investigate properties concerning temperature, such as optimum temperature and Q_{10} of cellulase activities in soils. Twenty-six soil samples were taken from following sites: Ishigaki(lat. 24.2°, annual temp. 23.8C°), Tokunoshima(lat. 27.4°, ann. temp.21.2C°), Kagoshima(lat. 31.3°, ann. temp.17.3C°) Chikugo(lat. 33.1°, ann. temp.16.0C°), Sapporo(lat. 43°, ann. temp. 8.3 C°) and Hamatonbetsu(lat. 45°,ann. temp.5.3C°). p-Nitrophenyl-β-cellobioside was used for assay of cellulase activity in soil. Optimum temperature of cellulase activity in soil ranged from 55.6 to 67.0℃. Significant correlation was observed between the optimum temperature of the cellulase activity and annual temperature at sampling site ($r = 0.602^{**}$). Q_{10} of cellulase activities between 20 and 30℃ ranged from 1.6 to 2.8. Correlation between Q_{10} and annual temperature was not significant. However, the lower in assay temperature of the enzyme activity, the Q_{10} value became larger. Following β-glucosidic substrate other than p-nitrophenyl-β-cellobioside was also hydrolyzed by soil extracts; p-nitrophenyl-β-cellotrioside, carboxymethyl cellulose and microcrystallin cellulose. Hydrolysis of p-nitrophenyl-β-cellotrioside by soil extracts was competitively inhibited by microcrystallin cellulose but not by carboxymethyl cellulose. Hydrolytic activity of the soil extracts to insoluble substrate was lower than that to soluble substrtate.

Key Words: Cellulolytic enzymes, Soil Enzymes, Agricultural soil, Optimum temperature, Q_{10}

Introduction

Cellulolytic enzyme system in soil is an essential process in humification of plant residue. Global production of cellulose by plant is calculated to be 10^{11} ton/year and most of the cellulose is supplied to soil as plant residue. Cellulase in soil is a key enzyme in decomposition of the plant residue because it operates at first step in the process that cellulose-C is mineralized to carbon dioxide and released to atmosphere. This study is aimed to predict the impact of global climate warming on cellulolytic activity in soil. For this purpose, we aimed to investigate the cellulase activity in soils sampled from various agricultural sites in the north latitude 25° to 33° in Japan. Especially, we investigated the properties concerning temperature, such as optimum temperature and Q_{10} of the cellulase activities in soils.

Materials and Methods

Soil samples were taken from Ishigaki, Tokunoshima, Kagoshima and Chikugo in August or

September. Characteristics of the fine material after the moist soil had been sieved (<2mm), was shown in Table 1. The cellulase activity of the soil samples was measured by using 1mM p-nitrophenyl-β-cellobioside as a substrate with various incubation temperature in ranging from 20 to 72.5℃. Optimum temperature of the cellulase activity of each soil sample was calculated from the intersecting point of Arrehenius plot. The Q_{10} of cellulase activities in soils were calculated from the data at 20 and 30℃. For a few soil samples, the organic debris remaining on the sieve consisted of decomposing litter and plant root was hand-picked from the <2mm fraction, each fractions were served for enzyme assay in order to investigate the localization of cellulase in soil. Cellulase -active extracts were obtained by phosphate buffer extraction at 40℃ (Hayano 1986). To assay of cellulase activity towards non-chromogenic substrate such as CMC, reducing sugars formed were determined after incubation by Somogyi-Nelson's(Nelson, 1944). Soil fungi were isolated by dilution plate method on Rose Bengal agar(Martin 1950). Cellulose decomposers were assayed on CMC agar (Hankin et al. 1974).

Results and Discussion

A cellulase component of *Trichderma viride* was reported to attack the aglycone linkage of p-nitrophenyl-β-cellobioside(Okada 1976). Optimum pH of the hydrolysis of p-nitrophenyl-β-cellobioside in an Andosol under tea field at Kagoshima and a Dark red soil under grassland at Ishigaki was about 4.5 and 5.0, respectively(Fig.1). The optimum pH of the cellulase activity in the soil was similar to those of fungal origin; cellulase activities of Aspergillus niger and Fusarium miniliiforme are reported to be optimal about pH 4.5-6(Clarke and Stone, 1965) and 5(Matsumoto et al., 1974), respectively. Hayano (1986) indicated that fungi were the more important source of cellulase in a tomato field soil from experiment of selective inhibition of microbes growth. Optimum temperature of cellulase activity in 28 soils measured at pH5.0 ranged from 58.5 to 65.5℃ (Table 1) and was well correlated with annual average temperature of the sites where soil was sampled ($r=0.602^{**}$)(Table 2 and Fig.2). Following regression equation was obained from the relationship between optimum temperature of the activity,f(x) and annual average temperature of sampling sites,x:$f(x) = 0.22x + 58.0$. The cellulase activity measured at 30℃ was negatively correlated with annual average temperature ($r=-0.612^{***}$). Q_{10} value of the cellulase activity ranged from 1.6 to 2.8(Table 1) and was not correlated with annual average temperature of the sampling sites(Table 2and Fig.3). However, the lower in assay temperature of the enzyme activity, the Q_{10} value became larger(Fig.4). Sarkar(1986) has prepared ^{14}C-cellulase-humus complex from cellulase and humic acid, observed effect of temperature on the stability and reported that ^{14}C-cellulase-humus complex retained 73% of the activity after 1h at 80℃, while humus free cellulase retained only 33% activity. Cellulase activityof a soil extract after protamine-treatement which has fairly removed humic substance, was reported to be sensitive to heat; the activity was lost after 10min at 80℃(Hayano 1986). These evidences suggest that elevation of climate temperature will accelerate hydrolysis of cellulose contained in plant residues without inactivation of cellulase component in soil.

Distribution of cellulase in soil was investigated on a Dark red soil under grassland at Tokunoshima(Fig.5). Although in soil samples, organic debris represented only about 1% of the dry weight, 14% and 38% of total cellulase activity was localized in this fraction in soil from Ishigaki and Tokunoshima, respectively.

Organic debris in the soil samples was composed of litter and root. Hayano (1986) has reported that more than 60% of CMCase cellulase activity was localized in organic debris fraction from a tomato field soil and that curvilinear relationship of the Langmuir type was observed between solution volume of phosphate buffer extractant and cellulase activity extracted from the organic debris fraction.

Sinsabaugh and Linkins(1988) observed that a cellulase component prepared from Trichoderma viride was adsorbed on litter analogs prepared by acid-detergent digestion of senescent Pinus strobus. Colony forming units of cellulolytic organisms in the organic debris

fractions of Yellow soil under sugarcane field in Ishigaki($5.1 \times 10^6 g^{-1}$) and of Dark red soil under Tokunoshima ($1.2 \times 10^7 g^{-1}$)were higher than those in mineral fractions($3.5 \times 10^5 g^{-1}$ and $3.9 \times 10^5 g^{-1}$, respectively) sieved through 2mm mesh, suggesting that organic debris is important site of cellulose hydrolysis in soil.

Hydrolysis of various cellulase substrate by the enzyme extracts from tea field soil and grassland soil was shown inFig.6. Following β-glucosidic substrate other than p-nitrophenyl-β-cellobioside was also hydrolyzed by soil extracts; p-nitrophenyl-β-cellotrioside, carboxymethyl cellulose and microcrystallin cellulose. CMC was most hydrolysable substrate for both soil extracts. Michaelis constant of the cellulase activity of the tea field soil and grassland soil towards p-nitrophenyl-β-cellotrioside was estimated to be 0.058mM and 0.10mM, respectively. Hydrolytic activity of the soil extracts to insoluble substrate was lower than that to soluble substrtate.

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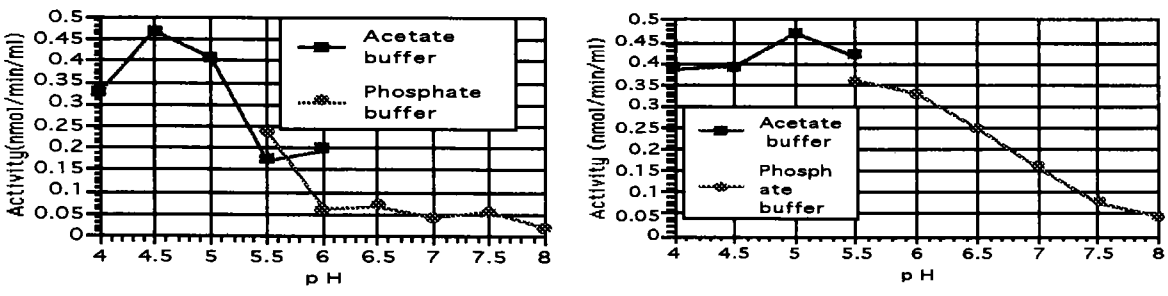

Fig.1. Optimum temperature of cellulase activirty in soils (Left:Kagoshima tea field; Right: Ishigaki Grassland)

Table 1. Annual climate temperature and properties of soils

Sampling site	Soil	Annual temp.	C (%)	N (%)	p H
Ishigaki	Grassland, Dark Red soil	23.8	3.92	0.35	7.1
	Grassland, Yellow soil	23.8	0.63	0.05	4.8
	Sugarcane field, Dark Red soil	23.8	0.33	0.03	4.7
	Sugarcane field, Yellowsoil	23.8	0.62	0.06	6.4
	Paddy field, Yellow soil	23.8	0.69	0.08	5.7
Tokunoshima	Grassland, Dark Red soil	21.2	1.64	0.19	4.8
	Grassland, Yellow soil	21.2	0.96	0.08	4.6
	Sugarcane field, Dark Red soil	21.2	0.97	0.12	6
	Sugarcane field, Yellowsoil	21.2	0.5	0.07	4.7
	Paddy field, Gray Lowland soil	21.2	1.54	0.14	5.3
Kagoshima	Grassland, Andosol	17.3	1.15	0.1	5.4
	Tea field, Andosol	17.3	6.98	0.35	5.6
	Sweet poteto field, Andosol	17.3	11.03	0.47	5.5
	Paddy field, Regosol	17.3	9.35	0.67	3.4
Chikugo	Wheat field, Gray Lowland soil	16	2.08	0.16	5.8
	Paddy field, Gray Lowland soil	16	2.19	0.17	5.8
	Paddy field, Gray Lowland soil	16	3.43	0.21	5.7
Ibaraki	Paddy rield, Andosol	13.3	2.3	0.21	5.8
	Paddy field, Gray Lowland soil	13.3	2.4	0.26	6.6
Sapporo	Paddy rield, Andosol	8.3	4.2	0.274	5.85
	Paddy field, Gray Lowland soil	8.3	2.12	0.156	5.9
	Corn field, Andosol	8.3	3.78	0.262	4.82
	Grassland, Andosol	8.3	4.05	0.313	5.1
Hamatonbetu	Grassland, Brawn forest soil	5.3	3.62	0.263	5.45
	Corn field, Brawn forest soil	5.3	3.29	0.237	6.72
	Grassland, Pseudogleyed Brawn Forest	5.3	3.69	0.269	5.62
	Grassland, Andosol	5.3	4.33	0.299	5.5

Table 2. Correlation coefficient among annual temperature and various soil properties

r	Annual temp.	C (%)	N (%)	pH	Activity(1)	Relative act.(1)	Activity(2)	Relative act.(2)	Opt.temp.	Q10
Annual temp.	1	-0.314	-0.389	-0.141	-0.619***	0.191	0.279	0.68***	0.602**	-0.12
C (%)		1	0.929	-0.125	0.324	-0.596	0.244	-0.576	-0.36	0.029
N (%)			1	-0.156	0.536	-0.532	-0.1	0.26	-0.326	-0.016
pH				1	-0.095	-0.302	-0.35	-0.274	-0.033	0.05
Activity(1)					1	0.116	0.487	-0.356	-0.09	0.103
Relative act.(1)						1	-0.114	0.767	0.399	0.031
Activity(2)							1	0.145	0.43	-0.136
Relative act.(2)								1	0.505	-0.138
Opt.temp.									1	0.186
Q10										1

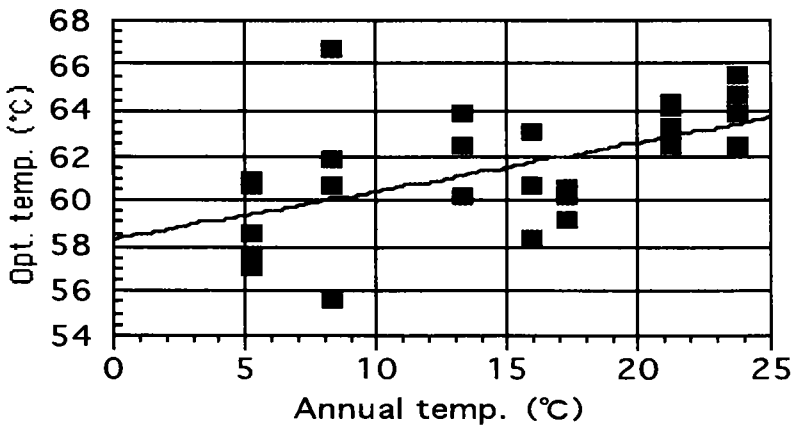

Fig.2. Correlation between optimum temperature of cellulase activitry in soil and annual temperature of sampling site.

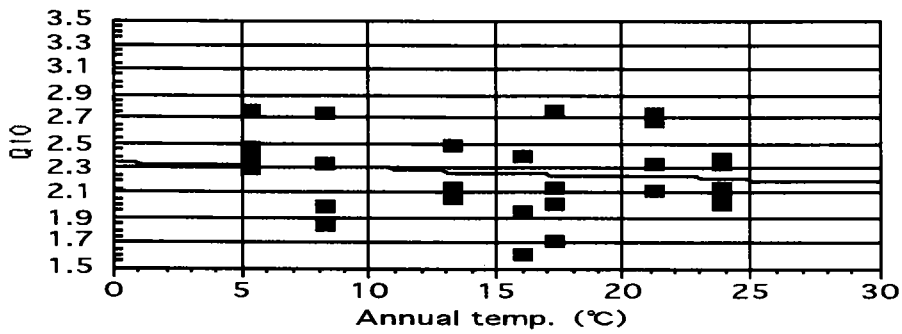

Fig.3. Relationship between annual temperature of sampling site and Q10 of the cellulase activity.

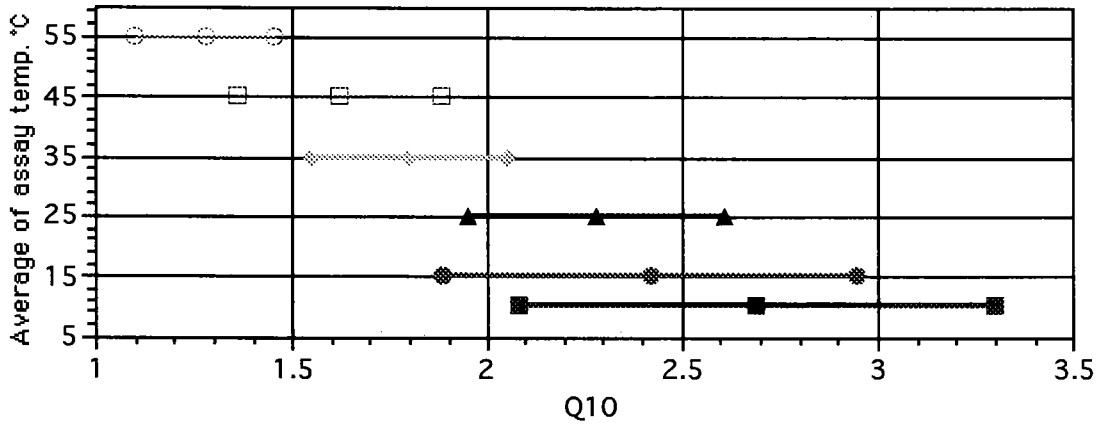

Fig.4. Relationship between Q_{10} of the cellulase activity and assay temperature. Bars denote SD.

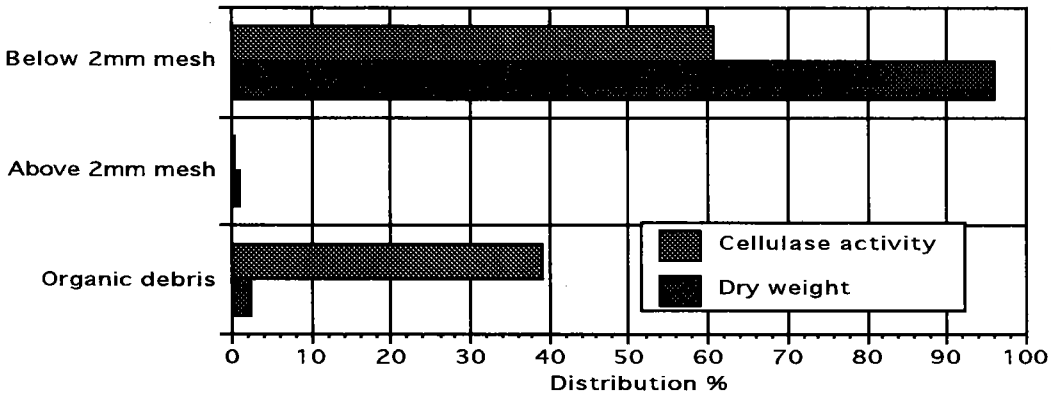

Fig.5. Distribution of cellulase activity in soil fractions

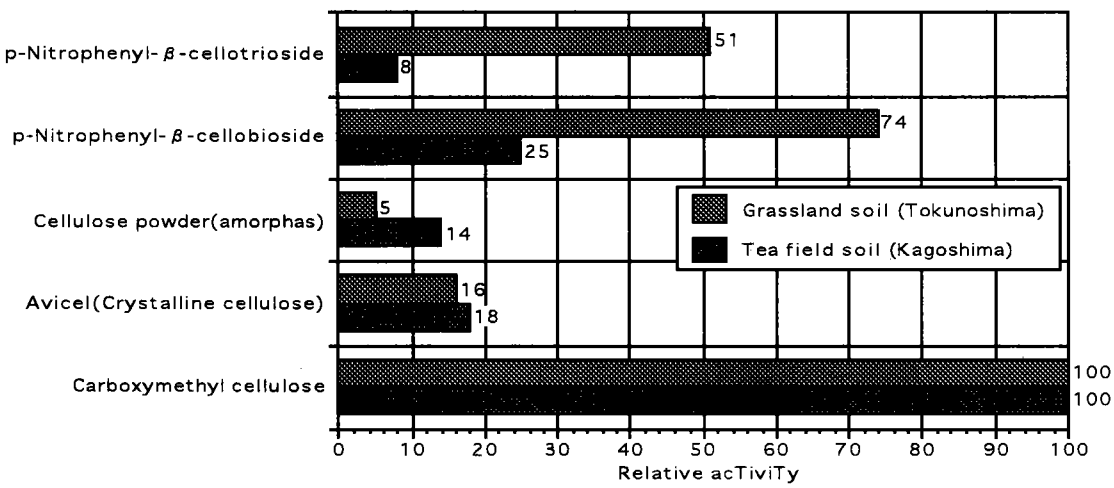

Fig.6. Substerate specificity of the cellulase activity in soil extract.