Comissão 2.4 - Química do solo

KINETIC PARAMETERS OF SOIL β-GLUCOSIDASE RESPONSE TO ENVIRONMENTAL TEMPERATURE AND MOISTURE REGIMES[1]

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SUMMARY

Soil β-glucosidase participates in the final step of cellulose biodegradation. It is significant in the soil C cycle and is used as an indicator of the biological fertility of soil. However, the response of its kinetic parameters to environmental temperature and moisture regimes is not well understood. This study tested the β-glucosidase response in the main agricultural soils (black soil, albic soil, brown soil, and cinnamon soil) of Northeast China. Incubation tests were conducted to measure the kinetic parameters K_m, V_{max} or V_{max}/K_m of soil β-glucosidase at environmental temperatures of 10, 20 and 30 ºC and at 10, 20 and 30 % soil moisture content. The insensitive response of the kinetic parameters to temperature changes indicates that soil β-glucosidase was present primarily in immobilized form. The significant response of the kinetic parameters of soil β-glucosidase to soil moisture rather than to environmental temperatures suggests that the catalytic ability of soil β-glucosidase was sensitive to changing soil moisture regimes.

Index terms: enzymatic kinetic, soil moisture, cellulose biodegradation.

RESUMO: *PARÂMETROS CINÉTICOS DA β-GLUCOSIDADE DO SOLO EM RESPOSTA À TEMPERATURA E A REGIMES DE ÁGUA NO AMBIENTE*

A β-glucosidade do solo participa do passo final da biodegradação da celulose. Ela tem contribuição significativa no ciclo do C no solo e é usada como indicador do componente biológico da fertilidade do solo. Contudo, a resposta de seus parâmetros cinéticos à temperatura

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e a regimes hídricos no ambiente não é bem conhecida. Este estudo testou a resposta da β-glucosidade nos principais solos agrícolas do norte da China (solo negro, álbico, castanho e castanho-amarelo-claro). Testes de incubação foram conduzidos para estimar os parâmetros cinéticos K_m, V_{max} *ou* V_{max}/K_m *da β-glucosidade nas condições de 10, 20 e 30 ºC e a 10, 20 e 30 % de água no solo. A resposta não significativa dos parâmetros cinéticos a mudanças na temperatura indica que a β-glucosidade do solo estava presente principalmente em uma forma imobilizada. A resposta significativa dos parâmetros dessa enzima à umidade, e não à temperatura, sugere que sua habilidade catalítica é sensível a mudanças no regime de água do solo.*

Termos de indexação: cinética enzimática, água no solo, biodegradação de celulose.

INTRODUCTION

β-glucosidase is widely distributed in the soil and has been detected in microorganisms, animals, and plants (Eivazi & Tabatabai, 1988). Soil β-glucosidase is a digestive enzyme for cellobiose/triose mineralization. It catalyzes the enzymatic hydrolysis of various polysaccharides and β-glucosides (Jiménez et al., 2007). Accordingly, it is viewed as an indicator of turnover of soil organic C compounds from crop residues, biotechnological byproducts, animal manure, and sewage sludge. It is the driving force in the decomposition of carbohydrates in soils. The resulting hydrolysis products (sugars) are important energy sources for microorganisms in soils (Eivazi & Tabatabai, 1988; Marx et al., 2005).

Soil β-glucosidase activity has been proposed as a soil quality indicator. It is sensitive to environmental changes caused by soil management (Kuperman & Carreiro, 1997; Bergstrom et al., 1998; Nannipieri & Gianfreda, 1998; Leirós et al., 1999; Bandick & Dick, 1999; Ndiaye et al., 2000; Madejón et al., 2001). Soil β-glucosidase is usually adsorbed on the surface of mineral particles and organic material in soil, and the heterogeneous nature of soil affects the kinetic diversity of immobilized enzymes (Skujins, 1976; Hayano & Katami, 1977). Enzymes in different locations in the soil (i.e., immobilized *vs*. free) may cause a change in K_m values (Paulson & Kurtz, 1970; Gianfreda & Bollag, 1994; Rao et al., 1996). Most soil enzymes are extracellular and are immobilized by soil components. These characteristics result in different catalytic properties, e.g., lower V_{max} and higher K_m values, compared with pure enzymes (Makboul & Ottow, 1979a,b; Gianfreda & Bollag, 1994).

Enzyme catalytic activities are markedly affected by site-specific factors such as environmental temperatures, moisture, nutrient availability and other site parameters (Bandick & Dick, 1999; Knight & Dick, 2004). Kasia et al. (1999) also suggested that the temporal variation of soil enzyme activities might be driven by environmental factors (e.g., temperature and moisture). In general, soil enzyme activities increase with increasing temperature up to the optimum catalytic value**.** Moyo et al. (1989) showed that the temperature for inactivating soil enzymes is about 10 ºC higher than that for inactivating free enzymes. Within the range of ambient temperature (2–30 ºC), the temperature response curves (Arrhenius plots) of soil enzymes are linear (McClaugherty & Linkins, 1990).

The soil moisture regime has definite effects on the catalytic potential of soil enzymes (García et al., 2002; Sardans & Penuelas, 2005). Engasser & Horvath **(**1976) reported that soil moisture content affected the movement of enzymes and their substrate concentrations in soil and that the diffusion limitation of the substrates may directly affect soil enzyme K_m. Several studies have also shown that changes in soil moisture content have significant effects on the kinetic parameters of soil hydrolases (Burns, 1978; Ladd, 1985; Boyd & Mortland, 1990).

Marx et al. (2005) showed that β-glucosidase followed simple Michaelis-Menten kinetics in all soil particle-size fractions. However, little information is available about the effect of environmental temperature and moisture on the kinetic properties of this enzyme in soils. Therefore, exploring the effects of incubation temperature and soil moisture fluctuations on kinetic characteristics of soil β-glucosidase will lead to a better understanding of the changes in the substrate affinity and the catalytic activity of soil β-glucosidase. Studies of this kind will further help to assess the effects of changing environmental factors on soil-C biochemical cycles. In this paper, kinetic characteristics of β-glucosidase in black soil (Phaeozem, WRB 1998), albic soil (Albi-Bori-Luvisol, WRB 1998), brown soil (Hapli-Udi-Luvisol, WRB 1998) and cinnamon soil (Hapli-Usti-Luvisol, WRB 1998) of Northeast China were studied under simulated environmental temperatures and soil moisture regimes in order to define their responses to changes in environmental factors.

MATERIALS AND METHODS

Four sampling sites were selected from black, albic, brown and cinnamon soil sites in Northeast China as

described by Zhang et al. (2009). In all, 60 soil samples (0 - 20 cm) over approximately 1 ha at each site were collected in early spring before sowing. Three plots of 50 × 80 m at each site were selected for soil sampling. A random sampling scheme was applied, with a distance of > 0.5 m between sampling points.

The 20 samples from each sampling plot were mixed to form a composite sample, transported to the laboratory in isothermal bags, and passed through a 2.0 mm sieve. Some sieved samples were air-dried for analysis of chemical and physical properties. Physical and chemical properties of the studied soils are given by Zhang et al. (2009). Parts of the subsamples (1,000 g; n = 3) of each composite sample were preincubated at ca. 60 % water-holding capacity (WHC) and 25 °C for 14 days to stabilize the biological and biochemical characteristics of the soil before the experiment. Distilled water was added daily to compensate for the water loss from incubation.

Incubation test

1) Temperature treatments: The preincubated soils were modified to 20 % (ca. 60 % WHC) moisture content with distilled water. They were incubated in incubation chambers set at 10, 20 and 30 °C for 21 days, respectively.

2) Moisture treatments: The preincubated soil was modified to 10, 20 and 30 % moisture content to simulate minimal, normal, and maximum soil humidity, respectively, and incubated at room temperature for 21 days. Each treatment was performed in triplicate. After incubation, the activities of test enzymes in each treatment were determined.

Soil enzyme activity measurement

p-nitrophenyl glucoside (AR) was purchased from Sigma-Aldrich Inc., and other reagents (AR) were purchased from Seebio Biotech Inc. and J&K China Chemical Ltd., respectively.

β–glucosidase (EC 3.2.1.21 pH 6, 37 °C) activity was determined using *p*-nitrophenyl β–glucopyranoside as substrate by incubating in a pH 6 modified buffer at 37 °C. After 1 h, 0.5 mol L^{-1} $CaCl_2$ and 0.5 mol L^{-1} Tris solution (pH 12) were added to precipitate humic molecules responsible for brown coloration and to extract *p*-nitrophenol. The *p*-nitrophenol produced was measured colorimetrically (Tabatabai, 1994). The enzyme activity of the controls was measured by the same procedures, but the substrates were added to the soil samples after incubation and prior to the analysis of the reaction product.

Kinetic parameter measurement

Five concentrations (0.005, 0.010, 0.020, 0.030, 0.050 mol L^{-1}) of *p*–nitrophenyl β-glucopyranoside solution were used as substrates of soil β-glucosidase, and the kinetic parameters V_{max} and K_m were calculated using the Lineweaver-Burk linearization of the Michaelis-Menten equation (Zhang et al., 2009, 2010).

Statistical analysis

All determinations were performed in triplicate. All values were reported as means ± standard deviation and expressed per gram of oven-dried soil (105 °C). Data treatment and statistical analysis were performed with SPSS 10.0 software. For each variable measured, the data were analyzed by one-way ANOVA. Least significant differences (LSD) at $p < 0.05$ were tested to determine the significant differences between treatment means.

RESULTS

Kinetic parameters of soil β-glucosidase under temperature and moisture regimes

The highest K_m values in black, albic and cinnamon soils occurred at 30 °C. However, the highest K_m value of β-glucosidase in brown soil occurred at 20 °C (Figure 1). The V_{max} values of β-glucosidase in black and albic soils were higher than those in brown and cinnamon soils. The V_{max} value of β-glucosidase increased with increasing temperatures in black soil. The trend was the same as in albic soil but no significant differences were observed. The response of cinnamon soil was reversed to that of the black soil. The highest V_{max} was observed at 20 °C in brown soil (Figure 1). The V_{max} of β-glucosidase was higher in black and albic soils than in brown and cinnamon soils (Figure 1).

The K_m value of β-glucosidase in all test soils increased with increasing soil water content (Figure 2). The V_{max} increased with increasing soil water content in black and albic soils and decreased with increasing soil water content in cinnamon soil. The highest V_{max} was observed at 20 % soil water content in brown soil. In general, the V_{max} values of β-glucosidase in black and albic soils were slightly lower than those in brown and cinnamon soils under different soil moisture regimes.

The V_{max}/K_m values of β-glucosidase were higher at 20 °C in black and albic soils and were higher at 10 °C in brown and cinnamon soils. The V_{max}/K_m values of β-glucosidase were higher at a soil water content of 20 % in black, albic and brown soils but higher at 10 % soil water content in cinnamon soil (Table 1).

Correlations between kinetic parameters of soil β-glucosidase under temperature and moisture regimes and soil physical and chemical properties

The V_{max} values of soil β-glucosidase for soil TOC and C/N ratios were significantly and positively

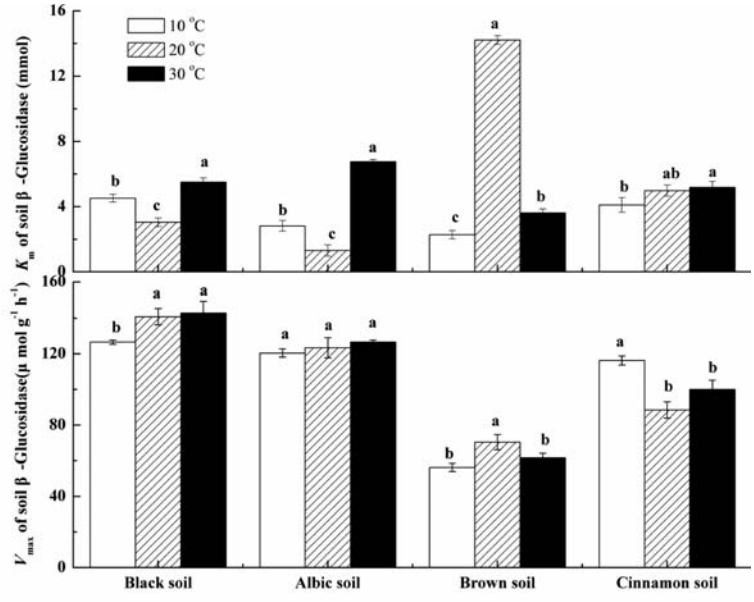

Figure 1. K_m and V_{max} of soil β-glucosidase (K_m – m mol L^{-1}; V_{max} – p – nitrophenol g^{-1} soil h^{-1}) at different incubated temperatures.

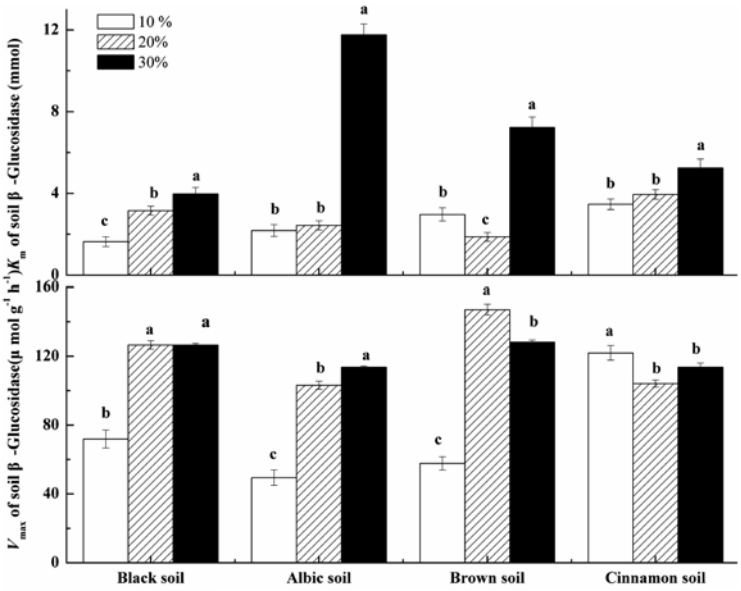

Figure 2. K_m and V_{max} of soil β-glucosidase (K_m – m mol L^{-1}; V_{max} – p – nitrophenol · g^{-1} soil h^{-1}) at different soil moisture contents.

Table 1. V_{max}/K_m of soil β-glucosidase under different environmental temperature and moisture regimes

Soil	Temperature			Moisture		
	10 °C	20 °C	30 °C	10 %	20 %	30 %
Black Soil	28.01 b	47.00 a	25.97 b	45.43 a	40.13 a	32.08 b
Albic Soil	42.74 b	94.34 a	18.73 c	22.87 b	42.50 a	9.68 c
Brown Soil	24.63 a	4.95 b	17.07 a	19.56 b	79.59 a	17.81 b
Cinnamon Soil	28.33 a	17.76 b	19.31 b	35.45 a	26.50 b	23.26 b

correlated. No significant relationships between the kinetic parameters of soil β-glucosidase and other soil physical and chemical properties were found (Figure 3).

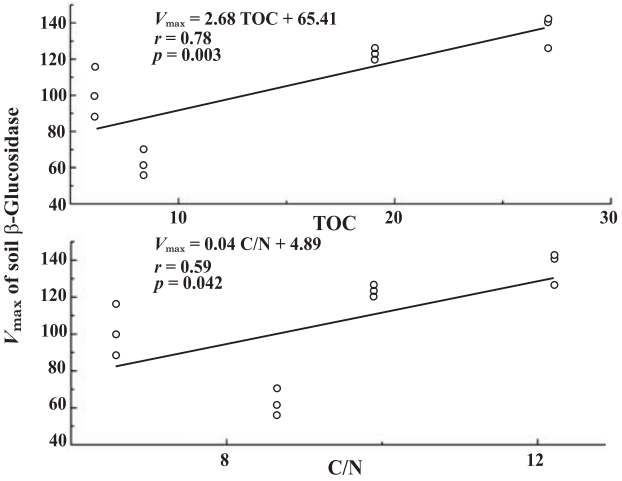

Figure 3. Relationships between Vmax of soil β-glucosidase and soil total organic carbon (TOC) and C/N ratio.

DISCUSSION

In general, the substrate affinity (K_m) of soil enzymes, as that of free enzymes, decreases with increasing environmental temperatures because of the enhanced thermal motion and translocation of substrate (Balkan & Ertan, 2007). In this study, the highest K_m value was observed at 30 ºC, perhaps due to lower binding between substrates and enzymes as the result of enhanced thermal motion at 30 ºC. The K_m of β-glucosidase in test soils increased with increasing soil water content. This result indicates that increasing water content could decrease the concentrations of soil substrate and soil β-glucosidase (Boyd & Mortland, 1990) and thereby decrease their possible affinity.

The V_{max} of free enzymes could double with a 10 ºC increase in temperature. However, the variation of soil β-glucosidase V_{max} with temperature was not linear. The response of V_{max} to temperature increase was not as sensitive as that of free enzymes. The response to temperature increase in cinnamon soil was even reversed (Figure 1), possibly owing to the coaction of temperature and soil physical and chemical properties (Lai & Tabatabai, 1992). These results also indicate that the dominant form of β-glucosidase in soil was immobilized rather than free. That V_{max} values of β-glucosidase were higher in black and albic soils than in brown and cinnamon soils is probably the result of the higher fertility (including biological fertility) of black and albic soils (Zhang et al., 2009). The higher V_{max} at 30 ºC in black and albic soils was possibly a consequence of the fact that 30 ºC was close to the optimum temperature for enzymes in soil of humid or subhumid areas (Moyo et al., 1989; Simihaian, 1998). However, the average annual temperature for cinnamon soil in semiarid regions is 8–9 ºC, near the lowest environmental temperature in this study. This temperature is the reason for the higher V_{max} observed at 10 ºC rather than at other environmental temperatures. Some studies showed that soil enzyme activity was strongly affected by the soil moisture regime (Skujins & McLaren, 1969; Kramer & Green, 2000; Yavitt et al., 2004). Dilly & Munch (1996) reported a positive relation between water content and β-glucosidase activity in litter. Increasing soil moisture enhanced dissolution and translocation of the substrates (i.e., increased movement of enzymes and their substrates). These factors produced increased β-glucosidase velocity (V_{max}) in test soils (Dannenberg et al., 1989; Simihaian, 1998). V_{max} increased with increasing soil water content in black, albic and brown soils. However, the highest V_{max} was found at a soil water content of 10 % in cinnamon soil. This result may be explained by the adaptability of soil β-glucosidase to a lower-rainfall environment in the cinnamon-soil region (Zhang et al., 2009).

The catalytic characteristics of β-glucosidases in the main agricultural soils of Northeast China were, however, to some degree affected by the soil moisture or temperature regime, depending on the organic matter content but not on the texture of these soils (Figure 3). In general, the stability of the enzymes in the face of seasonal climatic changes is based on a built-in protection mechanism that relies on the existence of clay and humus colloids (Lahdesmaki & Piispanen, 1992). Soil texture and organic matter content affect soil enzyme K_m and V_{max} (García et al., 1993). The protection of enzymes by organic matter sometimes enhances their catalytic reactions (Speir, 1977; Shi et al., 2006). The positive relationship between V_{max} and TOC and C/N ratios found in this study confirms these previous findings. This result suggests that V_{max} is dependent on the substrate rather than on other soil chemical and physical properties.

The V_{max}/K_m value is an indicator of the soil catalytic ability of a specific enzyme (Juan et al., 2009; Zhang et al., 2009). The higher V_{max}/K_m value found in brown and cinnamon soils at a lower temperature (10 ºC) than in black and albic soils indicates that lower temperatures mitigated the stress imposed by higher temperatures. Such effects can be expected in areas of brown and cinnamon soils. That the highest V_{max}/K_m value was found at a soil water content of 10 % in cinnamon soil rather than at the 20 % found for black, albic and cinnamon soils suggests that soil β-glucosidase is adapted to the water stress in its semiarid environment and not adapted to a humid environment.

The kinetic properties of soil β-glucosidase were affected more by moisture variation than by

temperature changes (Figures 1, 2). In typical agricultural soils used for the production of rainfed crops in Northeast China, the control of soil moisture conditions could be a feasible method for regulating the biochemical processes of soil carbon transformation catalyzed by soil β-glucosidase.

CONCLUSIONS

1. Temperature and moisture affected the kinetic parameters of soil β-glucosidase.

2. Compared with soil moisture regimes, environmental temperature conditions had less effect on soil C transformation processes related to β-glucosidase in Northeast China.

3. The apparent sensitivity of soil β-glucosidase to ambient moisture implied that controlling soil moisture could be a feasible method for regulating the biochemical processes of soil C transformation catalyzed by β-glucosidase.

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