Korean J. Ecol. 18(4) : 503~512, 1995

Enzyme Activities in the Soil of *Quercus mongolica* Forests

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신갈나무 산림토양에서의 효소활성도

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ABSTRACT

The present paper describes partial results of the study on the activities of microbes in the soil of *Quercus mongolica* forest from July, 1994 to April, 1995. To determine the relationship between structure and function of soil microbial ecosystem, the author investigated the seasonal change of physical environmental factors, microbial population and soil enzyme activities. The changes of pH was not significant and the temperature of surface soil was 2℃ higher than lower soil throughout the year. Moisture contents (%) of soil samples ranged from 7.64% to 42.11%. However, soils of site 3 at Mt. Kŏmdan in which vegetation is successional have higher moisture content than the others. The bacterial population increased in summer, but continuously decreased in autumn and winter, and then reincreased again in spring. Bacterial population of surface soil was higher than those of 30 cm depth all the year round. Dehydrogenase activity (DHA) was about two-fold higher throughout in surface soil compared to those of lower soil. And the correlation coefficient between DHA and bacterial population size was 0.713. It was suggested that DHA could be used as a primary index of soil microbial population and activity in soil ecosystem.

Key words: ATP, Enzyme activity, Soil microbial population, Vegetation

INTRODUCTION

Production and decomposition are major characteristics of soil ecosystems. It is well

This study was supported by a grant from Yonkang Foundation in 1994 and by the KOSEF research grant for SRC(Research Center for Molecular Microbiology, Seoul National University)

known to that decomposition is strongly related with soil microbial activities. Soil microbes take part in the cycling process of each kind of inorganic and organic materials as a primary decomposer. In the process, they exert an important effect on vegetation development and soil fertility (Alexander 1977, Brendecke *et al.* 1993). From these reasons, much research had been carried out on the soil microbes throughout the world.

Although many studies about microbial distribution and soil enzyme activities in particular area have been reported in Korea, studies on the dehydrogenase activity in accordance with heavy metal concentration (Kim and Lee 1993), on the relationship between microbial distribution and enzyme activities (Lee *et al.* 1994), on the microbial distribution in forest soil (Cho *et al.* 1988, Cho *et al.* 1992, Choi *et al.* 1994), and on the microbial distribution related to distinct vegetation types are only a few.

The *Quercus mongolica* forest is a typical forest vegetation in Korea. It has been evaluated as the dominant species of deciduous broadleaf vegetation in cool-temperate zone and of climatic climax at upland arid mountains in Korea (Lee *et al.* 1993a). Also, it is distributed at most of all the mountains in Korea, from the Kema Heights to Mt. Halla (Lee *et al.* 1993b).

This study was designed to analyze the relationship between population size of soil microbes and enzyme activities in soil of *Quercus mongolica* forest.

STUDY SITES

Soil samples were collected from three sites (Fig. 1): two sites (site 2 and 3) were located in Mt. Kŏmdan (650 m) and the other (site 1) was located in Banweol area.

Quercus mongolica was the dominant species in all the study sites. The average DBH of *Quercus mongolica* was 2 cm at site 1, 4.78 cm at site 2 and 13.89 cm at site 3. Vegetation of site 1 had more or less shrubby structure, that of site 2 was transitional from shrubby bushes to forest and that of site 3 was mature forest. Three sampling sites varied in successional age, especially in the cover of *Quercus mongolica*. Soil was collected seasonally in July, October, 1994 and January, April, 1995. Soil was sampled at surface and at the depth of 30 cm in *Quercus mongolica* forest. Randomly sampled soil at each site was mixed and carried to the lab for analysis.

MATERIALS AND METHODS

Environmental factors

Temperature was determined by glass thermometer. pH was determined by soil pH tester (DM-15, Takemura Electric Works, Ltd., Japan). Moisture content was determined by soil-drying method (Choi 1982).

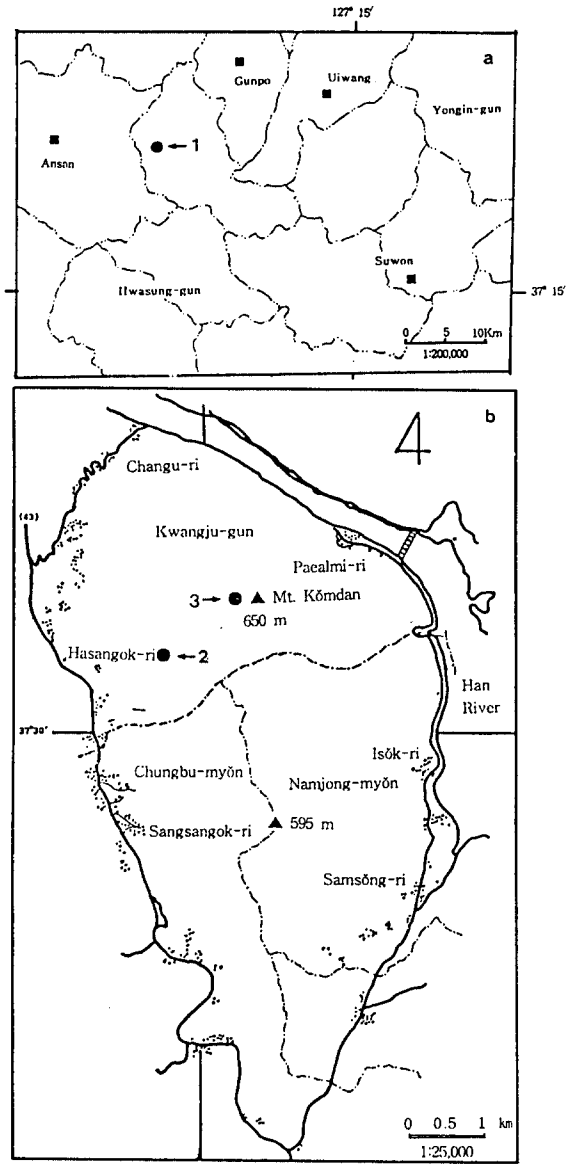

Fig. 1. Location of the sampling sites.
a: Banweol, site 1.
b: Mt. Kŏmdan, sites 2, 3.

Total microbes

Soil suspension was made of 1 g of air dried soil added with Tris buffer (pH 7.5, 50 mM), and then was added with 10 ml of 25% Ringer's solution. After agitation for 1 minute, the soil suspension was pre-filtered (GF /C, ϕ47 mm, Whatman Co.). By the method of Hobbie *et al.* (1973), pre-filtered samples were fixed by adding 4 ml of 4% formalin, and then samples were passed through membrane filter (0.2 μm, ϕ25 mm, Nucleopore Co.) that pre-stained with Sudan black B. After the final staining with acrydine orange solution (1: 10,000 in 6.6 mM phosphate buffer, pH 6.7), stained soil bacterial cells were counted under the fluorescence microscopy (Axioplan, Zeiss, Germany).

ATP determination

ATP was extracted by the method of Arnebrant and Bääth (1991). Moist soil (1 g wet wt.) was shaken with 10 ml of ice-cold 500 mM H_2SO_4 and 250 mM Na_2HPO_4 for 30 minutes. And then, ATP was determined by the procedures of Sigma Technical Bulletin (Sigma Co. 1988). 0.1 ml of ATP assay mix solution was put into reaction vial, swirled and stood at room temperture for 3 min. And then 1 ml of the sample was added rapidly to reaction solution and was shaken for 10 sec before ATP measurement. The amount of ATP was determined with luminometer (Lumat LB 9501, Berthold) as the luminescent light output (RLU, relative light unit) equivalent to an

integrated value for 10 sec after enzyme addition.

Enzyme activities

1) Dehydrogenase

Dehydrogenase activity was determined according to the method of Beyer *et al.* (1993). Moist soil was sieved through a 2 mm sieve, and then 5 g of the soil was put into 25 × 150 mm test tube, and treated with 5 ml of TTC(2,3,5-triphenyltetrazolium chloride)-tris buffer (pH 7.4). That solution was mixed on a vortex and incubated for 24 hr at 30℃ in the dark. After incubation, the triphenyl-formazan formed by the reduction of TTC was extracted twice with 20 ml aceton by shaking for 2 hr at 200 rpm. Reactive products were measured at 485 nm with a spectrophotometer (Biochrome 4060, Pharmacia LKB).

2) Phosphatase

10 g of moist soil and 1/4 spoon of active carbon were put into a flask. After suspended with 50 ml of Morgan's solution, the suspension was filtered and shaken for 30 min. After 1 ml of filtrate was added to reaction solution, the mixture was shaken for 10 min at 37℃. Reaction solution was made with 5 mM p-NPP, 10 mM $MgCl_2$, 10 mM KCl and 0.1 M Tris-HCl buffer (pH 7.0) (Ernst 1975). Enzyme activity was stopped by 2~3 drops of 37.5% TCA solution. After 10 min, 2.5 ml of 1 N NaOH was put into mixture and centrifuged for 10 min at 5,000 rpm. Absorbance was recorded at 410 nm.

RESULTS AND DISCUSSION

Environmental factors

Fig. 2 shows the seasonal changes of the environmental factors in the soil of all the study sites. The pH was ranged about 6.4~7.0 in the surface soils and 6.6~6.8 in the depth of 30 cm soils. On the whole, seasonal changes of soil pH were very slight as shown at site 2 with pH 6.6~6.8 at the surface and 6.7~6.8 at 30 cm deep soil. At the sites 2 and 3, acidity was commonly higher in the surface than the lower soil and in older vegetation. From the result, population size of microbes and soil enzyme activities do not seen to be affected by variation by pH changes in the study sites. Soil temperature ranged −4℃ ~25℃ in the surface soil and −3℃~23℃ in the lower soil. Generally, soil temperature was higher by 2℃ in the surface than in the lower soil. Moisture content showed seasonal variation from 8.80 to 42.10%, throughout the year. It ranged 8.80~42.10% in the surface soil and 11.20~38.50% in the lower soil. Average moisture content was higher at site 3 (35.75% in the surface, 34.41% in the lower) than in the site 1 (13.79% in the surface, 12.56% in the lower soil) and at the site 2 (20.00% in the surface, 21.61% in the lower soil). Average moisture content was higher in spring (26.00%) than in summer (19.00%). Generally, it is known that the optimum level of moisture content for the activities of

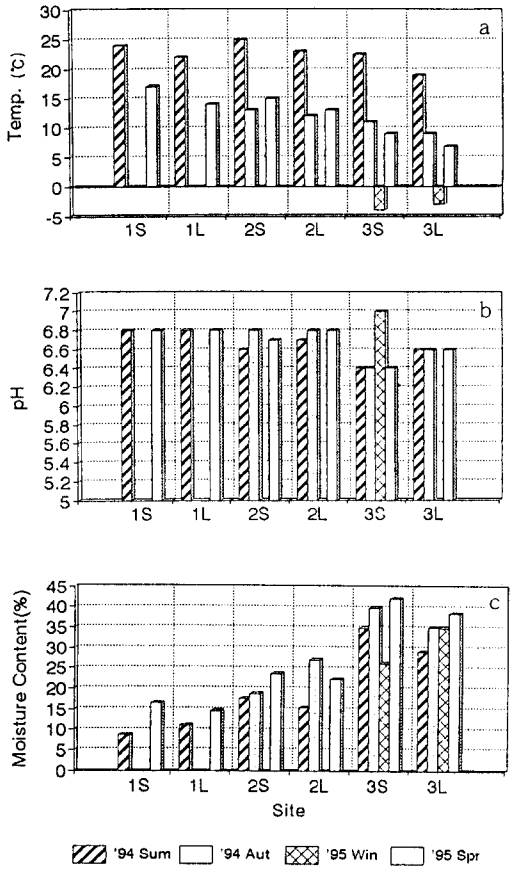

Fig. 2. Seasonal changes in environmental factors at the study sites. a: Temperautre, b: pH, c: Moisture content

* S: surface soil, L: lower soil (soil at 30 cm-depth) from the surface.

** Numbers 1, 2 and 3 indicate the study sites.

aerobic bacteria is about 50~75% at any soil texture (Alexander 1977). Moisture contents of soil at the *Quercus mongolica* forests as a climax community were 25.55~32.19% in Mt. Palwang (Cho *et al.* 1992), 26.48% in Mt. Gyebang (Choi *et al.* 1994) and 24.00~31.40 % in Mt. Minjuji (Cho *et al.* 1988). These different contents can be resulted from variations in developmental level of forest ecosystem and microbial degradation of organic matter. It is considered that the soil moisture contents increased by the result of adsorption effect of accumulated organic matters. And it is supposed that moisture contents at the sites 1 and 3 increased by development of vegetation, accumulation of organic matters and activities of microbial degradation.

Microbial population

1) Total bacteria counts by acrydine orange stain

Bacterial population in soil ranged 0.48~24.20 × 10^6 cells/g soil in the surface soil and 0.16~10.70 × 10^6 cells/g soil in the lower soil (Fig. 3). Distribution of soil bacteria showed that bacterial population size was larger at the surface soil than at the 30 cm deep soil throughout the year. And the extent of change in seasonal population size was higher at the surface than at the lower soil, commonly in the sites 2 and 3. The largest variation in bacterial population size appeared at the surface soil of site 3: about 5-fold larger in summer than in autumn. The bacterial population size appeared $\leqq$ 5 × 10^6 cells/g soil on the whole, except the surface soil in summer, the lower soil in spring and summer at site 3, and the surface soil in summer and autumn at site 2. And there was a tendency that the increased bacterial population in summer continuously decreased until autumn and winter, and then reincreased again in spring. These results were similar to the reports that the bacterial biomass differed considerably between the sampling dates and between the soil

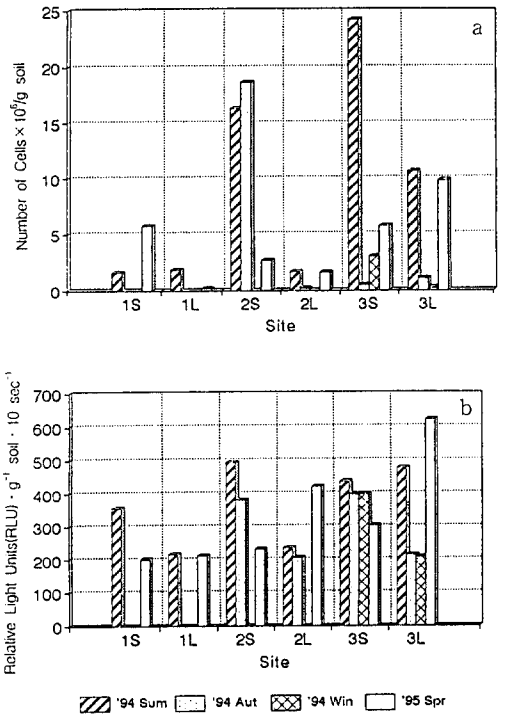

Fig. 3. Seasonal changes in variation of microorganisms at experimental sites.
a : Total bacteria stained by Acridine orange(AO).
b : RLU value estimated by ATP assay.
* S and L are the same as in Fig. 2.

layers in the pine forest soil (Lundgren and Söderström 1983). These results suggest that population size of bacteria are closely related to nutritional factors such as pre-existing organic matters and degradated products by microbial activities in soil (Alexander 1977).

The accuracy of determining bacterial population is very important to understand the flow of bacterial carbon sources and to account the bacterial biomass. The related papers report that mean ratio between CFU (Colony Forming Unit) and direct count by acrydine orange (AO) stain is 1.55 (Lebaron *et al.* 1994). To analyze the population size of bacteria, therefore, AO stain method was seed in this study.

Bacterial survival in soil can be influenced by a number of factors such as moisture, plants (rhizosphere), bacterial movements, surfaces, clay type and content, and predation by protozoans (England *et al.* 1993). Also, it can be affected by nutrient availability, temperature, pH, microbial interactions, radiation, light, antibiotics and toxic compounds. In this study, the correlation coefficient between bacterial population and some environmental factors was calculated. They were 0.108 with moisture content, −0.329 with pH and 0.326 with temperature. Population size of bacteria correlated more with temperature (positive) and pH (negative) than with moisture content in this study.

2) Population size of microbes by ATP determination

ATP plays a central role in metabolism and it is maintained at a fairly constant intracellular concentration. Also, it is rapidly destroyed by enzymes on an organism's death: the half life of ATP in a typical living microorganism is ≈1 s. Therefore, intracellular nucleotides such as ATP is used as an index of metabolic activity and biomass in environment (Pangburn *et al.* 1994).

RLU (Relative Light Unit) value, expressed as the relative amounts of ATP in soils of each site, was shown in Fig. 3. The values showed a variation from 201 to 624 RLU · g

soil · 10 sec^{-1} at the site 1 and 3, respectively. In the surface soil, the range of RLU was 201~495 RLU · g soil · 10 sec^{-1}. Most of 30 cm lower soils showed near 200 RLU except soil of spring season both at the sites 2 and 3. Most of the surface soils contained 300~600 RLU. These results strongly suggests that microbial distribution and activities occur mainly near the surface more than those of lower soil. According to single regression analysis between microbes and environmental factors, population size of soil microbes and activities were affected by the order of moisture content ($r=0.286$), temperature ($r=0.013$) and pH ($r=-0.29$). It is believed that maximal biological activity in soil ecosystem is maintained when moisture content is about 40~50% as a whole (Alexander 1977).

Enzyme activities

Soil enzymes can be derived from animals, plants and microbes, but primarily from soil microbes. Soil enzymatic analysis may used as an index of size and activity of total microbial community (Bolton *et al.* 1985). Dehydrogenase and phosphatase are the representative soil enzymes that express the size of total soil microbial population and the activity. Fig. 4. shows the variation of activities of these two enzymes.

1) Dehydrogenase activity (DHA)

The measurement of intracellular dehydrogenase activity in soil is widely used as a common method of estimating soil microbiological activity (Beyer *et al.* 1993). Dehydrogenase is mainly linked with microbial respiratory processes and it's assay has a potential advantage because no additional substrate is added to the soil (Bolton *et al.* 1985). The value of DHA showed range of 3.0~12.0 triphenyl formazan $\mu g \cdot g^{-1}$ soil · day^{-1} in the surface soil and 0.7~5.0 triphenyl formazan $\mu g \cdot g^{-1}$ soil · day^{-1} in the lower soil (Fig. 4).

The value of DHA showed closer relationship with soil bacterial population ($r=0.713$), more than with total soil organisms ($r=0.155$) measured by ATP contents. From these analysis, it can be concluded that bacterial population plays a significant role in secretion of the enzyme and enzyme activities in soil environment. The value of DHA is evaluated less significantly correlated with environmental factors such as temperature ($r=0.239$), moisture content ($r=0.236$) and pH ($r=-0.236$). Surface soil was two-fold higher in the value of DHA than 30 cm deep soil. The higher value of DHA appeared in the soil of spring, generally. It was assumed that the metabolically depressed microorganisms during the winter were rapidly reactivated in spring. Ross (1970) stated that DHA appeared to be more dependent on the metabolic state of the soil or on the biological activity of the microbial population than any other free enzymes present. From these results, that DHA can be seed as a primary index of microbial activity in soil environment. Especially, soil at the site 2 had lower microbial population size but had higher DHA throughout, compared to the site 3 where the vegetation cover is in a successional state. Therefore, it is assumed that population size and activity of soil microbes are closely related with the development of vegetation cover.

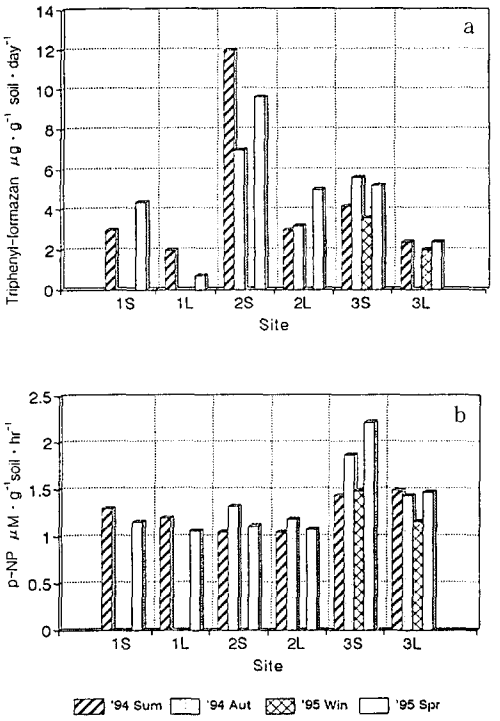

Fig. 4. Seasonal changes in enzyme activity at experimental sites.
a : Dehydrogenase
b : Phosphatase
* S and L are same as in Fig. 2.

2) Phosphatase activity

Soil phosphatase has been used to describe a broad group of enzymes that hydrolyze organic phosphorus compounds, pyrophosphates, metaphosphates, and inorganic polyphosphates which occur in soil (Malcom 1983). Soil phosphatase activity has two pH optima when measured with artificial substrates such as p-nitrophenyl phosphate. Acid phosphatase has an optimal pH near 6.5, whereas alkaline phosphatase shows optimal activity at pH 11 (Frankenberger and Johanson 1982). Referring to pH range (6.4~7.0) at all the study sites, it can be concluded that cycling of inorganic and organic phosphates were mainly affected by both acid phosphatase and neutral phosphatase activities. Phosphatase activities ranged 1.05~2.22 p-NP $\mu M \cdot g^{-1}$ soil $\cdot hr^{-1}$ in the surface soil and 1.05~1.49 p-NP $\mu M \cdot g^{-1}$ soil $\cdot hr^{-1}$ in the lower soil (Fig. 4). Phosphatase activity was higher in summer and spring than that of other seasons. These values represent similar tendency to those of DHA. But phosphatase activities showed a different pattern compared with DHA along the soil depth. The value of DHA was about two fold higher in the surface soil than the soil of 30 cm depth. Activities of phosphatase were higher in the surface than the lower soil throughout, but the the changes of enzyme activities between the surface soil and the lower soil were slight by different. Phosphatase activities were interpreted to have a close relationship with moisture content ($r=0.607$) among the three environmental factors. And also, secretion of the enzyme had closer relationship with total microbes ($r=0.267$) than with bacterial population ($r=0.096$). There are possibilities that phosphatase could be originated from plants and animals such as nematodes. Total enzymatic reaction of phosphatase in soil are believed to be dependent mainly on secretion from other organisms than soil microbes. Phosphatase activities showed slight seasonal changes ranging 1.05~1.19 microbes p-NP $\mu M \cdot g^{-1}$ soil $\cdot hr^{-1}$ (Fig. 4), but soil microbial population as expressed by RLU value showed a large variation(Fig. 3).

적 요

본 연구는 신갈나무 식생 토양의 물리적 환경요인과 토양내 미생물의 군집크기의 변화 및 이에 따른 토양내 효소의 활성도의 변화를 계절별로 조사하여 토양미생물의 구조와 기능에 대한 연관관계를 분석하기 위해 1994년 7월부터 조사된 일련의 연구중 1995년 4월 까지의 일부 결과를 기술하였다. pH는 연중 변화가 거의 없었으며 연중토양온도는 표층의 토양이 하층에 비해 평균 2℃ 높았다. 함수량은 연중 7.64~42.11%의 다양한 변화를 보였으나 식생이 안정되어 있는 검단산 정상 하부에 소재하는 신갈나무 식생의 토양 (정점 3)은 연중 평균함수량이 표층 35.75%, 하층 34.41%로 높게 나타났다. 세균의 개체군 크기는 하층에 비해 표층에서 크게 나타났으며, 여름에 크기가 증가하여 겨울까지는 계속 감소하고 봄에 다시 증가하는 양상을 보였다. 탈수소효소의 활성도는 전체적으로 표층이 하층에 비해 2배 높고 봄에 활성도가 높게 나타났다. 세균의 개체군 크기와는 상관계수 0.713으로 높게 나타났으며 이는 일차적으로 토양미생물의 개체군 크기와 활성에 대한 지표로 이용될 수 있음을 시사하며 그외 몇가지 효소의 추가적 분석을 통하여 정확한 지표설정을 할 수 있을 것으로 사료된다.

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(Received 14 November, 1995)