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Article

Long-term Composting and Fertilization Impact on Dehydrogenase-producing Bacteria and Dehydrogenase Activity in Rice Paddy Soil

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A long-term rice paddy field, which is located in the National Institute of Crop Science (Suwon city, Korea) has been managed for studying of fertilization and composting impact on paddy soil fertility since 1954. The objective of this research was to evaluate soil quality through dehydrogenase analysis in long-term paddy soil treatment plots, such as control, N fertilization (N), NPK fertilization (NPK), and rice straw compost with NPK (CNPK). Dehydrogenase-producing bacterial population developing red-colored triphenyl formazan (TPF) was highly correlated to the dehydrogenase activity in rice paddy soils sampled prior to waterlog. The dehydrogenase-producing bacterial population and dehydrogenase activity was comparatively high in plots of NPK, and CNPK, which organic matter content was relatively high.

Key words: Microbial activity, Dehydrogenase, Triphenyl formazan, 2,3,5-Triphenyl tetrazolium chloride

Introduction

Microbial communities play important roles in many soil processes. Due to the importance of microorganisms for soil process, many studies have been performed to determine the ecological factors regulating microbial community and diversity (Kirk et al., 2004; Nielsen et al., 2002). Beyer et al. (1999) reported that microbial biomass, dehydrogenase, and alkaline phosphatase were not necessarily indicators of soil fertility with a high fertilization level without forage production and manure application. However, season and site effects were better determinants of dehydrogenase activity than management practices (Quilchano and Marañón, 2002). Otherwise, Saviozzi et al. (2002) reported that biochemical parameters may be potentials used to predict long-term trends in the soil quality. Enzyme activities are considered effective indicators of soil quality changes resulting from environmental stress or management practices, the activities are very sensitive to and show a quick response to the induced changes on both natural and anthropogenic disturbances such as non-tillage, organic amendments, crop rotation, and organic cultivation.

The microbial oxidation of organic compounds under aerobic conditions is a dehydrogenation process mediated by many different intracellular and specific dehydrogenases. Therefore, dehydrogenase (DHA) activity by microorganisms can be considered as an index of the oxidative metabolism in soils due to the properties of which microorganisms are exclusively intracellular (Quilchano and Marañón, 2002; Nielsen and Winding, 2002). Dehydrogenase assays are based on the use of 2,3,5-triphenyl tetrazolium chloride (TTC), a water-soluble compound, which is reduced to triphenyl formazan (TPF), a red-coloured compound (Ross, 1970; Ross, 1971). The activity estimation is based on use of redox-sensitive tetrazolium dye, which is reduced to insoluble formazan inside cells due to respiratory activity. However, TTC-based methods generally have low sensitivity and poor reproducibility, which are attributable partly to inhibition by oxygen of TTC reduction due to problems maintaining anaerobic conditions during the assay, and partly to the fact that TTC and its reduced form are toxic to soil microorganisms (Camiña et al., 1998); however, these assays have been extensively used to determine microbial activity.

TTC was used as an electron acceptor estimating dehydrogenase activity by culturable soil bacterial colonies, whose growth was induced by applying glucose. Praveen-Kumar and Tarafdar (2003) reported that by spraying TTC on microbial colonies, whose growth was induced by applying glucose. Also they

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observed that from 72 to 100% of the bacterial and actinomycetes colonies in different soils were capable of using TTC as an electron acceptor.

Therefore, the objective was to study the use of TTC as a substance for direct counting of dehydrogenase-producing bacteria and dehydrogenase-producing *Bacillus* growing on plates by the spread plate method, and the relation between dehydrogenase-producing bacterial population and dehydrogenase activity in a long-term composting and fertilization paddy field.

Materials and Methods

Site description and field experiment This research was carried out in the field experiment on rice cropping established in 1954 to assess the long-term impact of both organic and fertilizer. Each plot was applied with chemical fertilizer (N or NPK), and compost and NPK (CNPK), or neither. An annual rate of N, P and K was 110, 70, and 80 kg ha^{-1}, respectively. Application rate of compost was 7.5 MT ha^{-1} soil. Soils were sampled from 0 to 20cm depth from under different treatments on April 2006 before water irrigation for planting. Samples were immediately sieved (2 mm) and stored at 4˚C until analyzed.

Culture conditions for microbial populations Soil samples were serially diluted and plated on yeast extract glucose (YG) agar (yeast extract 1.0g; glucose 1.0g; K_2HPO_4 0.3g; KH_2PO_4 0.2g; $MgSO_4 \cdot 7H_2O$ 0.2 g; agar 15.0g; distilled water 1L; pH 6.8) for aerobic culturable bacteria, and *Bacillus*. The diluent for *Bacillus* was heated for 30 minutes in the water bath maintained 80˚C. All microbes were counted using spread plate method, and incubated the plates in the dark at 30 for 3 days. Dilutions yielding 30-300 colonies of bacteria per plate were counted.

Dehydrogenase-producing bacteria and *Bacillus* A modified method was used to determine DHA-producing bacteria in soils (Praveen-Kumar and Tarafdar, 2003). Briefly a 1:1 of the aqueous solutions of 2,3,5-triphenyltetrazolium chloride (0.5% w/v) and glucose (0.5% w/v) was added to the each plate after inoculation of diluents. And then the plates were slightly dried until the liquid on the surface of agar plate was disappeared (Suh et al., 2006). The plates were incubated and counted same as bacteria and *Bacillus*. After incubation, red-colored colonies were counted as DHA-producing bacteria and DHA-producing *Bacillus* (Fig. 1).

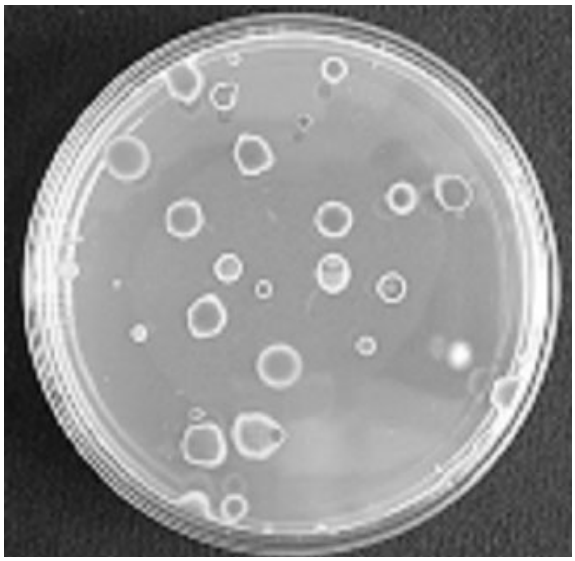

Fig. 1. Red-coloured dehydrogenase-producing bacterial colonies on medium.

Dehydrogenase activity Soil DHA activity was estimated using 2,3,5,-triphenyltetrazolium chloride (TTC) as a substrate. Five g of soil sample was mixed with 50 mg of $CaCO_3$ and 1 ml of 3% (w/v) TTC, and then the mixture was incubated at 37˚C for 24 hours. Dehydrogenase converts TTC to TPF. The TPF formed was extracted with methanol (3 × 50 ml), and then the extracts were filtered on Whatman No. 5 filter paper. Absorption was measured at 485 nm with a spectrophotometer (Sukul, 2006). Enzyme determination was replicated three times and expressed g^{-1} of oven dried soil 24 h^{-1} of incubation

Statistical analysis Statistical analysis was done with SAS enterprise guide 3. Statistically significant differences between data were analyzed using analysis of variance (ANOVA) and least significant difference (LSD) calculations at a 5% significance level. The relationship among each parameter was determined by regression equations.

Results and Discussion

Dehydrogenase-producing bacteria and *Bacillus* After incubation, the red-coloured colonies on agar plates

were counted as DHA-producing bacteria and *Bacillus*. Colonies of DHA-producing bacteria were generally creamy red color, but those of DHA-producing *Bacillus* were not (Fig. 1). The range of DHA-producing bacteria was from 4.7×10^4 to 34.0×10^4 cfu g^{-1} in rice paddy soils. The number of DHA-producing bacteria was significantly correlated to the total culturable bacteria in soils (Fig. 2). Population of DHA-producing *Bacillus* ranged from 4.1×10^3 to 15.8×10^3 cfu g^{-1}. However, there was no significant correlation between DHA-producing *Bacillus* and mesophilic *Bacillus* in this experimental soil (Fig. 3).

Dehydrogenase activities of soils were positively correlated to DHA-producing bacterial number (Fig. 4). However DHA-producing *Bacillus* was not significantly correlated to dehydrogenase activity, the activity pattern was slightly similar to bacteria (Fig. 5).

In spite of the fact that enzymes can be also active in the extra-cellular soil environment, the correlation coefficiencies between these enzyme activities and the basal respiration or the dehydrogenase activity (both indicators of microbiological activity) are high. There is also a very significant correlation between basal respiration and dehydrogenase activity, confirming that intracellular enzyme activity can be used as an indicator of microbial activity in the soil (de la Paz Jimenez et al., 2002). Also dehydrogenase has been proposed as a valid biomarker of soil management under different agronomic practices and climate (Ceccanti et al., 1994). A highly significant correlation between basal respiration and dehydrogenase activity confirmed that intracellular enzyme activity could be used as an indicator of microbial activity in the soil (de la Paz Jimenez et al., 2002).

With these reports, we can suggest that there is no complete enumeration method but specific microbes such as DHA-producing bacteria may possible one of microbial indicators to assess biological properties in soils.

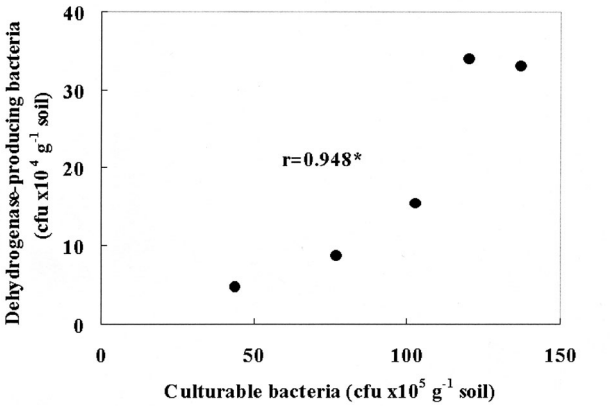

Fig. 2. Correlation between culturable bacteria and dehydrogenase-producing bacteria in soils. Significant at 5% level of significance.

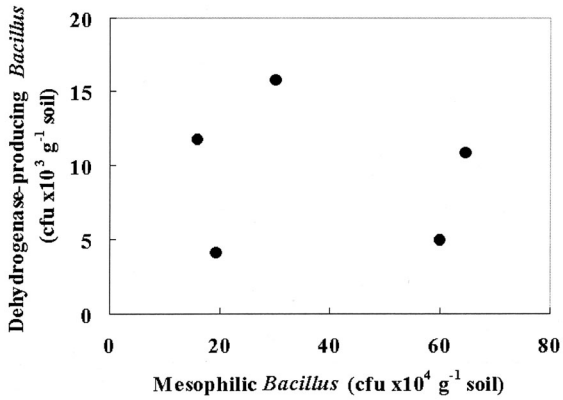

Fig. 3. Correlation between *Bacillus* and dehydrogenase-producing *Bacillus* in soils.

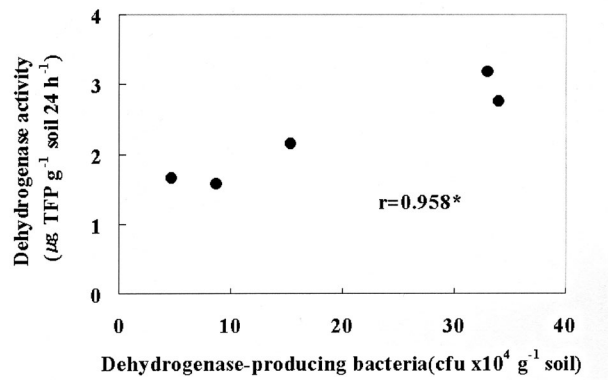

Fig. 4. Correlation between dehydrogenase-producing bacteria and dehydrogenase activity in soils. Significant at 5% level of significance.

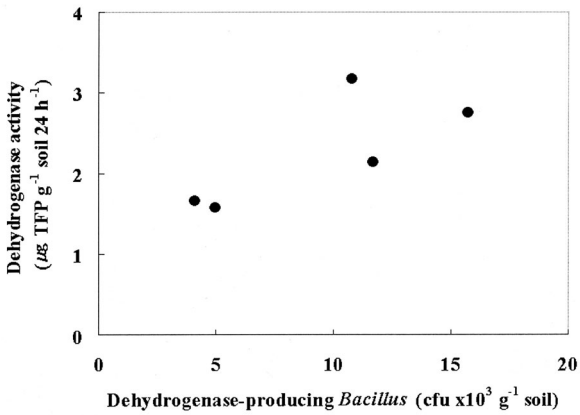

Fig. 5. Correlation between dehydrogenase-producing *Bacillus* and dehydrogenase activity in soils.

Dehydrogenase activity in soils The range of soil pH reported previously was between 5.6 and 6.3 (Suh et al., 2006). Soil phosphate content was high in the plots of NPK and CNPK received phosphate fertilizer, organic contents of soils were high in the plots treated with N fertilizer. It is a general concept that addition of organic materials to soil stimulates microbial and enzyme activity. But it is also well known that addition of organic matter into soils increases the content of soil organic matter very slowly, which means that many years are required to measure significant changes in soil property. However, soil organic matter contents of the plots of control, N, NPK, and CNPK were 17, 20, 23, and 29 g kg^{-1}, respectively (Fig. 6). The range of soil dehydrogenase activities was from 1.5 to 3.2 μg TPF g^{-1} soil 24 h^{-1} (Fig. 7).

Dehydrogenase activity is closely linked with soil microbial activity, which may be involved in initial breakdown of organic matter (Ross, 1971). Compost treatment influences enzymatic activities in the soil because the added organic fractions contain intra- and extra-cellular enzymes, which may also stimulate soil microbial activity (Ross, 1971). This means that the level of soil enzyme activity increases with soil organic matter content increasing, which may be correlated to the population dynamics of the soil microorganisms. As the reports, dehydrogenase may be widely used in evaluating the metabolic activity of soil microorganisms. But the activity was higher in the soil treated with rice straw compost such as CNPK> NPK> control> N. Organic matter content of N plot was higher than control but the activity was not. This result is similar to the report of Parham et al. (2002), which the dehydrogenase activity was significantly higher in the soil treated with cattle manure, such as manure > P > NPK > NPKL > control > NP.

Generally, high correlation coefficients between enzymes activities were probably due to the fact that each enzymatic activity is correlated with the organic C content (de la Paz Jimenez et al., 2002).

In this study, dehydrogenase-producing bacterial number and dehydrogenase activity were relatively high in plots of high level of organic matter contents such as NPK, and compost and NPK plots, which have high content of available phosphate. Thus, DHA-producing bacterial number and DHA activity may be useful microbiological indicators.

Conclusions

Long-term composting and fertilization research rice field used to study dehydrogenase for soil quality assessment with four treatments: control, fertilizer N, fertilizer NPK, and rice straw compost and NPK.

Dehydrogenase-producing bacterial population developing red-colored triphenyl formazan (TPF) was significantly correlated to the dehydrogenase activity in rice paddy soils sampled prior to waterlog. The dehydrogenase-producing bacterial population and dehydrogenase activity was high in plots of NPK, and CNPK that available phosphate and organic matter contents were relatively high. Therefore, dehydrogenase-producing bacteria may be an effective microbial indicator for soil quality assessment. However, more frequent monitoring and better indices for soil microbial community might be needed to assess soil microbial activity.

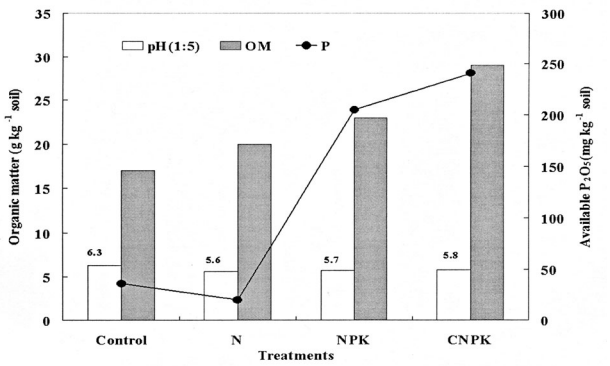

Fig. 6. Soil chemical properties of treatments. C: rice straw compost

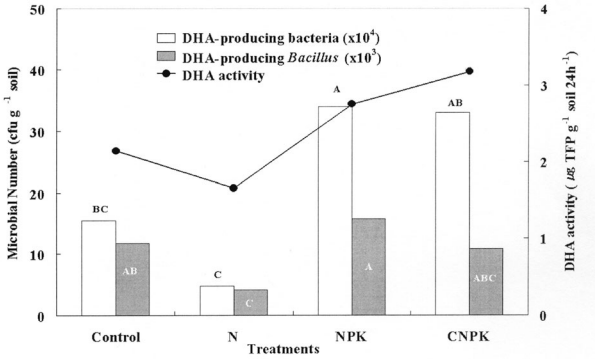

Fig. 7. Soil dehydrogenase activities and dehydrogenase-producing bacterial numbers of treatment. C: rice straw compost. Values in each vertical column followed by the same letter do not differ significantly at p<0.05.

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동일비료 장기연용 논토양의 탈수소효소 생성균과 효소활성

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탈수소효소(Dehydrogenase)생성균과 탈수소효소활성의 논토양 건전성평가 생물지표로서의 기능을 평가하고자, 동일 비료 장기연용 논토양의 무처리구, 질소시용구 (N), 화학비료시용구 (NPK), 그리고 퇴비와 화학비료 혼합시용구 (CNPK)를 대상으로 담수전에 시료를 채취하여 탈수소효소를 분비하는 세균과 *Bacillus* 그리고 탈수소효소활성을 조사하였다. 논토양의 탈수소효소활성은 유기물함량이 비교적 높고 유효인산 많은 화학비료시용구(NPK) 및 퇴비와 화학비료 혼합시용구(CNPK)에서 높은 경향이었다. 토양의 탈수소효소생성균 밀도와 탈수소효소활성간에는 유의성이 높은 정의 상관관계를 보였다.