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Soil Fluorescein Diacetate Hydrolase Activity in Natural and Degraded Soil—A Review

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Abstract

Many soil microbiological properties have been used and were studied to measure the status of soil quality. In this review paper we have used one of the fluorescein diacetate (FDA) hydrolase enzymatic assays to measure the total microbiological potential of soil. Fluorescein diacetate (FDA) hydrolysis is widely accepted as an accurate and simple method for measuring total microbial activity in a range of environmental samples, including soils. Colorless fluorescein diacetate is hydrolysed by both free and membrane bound enzymes, releasing a colored end product fluorescein which can be measured by spectrophotometry. FDA hydrolase activities were found predominantly higher in natural soils than degraded soil. FDA hydrolase activities depend on soil pH, effect of incubation time, termination reaction, substrate concentration and temperature.

Key words : Fluorescein diacetate hydrolase, Soil, Natural, Dagraded.

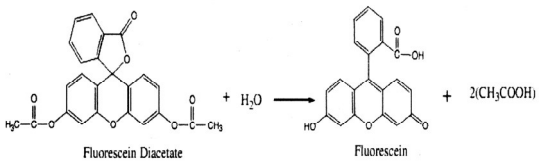
Soil plays a fundamental role in ecosystem functioning, and it is thus critical to safeguard soil health. Soil health is related to the soil quality and its assessment is crucial for determining the sustainability of land management systems. Soil quality has been defined as the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health (1). The functions under-lying soil qualities are secured by the presence and activity of microorganisms. Given the important role that microorganisms play in terms of soil quality, soil biological properties such as enzyme activities has been proposed as a suitable indicators of soil quality. The enzymatic activities play a key role in soil nutrient cycling, its activity is essential in both the mineralisation and transformation of organic matters and plant nutrients in soil ecosystem (2). Enzyme activity in soil results from the activity of accumulated enzymes and from enzymatic activity of proliferating microorganisms (3). They are usually associated with viable proliferating cells, but enzymes can be excreted from a living cell or released into the soil solution from dead cells. Study of soil enzymes gives information about the release of nutrients in soil by means of organic matter degradation and microbial activity as well as indicators of ecological change. Soil enzymes analysis helps to establish correlation with soil fertilization, microbial activity, biochemical cycling of various clements in soil, degree of pollution (heavy metals) and to assess the succession stage of an ecosystem. So, measurements of enzyme activity in degraded soils have useful in examining impacts of environmental change or management on soil enzyme activities. Several works have been reported the potential use of enzyme activity as an index of soil productivity or microbial activity (4, 5). One of the general criteria used here in this review paper is the determination of Fluorescein diacetate (FDA) hydrolases enzymatic activities in soil. In this review paper, measurement of phosphates activity, factors affecting enzyme activity and its status in natural and mine soil are discussed.

FDA Hydrolase Enzyme

The use of fluorescein esters as a measure of enzyme activity was first reported by Kramer and Guilbault (6) where a simple procedure was described for the assay of lipase activity in the presence of other esterases. Swisher and Carroll (7) demonstrated that the amount of fluorescein produced by the hydrolysis of fluorescein diacetate (FDA) was directly proportional to the microbial population growing on

Douglas Fir foliage and a standardized method was developed. This method was later evaluated by Schnurer and Rosswall (8) who used FDA hydrolysis to determine total microbial activity in soil and straw litter as well as cell density in pure microbial cultures.

Fluorescein diacetate (3, 6-diacetyl-fluorescein) is a fluorescein conjugated to two acetate radicals. This color-less compound is hydrolysed by both free (exoenzymes) and membrane bound enzymes (9), releasing a colored end product, fluorescein. The enzymatic conversion of FDA to fluorescein which appears to be primarily a hydrolysis followed by a dehydration reaction. This end product absorbs strongly in the visible wavelength (490 nm) and can be measured by spectrophotometry. The enzymes responsible for FDA hydrolysis are plentiful in the soil environment. Non-specific esterases, proteases and lipases, which have been shown to hydrolyse FDA, are involved in the decomposition of many types of tissue. The equation of the reaction is given below :



Equation of reaction Source : Green et al. (10).

Fluorescein diacetate (FDA) hydrolysis is widely accepted as an accurate and simple method for measuring total microbial activity in a range of environmental samples, including soils. The use of fluorescein esters as a measure of enzyme activity was first noted by Kramer and Guilbault (6) where a simple procedure was described for the assay of lipase activity in the presence of other esterases (11). Since 1982, many authors have reported that the FDA hydrolysis has been used to measure total microbial activity in a range of samples from mould growth on wood and other building materials, to plant residues, to stream sediment biofilms, activated sludge and deep sea clay and sediment profiles (11).

Measurement of FDA Hydroolysis

Total microbial activity potential was measured through fluorescein diacetate (FDA) hydrolysis assay, which hydrolyze colorless FDA to release a colored end product fluorescein (Green et al., 2006). Soil samples and controls (300 mg air-dried) were pre-incubated in 30-ml glass tube. Samples were mixed with 10 ml buffered solutions (pH 6.0) and 100 ml, 2M FDA substrate solution, while controls received only the buffer. The soils were swirled briefly and then incubated for 2 h at 37 C on an orbital shaker. Then, 100 ml FDA substrate solutions were added to controls, and 400 ml of acetones were added to all suspensions to terminate FDA hydrolysis. Soil suspensions (2 ml) were centrifuged at 15,000 rev/min and 4C for 3 min. Absorbance of the supernatants were measured on a spectrophotometer at 490 nm.

FDA Hydrolysis Measurement in Natural Soil

With the increased interest in integrated soil bioecosystem studies, there is a need to have a method of measuring overall microbial activity potential. Hydrolysis of fluorescein diacetate (3, 6-diacetyl fluorescein (FDA) has been suggested as a possible method because the ubiquitous lipase, protease, and esterase enzymes are involved in the hydrolysis of FDA. Udawatta et al. (12) studied at the center for agroforestry, School of Natural Resources (USA) about the variations in soil aggregate stability and enzyme activities in a temperate agroforestry practice. They have found that FDA hydrolase activity was lowest in the row crop treatment which released an average of 8.49 ± 0.61 µg fluorescein/g dry soil under assay conditions, and for the remaining three treatment areas, the grass buffer contained the greatest enzyme activity releasing, on average, 13.55 ± 0.86 µg fluorescein/g dry soil/h. The FDA activity was significantly greater in grass buffer compared with the other three treatments. The agroforestry buffer and grass waterways treatments contained similar amounts of enzyme activity releasing an average of 11.64 ± 0.86 and 10.53 ± 0.86 µg fluorescein/g dry soil/h, respectively. The landscape position from which soil samples were taken had no significant influence on FDA activity within the sampling area. In another experimental work was conducted by Udawatta et al. (13) to study the FDA hydrolase activity and physical properties in a watershed managed under agroforestry and row-crop systems Missouri

Table 1. Status of soil FDA hydrolase activity in different types of land use.

Site description	Types of soil	FDA hydrolase activity (μg fluorescein/g dry soil/h)	Reference
Temperate agroforestry practice (USA)	Crop treatment soils	8.49 ± 0.61	Udawatta et al. (12)
	Grass buffer	13.55 ± 0.86	
	Agroforestry buffer	11.64 ± 0.86	
	Grass waterways treatments	10.53 ± 0.86	
Watershed managed under agroforestry and row-crop systems Missouri (USA)	Crop area	8.0	Udawatta et al. (13)
	Grass buffer area	14.0	
	Agroforestry waterways treatments	12.0	
	Grass waterways treatments	11.0	
Eucalyptus grandis (Hill ex Maiden) plantations land use conversion (Uruguay)	Summer seasons :		Sicardi et al. (14)
	Forest soil	220.0	
	Pasture soil	122.0	
	Winter Season :		
	Forest soil	86.0	
	Pasture soil	106.0	
	Spring Season :		
	Forest soil	86.0	
	Pasture soil	97.0	
Mediterranean ecosystem in different successional stages, natural reserve of Castel Volturno (Italy)	Shurbland	24.0	Fioretto el al. (15)
	Maquis	-	
	Meadow	-	
Polluted site due to acid metals stress, (China)	Agricultural field Grassland sites	Average 0.9 value in both site	Li et al. (17)
Soil health evaluation in an Italian polluted site	sites la	4.48	Fabiani et al. (18)
	sites lb	-	
	sites 2a	-	
	sites 2b	3.23	
Kraft mill wastewater treatment as improver of volcanic soils, (Chile)	Gorbea soil, treated with sludge application.	180.0	Gallardo et al. (20)
	Collipulli soil, treated with sludge amendment.	240.0	
Agricultural soils in Northern Italy used for maize cultivation, contaminated with Zn	Treated soil samples with Zn	6.6 to 12.6	Coppolecchia et al. (21)
Post-mining sites (Sokolov)	Open cover	0.2	Frouz et al. (22)
	Shrub land	0.8	
	Forest cover	0.6	

(USA).The have reported that the FDA activity was lowest in the crop area 8 μg fluorescein/g dry soil/h and highest in the grass buffer area 14 μg fluorescein/g dry soil/h, and differed significantly between crop areas and the other three treatments. The agroforestry and grass waterways treatments showed similar activities releasing and average of 12 and 11 μg fluorescein/g dry soil/h, respectively.

Another experimental work has been conducted by Sicardi et al. (14) to see the status of soil microbial activities from pastures to commercial Eucalyptus grandis (Hill ex Maiden) plantations land use conversion (Uruguay). The influence of changing land use from pasture soil (PS) to forest soil (FS) on FDA hydrolysis found that in summer season the FDA hydrolysis determined at 0—10 and 10—20 cm soil

depths were greater than winter and spring. During the summer seasons the enzymatic activity of FDA hydrolysis was 220 μg fluorescein/g soil/hr in forest soil and 122 μg fluorescein/g soils/hr in pasture soil. The lowest value recorded during the spring seasons, 86 μg fluorescein/g soil/hr in forest soil and 97 μg fluorescein/g soil/hr in pasture soil. The winter seasons soils have averaged vale of 86 μg fluorescein/g soil/hr in forest soil to 106 μg fluorescein/g soil/hr in pasture soil of FDA hydrolysis activity compared to summer and spring.

To show the status of different microbial activities in soils of a Mediterranean ecosystem in different successional stages were studied by Fioretto et al. (15) in the Natural Reserve of Castel Volturno (Italy). They have selected four plots with high maquis (M), shrubland (SH) and meadow (ME) for soil sampling and measurement of FDA hydrolase activities during the spring and autumn season. They have showed that in all three plots shurbland has high FDA activity than other plots. In the spring seasons the FDA hydrolases in shurbland is 24 μmol FDA realeased/g dw/h and in autumn is 22 μmol FDA released/g dwh compared to maquis and meadow plots. Sant' Anna et al. (16) reported the highest activities of FDA hydrolases in forest area and lowest value reported in burned sugarcane area during the both rainy and dry season and lowest in sugarcane management practices of Brazil. The decreases in FDA activity in soils in response due to the shift in land use from forests to burned sugarcane and also associated with the concomitant reduction in soil organic matter content after deforestation of the area.

FDA Hydrolysis Measurement in Degraded Soil

A studied was conducted at College of Natural Resources and Environment, South China Agricultural University, China by Li et al. (17). The have studied the microbial biomass, enzyme and mineralization activity in relation to soil organic C, N and P turnover influenced by acid metal stress. They have selected different sites for soil samples collection, one from agricultural field which are close to mine area, and second one from grassland sites are also closed to mine areas. Three sediment samples were collected beside each rice field in at one site. The mean of FDA expressed as total enzyme activity was 0.9 μg fluorescein/g soil/h for all sites. They have found that the acid metals have adverse effect on FDA hydrolase activities. Another studied were conducted by Fabiani et al. (18) to show the microbiological polyphasic approach for soil health evaluation in an Italian polluted site. They have recorded the cleavage of fluorescein diacetate with the concomitant release of fluorescein provides a measure of the overall microbial activity in soil. Low levels of FDA hydrolases enzymatic activities were recorded for samples from sites 1b found as 4.48 μg fluorescein/g of dry soil/h and at 2b found as 3.23 μg fluorescein/g of dry soil/h, while the samples from sites 1a and 2a did not show any activity. This provided a preliminary indication that the samples from sites 1b and 2b contained a greater number of active microorganisms.

An experimental work were carried out to show the effect of various amendments on heavy mineral oil bioremediation and soil microbial activity by Lee et al. (19)at the Korea Rural Community and Agricultural Corporation, Republic of Korea. They have reported that FDA was hydrolyzed at significantly higher rates in hay and compost-amended soils than in mineral nutrient and sawdust-amended soils. FDA hydrolysis activity was higher in soils that had a high content of easily degradable carbon such as the hay- and compost-amended soils. Gallardo et al. (20) studied the use of sludge from kraft mill wastewater treatment as improver of volcanic soils effect on FDA hydrolase activity as soil biological parameters. They have reported that the FDA activity in Gorbea soil increased with the increment of sludge application, and the higher level 180 μg FDA/g/h was obtained with the application of 50 t/ha of sludge, after 30 days of incubation. Similar to those results of FDA obtained with Gorbea soil throughout the all incubation period (0—60 days), the FDA hydrolysis in sludge-amended Collipulli soil was significantly different ($P < 0.05$) compared to unamended Collipulli soil. They have found that High rates of sludge application contribute to increase the FDA reaching the maximum levels 30 days after application of sludge. They have reported that the FDA activity was about 4.8 times for Collipulli soil with 50 t/ha of sludge application compared to unamended soil.

Coppolecchia et al. (21) studied the relative sensitivity of different soil biological properties to zinc in the soil samples were collected from the surface layer

(0—10 cm) of a field in Alseno, Northern Italy. They have reported that there was no significant effect of Zn on the response of FDA activity at any of the concentrations applied. The minimum and maximum value reported as 6.6 to 12.6 μg fluorescein g/h in the treated soil samples with increasing the concentration of Zn. With the increment of WA from 5 t/ha to 20 t/ha for a period of 8 months and suddenly decrease in FDA activity was resulted for another higher duration of treatment periods. FDA values similar to the control for the entire sampling period for the WA addiction at lower dose (5 t/ha). A wide spectrum of soil microbial parameters was studied in the chronosequences of 1-to 41-year-old plots of spontaneous succession on post-mining sites near the town of Sokolov by Frouz Jan and A. Nova'kova (22). It has been found that FDY activity per gram of soil rapidly increased during the first 10—15 years of succession. In older plots, the FDA activity remained on a similar level. Higher FDA activity was found in the depressions than in the elevations. The FDA hydrolase activity reported in the open cover is 0.2 dA/g/h reported as lowest value of FDA activity, under shrub land is 0.8 dA/g/h which have been reported the highest value of FDA activity and under the forest cover 0.6 dA/gh.

Factors Affecting FDA Hydrolase Enzyme Activity in Soil

Effect of pH. The optimal pH value is to be ranged from 5.5—8.5 at which the FDA hydrolases act as a stain for metabolically active bacteria in soil. It has been reported that FDA hydrolases spontaneously at slightly alkaline condition in soil. At low pH values (%5.0), non-biological hydrolysis of FDA may occur (8). The effect of pH buffer on FDA hydrolysis is critical because the HC concentration in the reaction solution affects the ionization groups of the enzyme protein and influences the substrate's ionization state. For effective interaction between the substrate and enzyme, the ionizable groups of both the substrate and the active site of the enzyme must be in their proper states to maintain the correct conformations.

Effect of Incubation Time. Schnu''rer and Rosswall (8) reported a linear relationship between FDA hydrolysis in soils and incubation time (0 to 3h) based on one soil. Adam and Duncan (11) show a linear relationship up to 40 min. An incubation time of 3h allows sufficient time for hydrolysis to take place and provides better differentiation between soils, yet is still not limited by the amount of substrate. Enzyme-catalyzed reactions typically show linear relationships between the amounts of products formed and the time of incubation (23). Skujins (24) suggests that an assay for soil enzymes should not require incubation times longer than 24h, due to the risk of error through microbial activity increases with increasing incubation time. The 3h incubation time chosen fits these guidelines.

Effects of Substrate Concentration. Schnu rer and Rosswall (8) used a final substrate concentration (concentration of substrate in incubation solution) of 10 mg ml (2.9 μM) for measuring FDA hydrolysis in litter and pure cultures, respectively while Adam and Duncan (11) used a substrate concentration of 67 mg/ml (32 μM).

Effect of Temperature. The rate of hydrolysis of a substrate by an enzyme depends on the temperature of incubation. A study of FDA activity in soil as a function of temperature showed maximum activity occurred at 30 C. This is in agreement with findings by Breeuwer et al. (25) who observed maximum FDA activity by yeast esterases at this temperature. The activity rapidly decreased just above 30 C suggesting inactivation of the enzymes involved at this elevated temperature. At high temperatures considerable spontaneous hydrolysis of fluorescein esters can occur (26), adversely affecting the accuracy and reproducibility of the method. No spontaneous hydrolysis of FDY occurred between 20 and 40 C which covers the range around the temperature chosen for this assay.

Effect of Reaction Termination Method. Different methods have been used to terminate the FDA hydrolysis reaction. Schnu``rer and Rosswall (8) used acetone (50% vol/vol final concentration) while Adam and Duncan (11) added 15 ml chloroform/methanol (2: 1 vol/vol) to the 15 ml soil solution. It is important to stop the reaction long enough to measure the absorbance.

Importance of FDA Hydrolase Activity

The potential of fluorescein diacetate (FDA) hy-

drolysis as a measure of total microbial activity has been recognised by many authors and used on a wide range of samples. FDA hydrolysis is widely accepted as an accurate and simple measurement of total microbial activity in soils (11), which include the ubiquitous free and membrane-bound digestion enzymes, such as lipase, protease, and esterase enzymes (8, 10). FDA hydrolysis has been successfully used to measure hydrolases activity in different organic matrices such as composts (27). The FDA hydrolysis method was both simple and rapid to perform.

In addition, FDA hydrolysis has been found to be significantly correlated with microbial biomass in pure and mixed microbial cultures, in pastures and cultivated soils (28) and in soil amended with municipal refuse (29) and therefore could be used as alternative estimate of the content of the size of soil microflora. It has been reported by many authors that FDA assay is better for estimates of decomposer activity.

Conclusion

The assessment of fluorescein diacetate (FDA) hydrolase enzymes activity is essential to measure the total microbiological potential of soil. Hydrolysis of FDA has been suggested as a possible method because the ubiquitous lipase, protease, and esterase enzymes are involved in the hydrolysis of FDA. In forest soil and grassland the FDA hydrolase activity were found higher than agricultural lands. In summer seasons FDA hydrolase activity was greater than spring and winter. In polluted sites, the acid metals have adverse effect on FDA hydrolase activities. FDA hydrolase activity of soil rapidly increased during the first 10—15 years of succession in spoil dumps from coal mined area. Soil pH, incubation time, substrate concentration, temperature and reaction termination have significant effects on FDA hydrolase activity in soil.

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