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The determination of soil chitinase activity: Conditions for assay and ecological studies

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Summary Chitinase activity was determined by incubating a mixture of toluene-treated soil with 1% (w/w) colloidal chitin suspension for 18 h at 37°C and then, after dilution, assaying the amount of N-acetyl-glucosamine released. Maximal chitinase activity was observed at 45°C and optimal pH for enzymatic reaction was 5.0–5.5. Soil chitinase activity decreased with increasing soil depth and was significantly affected by crop cover and fertilization regime. Chitin added to soil stimulated chitinase activity. Enzyme activity was correlated with the soil fungal population but not with numbers of actinomycetes or bacteria. A specialized mycoflora was associated with chitin decomposition.

Introduction

Chitin, a polymer of N-acetyl glucosamine, is common in nature. It is a constituent of cell walls or structural tissues of insects, crustaceans, and many species of fungi and other organisms[12]. Enzymatic hydrolysis of chitin to acetylglucosamine is mediated by 2 hydrolases: chitinase (chitin glycanohydrolase, E.C. 3.2.1.14) and chitobiase (Chitobiose acetylamidodeoxyglucohydrolase, E.C. 3.2.1.14). Chitinases are common in nature and are produced by bacteria, fungi and the digestive glands of animals that consume chitin-containing materials[12].

The addition of chitin to soil has been studied as a possible method for control of fungal pathogens[11] and plant parasitic nematodes [9,10]. The effect of chitin amendments on the soil microflora has also received some attention. Veldkamp[18] found that a number of bacteria, actinomycetes, and a limited number of fungal species with chitinolytic properties developed in chitin-treated soils. Mitchell and Alexander[11] suggested that chitin degradation and chitinase formation in soil resulted largely from the metabolism of actinomycetes. Indeed, culture media containing chitin have been proposed for isolation of soil actinomycetes[8]. There is, however, no information on the exact relationship between soil microorganisms and soil chitinase activity. One of the difficulties in studying the relationship has been the lack of adequate methods for the assay of soil chitinase activity [6,7,15]. This paper presents a method for determination of soil chitinase activity and information on optimal conditions for the assay, distribution of

chitinase activity in some Alabama soils, and the relationship between chitinase activity and soil microflora.

Materials and methods

A series of experiments were conducted to determine conditions for the assay of soil chitinase activity. Soil for the experiments was a loamy sand [pH = 6·0; org. matter < 1.0% (w/w)] from a field with corn (*Zea mays* L) that received a complete fertilization regime. The soil was collected to a depth of 20 cm, sieved (1 mm) and allowed to dry at 25°C. The dry soil was stored in the dark at 4°C until used in the experiments.

Preparation of chitin suspension

A colloidal chitin suspension was prepared by dissolving 200 g of ground (0·25 mm) crustacean chitin (U.S.B. Corporation, Cleveland, OH) in 1.8 litre of conc. HCl. The acid was added to the chitin with stirring and the mixture was allowed to stand at 20°C with intermittent stirring until dissolved (1.5–2 h). The solution was poured with stirring into a 60-l container half-filled with tap water. A suspension of chitin in water was formed and additional water was added to have a final volume of 50 l. The suspension was allowed to stand overnight to allow the chitin to settle and form a concentrated suspension. The supernatant liquid was slowly siphoned off and tap water was added to resuspend the chitin. This process of washing with tap water was repeated 4 times , followed by 3 washes with demineralized water. After the final washing, the suspension was passed through a triple layer of 0·1-mm-mesh nylon cloth to remove large particles. The resulting chitin suspension had a pH of 5.5–6.0 and was stored in the dark at 4°C until used. Chitin content of the suspension was determined gravimetrically after drying 10 ml-samples at 80°C for 24 h. The stock suspension was used to prepare others of lower concentration by diluting with demineralized water immediately prior to use.

Assay of soil chitinase activity

In the standard procedure, 10 g of dry soil in a 30-ml, square (3.0 × 3.0 cm) glass bottle was treated with 1.5 ml toluene for 15 min. The mixture then received 10 ml of a 1.0% (w/v) chitin suspension and, after gentle mixing, the bottle was stoppered and placed in an incubator at 37°C for 18 h. Following incubation, 10 ml of demineralized water were added and, after thorough mixing, 10 ml of the resulting soil suspension were centrifuged (15 min, 5000 × *g*). The amount of N-acetyl-glucosamine in 1 ml of clear supernatant was determined using the method of Aminoff and associates[1].Each determination was performed in triplicate and included controls with water instead of substrate and others with twice-autoclaved (no enzymatic activity) soil. Chitinase activity was expressed as nanomoles of N-acetyl-glucosamine released per h/gm of soil.

Conditions for the assay of soil chitinase activity

The effect of the amount of soil on chitinase activity was determined by the standard assay procedure. Field soil was mixed with twice-autoclaved soil to give 0, 2, 4, 6, 8, or 10 g of field soil in the 10-g sample assayed.

The effect of incubation time on chitinase activity was studied by assaying the activity every 8 h for a period of 72 h following the standard assay procedure in all other details. Each incubation time was represented by 3 replications.

The effect of substrate concentration on chitinase activity was studied using the standard assay procedure modified to provide substrate suspensions with 0, 0.5, 1.0, 1.5, 2.0, 2.5, or 3.0 % (w/v) chitin. Each substrate concentration was represented by 3 replications.

The effect of incubation temperature on chitinase activity was examined following the standard assay procedure but with incubation temperatures of 16.5, 18.0, 22.0, 29.0, 34.9, 40.2, 45.5, 50.0, 54.5, 59.0, 65.0, and 69.2 °C. Each temperature was represented by 4 replications.

The effect of pH on chitinase activity was studied by adding 10 ml of 1·0% chitin suspension and 10 ml of 0·5 *M* buffer to 10 g of toluene-treated soil. The mixture was incubated for 72 h at 37°C. After incubation, 20 ml of water were added to the mixture and 10 ml of the resulting suspension were centrifuged (5000 × *g*, 15 min). Five ml of the clear supernatant were then mixed with 15 ml of 0.053 *M* $K_2B_4O_7 \cdot 8H_2O$ (pH = 9.1) and the amount of N-acetyl-glucosamine in 1 ml of the resulting solution was determined [1]. Buffer solutions were prepared using sodium salts and were: pH 3.5–5.0, acetate; pH5.0–5.5, propionate; and pH 6.0–7.5, phosphate. Each pH was represented by 4 replications, and appropriate controls were included to determine the effect of pH on non-enzymatic hydrolysis of chitin. A standard curve for determination of N-acetyl-glucosamine was prepared for each buffer to correct for any effect of the buffer on color development.

The effect of heating soil on enzymatic activity was studied by suspending 10 g soil in 10 ml of water in the standard assay bottles. The mixture was placed in water baths at 50°C or 70°C for 0, 10, 20, 40, or 60 min. Following heating, the soil suspension was treated with 1·5 ml of toluene for 15 min and then received 10 ml of 1·0% (w/v) chitin suspension, and chitinase activity was determined following all other details of the standard assay procedure. Each temperature was represented by 4 replications and appropriate controls to account for non-enzymatic degradation of chitin at each heating treatment were also included in quadruplicate.

The relationship between soil particle size and chitinase activity was studied by determining enzymatic activity of several particle size component fractions of soil. Dry soil was passed through a nest of sieves with mesh sizes of: 0.025, 0.063, 0.113, 0.198, and 0.480 mm. Chitinase activity of all fractions but the 0.480 mm fraction was then determined following the standard procedures; the fraction retained in the 0.480-mm sieve was discarded since it contained large mineral particles and crop debris.

Field studies

The effect of fertility and crop cover on soil chitinase activity was studied by collecting soil samples from a field under a rotation of corn, cotton (*Gossypium hirsutum* L.), and soybean [*Glycine max.* (L.) Merr.] as summer crops, and rye (*Secale cereale* L.), and a mixture of crimson clover (*Trifolium incarnatum* Gibelli & Belli)+common vetch (*Vicia sativa* L.) as winter crops. The rotation was established in 1905 and was designed to study the long-term effects of several fertilization regimes on the crops. The rotation scheme have remained unchanged during the past 10 years. A detailed description of the rotation has been published[13]. Soil samples from plots with fertilizer regimes chosen for the present study (Table 1) were sampled in December 1981. The samples were obtained from the root zone of the plants to a depth of 20 cm using a standard 2.54-cm-diam soil probe. Twenty-five soil cores were collected from each plot. The cores were composited and the soil was air-dried (25°C), sieved (1 mm) and stored in the dark at 5°C until analyzed following the standard assay procedure. Each fertilization regime was represented by 4 replications. Yields for the crops in 1981 (corn, cotton, and soybean) or 1982 (rye) were obtained from the center of each plot at maturity.

Plots that received NPK +(+ =with winter legume combination, and − =no winter legume combination in the fertilization regime), and others that received PK+, or no fertilization were chosen for a study of the effect of soil sampling depth on chitinase activity. In each plot, separate soil samples were collected at depths of: 0–10 cm and then at 5-cm intervals to a depth of 40 cm. The samples were processed as described for the rotation study. Three samples from each depth were assayed for chitinase activity following the standard procedure.

Decomposition of chitin in soil

A field experiment with micro-plots was established to study the effects of chitin amendments to soil on chitinase activity and the soil microflora. Soil for the study was a sandy loam (pH = 6·0; org. matter content < 1%) similar in properties to that used for the other experiments. The soil was collected from a peanut (*Arachis hypogaea* L.) field near Headland, Alabama, and was screened (1 mm) and mixed 1:1 (v/v) with sand. This mixture will, henceforth, be referred to as soil. Microplots

Table 1. Fertilization regimes employed in a 3-year rotation program with corn, soybean, cotton and wheat

Fertilizer treatment*	Winter legume**	Factor studied
NPK	+	Mineral and legume N
NPK	−	Mineral N
PK	+	Legume N
PK	−	No N
NK	+	No P
NP	+	No K

* Lime was applied to all plots at 0.36 MT/ha following soil-test recommendations: N was applied as NH_4NO_3 at (kg/ha): 135 to cotton, 67 to corn, 67 to wheat, 135 to corn on plots with NPK but no winter legume; P was applied at 224 kg P_2O_5/ha per 3-year rotation; minor elements were applied at (kg/ha): 5.6 cupric sulfate, 11.2 manganous sulfate, 1.1 sodium borate, 16.8 zinc sulfate, and 0·6 sodium molybdate. One-half of the mineral fertilizer was applied broadcast just prior to planting wheat and one-half just prior to planting cotton.

** + and − refer to the presence or absence, respectively, of a winter legume combination, consisting of common vetch and crimson clover, included in the 3-year rotation following cotton and before corn.

were prepared by burying 30-cm-long sections of 15-cm-diam polyvinyl chloride (PVC) pipe 22 cm into the ground in a field on the Agronomy farm near the Auburn University campus.

Ground (1 mm) crustacean chitin was mixed with the soil to give chitin concentrations of 0, 0.2, 0.4, 0.8, 1.0, 2.0, or 4.0% (w/w). Each treatment was thoroughly mixed in a cement mixer to assure even distribution of the chitin in the soil. Each microplot received 5 kg of the amended soil. Each chitin concentration was represented by 8 microplots arranged in a randomized complete-block design. The plots were kept moist (approx. 60% of field capacity) and free of weeds during the experiment. Two-hundred-gram soil samples were collected from each plot at 2, 4, 6, 8 and 10 weeks after addition of the chitin. The samples consisted of soil cores obtained as described for the rotation experiment. One hundred and fifty grams of each sample was air dried and stored as described for enzymatic analysis. The rest of each sample was used to determine microbial populations within 24 h after collection of the sample. Numbers of actinomycetes, Bacteria, and fungi were determined as described before[10] using mineral salts-chitin agar media[4].

Statistical analyses

All data were analyzed following standard procedures for analysis of variance; regression analyses and calculation of linear correlation coefficients were also according to standard procedures[17]. Differences between means were evaluated for significance following a modified Duncan's multiple range test[17]. Unless otherwise stated differences referred to in the text were significant at the 1% or lower level of probability.

Results

Condition for the assay of soil chitinase activity

Chitinase activity (Yc) increased directly with the amount of soil (Xs) in the reaction mixture (Fig. 1A).

Increasing the concentration of chitin in the reaction mixture resulted in increases in chitinase activity when chitin concentration was increased from 0–1.5% (Fig. 1B); concentrations above 1.5% resulted in no further increases in activity.

The relation between soil chitinase activity and reaction time (t) was linear and direct (Fig. 1C). A significant amount of nonenzymatic hydrolysis of chitin was recorded for twice-autoclaved soil with reaction times longer than 16 h; however, the degree of non-enzymatic decomposition was 3.7-times less than that observed with non-autoclaved field soil.

Maximal chitinase activity was observed at pH values between 5·0 and 5·5 (Fig. 1D). Activity declined sharply from this maximum in response to pH values above and below the range 5.0–5.5.

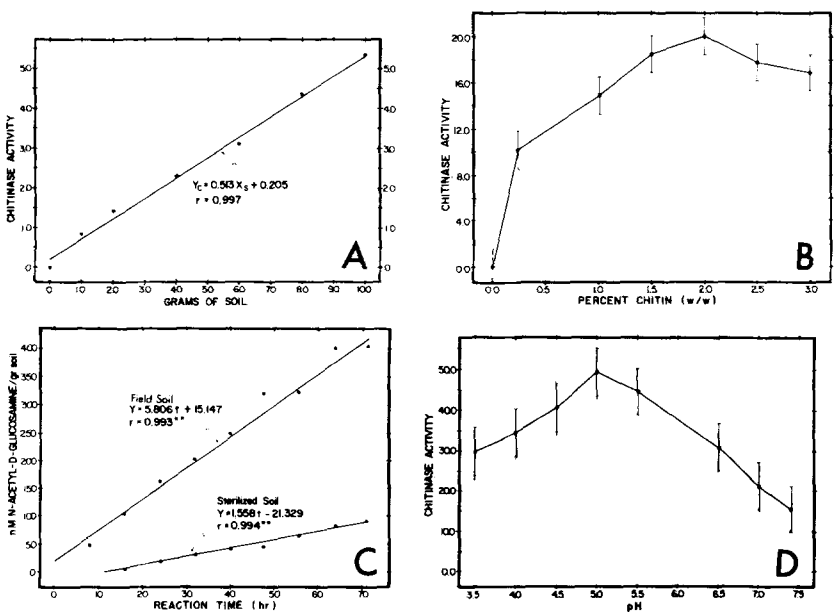

Fig. 1. Relation between amount of soil (**A**), substrate concentration (**B**), reaction time (**C**), or pH (**D**), and soil chitinase activity (n*M* N-acetyl-D-glucosamine/h/g soil).

Increasing the temperature of incubation (T) from 16.5 to 45.5°C resulted in corresponding increases in chitinase activity (Fig. 2A). For $16{\cdot}5 \leq T \leq 45.5$ chitinase activity was related ($r = 0{\cdot}980$) to temperature as described by $Yc = 0.273T - 2{\cdot}410$. However, the relation between Yc and T for $45.5 \leq T \leq 69.2$ was inverse and corresponded ($r = -0{\cdot}973$) to $Yc = -0.380T + 29.021$.

Heating the soil at 50°C for up to 60 min prior to determination of chitinase activity had no significant effect on activity (Fig. 2B); however, with heating at 70°C, a sharp decline in activity was observed in response to heating times (t) of 0–20 min followed by small additional declines with exposures beyond 20 min. The relation between chitinase activity and heating time (t) at 70°C followed ($R^2 = 0{\cdot}964$) the function

$$Yc = \frac{6.623}{(t+1)^{0.278}}$$

Chitinase activity decreased with increasing soil particle size (Fig. 2C).

Field studies

Chitinase activity diminished with soil depth in all plots studied (Fig. 3A). The relation between the activity and soil depth could be described with linear equations for all 3 soils sampled. The decline in activity with depth was 1.73 times more pronounced in soils that received NPK + than in those with PK +, and 3.60 times more than in soil with no fertilization.

Highest chitinase activity was detected (Fig. 3B) in soils with corn or soybean and a complete fertilization regime and winter legume combination (NPK +).

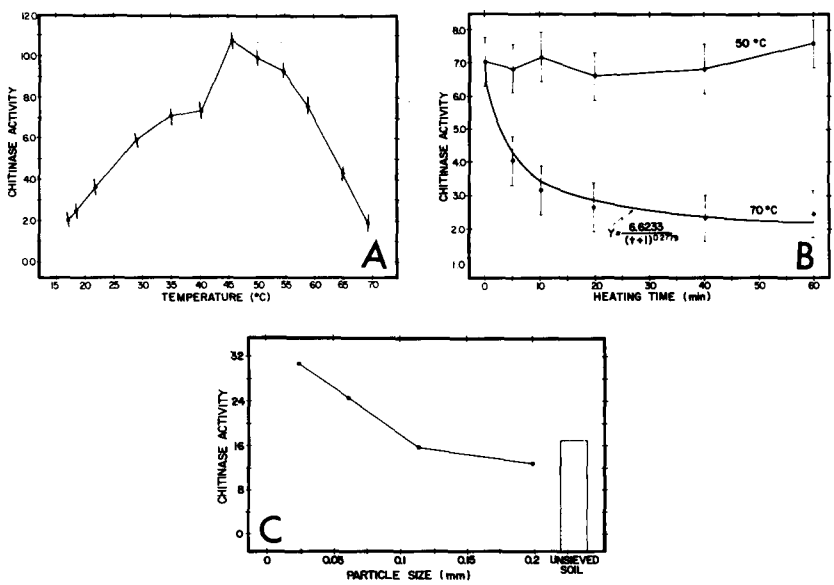

Fig. 2. Effect of assay temperature (**A**), heating of soil prior to determination of enzymatic activity (**B**), or soil particle size (**C**) on soil chitinase activity (n*M* N-acetyl-D-glucosamine/h/g soil).

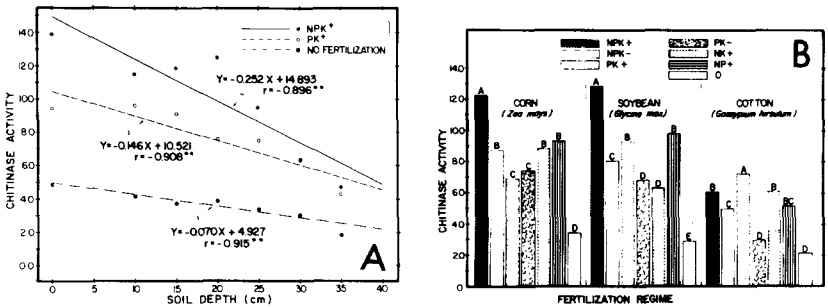

Fig. 3. Relation between soil depth (**A**), or fertilization regime (**B**), and chitinase activity (n*M* N-acetyl-D-glucosamine/h/g soil).

The absence of the winter legume from the regime (NPK−) resulted in significant reductions in soil chitinase activity. Elimination of any major nutritional element in the fertilization regime in plots with corn or soybeans resulted in significant declines in chitinase activity as compared to the activity in plots with NPK+; this relationship was not true for plots with cotton where no clear pattern was observed.

Chitinase activity was lowest in soils with cotton, and those with soybean or corn had approximately the same level of activity.

Yields of corn, cotton, soybean and rye are presented in Table 2. The relation between chitinase activity of plots and corresponding relative yields (NPK+ = 100%) was significant (r = +0.405) at the 5% level of probability.

Table 2. Yield of crops (kg/ha) from the 1981–1982 season in a long-term rotation on the effect of fertilization regimes

Fertilization regimes*	Corn	Cotton	Rye	Soybeans
NPK+	1765	2303	1771	2320
NPK−	2145	2692	2133	2256
PK+	2042	2430	1159	2264
PK−	399	2663	315	2137
NK+	373	1413	475	917
NP+	1117	662	882	1693

* + or − refer to the presence or absence of a winter legume combination (crimson clover + common vetch) in the fertilization regime.

Effect of chitin amendments to soil

Data for chitinase activity were collected for all samplings but the 2-week sampling. The addition of chitin to soil at rates of 0·8% or higher resulted in sharp increases in soil chitinase activity (Fig. 4A); soils with less than 0·8% chitin showed no change in activity compared with unamended soil. Chitinase activity during the first 8 weeks increased with time in soils treated with 1·0% or more of the polymer. With one exception (2% treatment) maximal activity was observed 6 or 8 weeks after addition of chitin to soil with little or no change in activity detected between the last 2 weeks of the experiment; soils with the 2% rate showed a marked increase in chitinase activity in this period. Microbial analysis of the soils were performed for all samples but the 4th-week sampling.

A sharp increase in bacterial numbers in response to chitin amendments was observed 2 weeks after sampling (Fig. 4b); however, there were no differences between treatments at any other sampling time (Data not presented). At the 2-week sampling, maximal numbers of bacteria were in soils with 0.8% chitin followed in decreasing order by soils with 1.0 and 2.0% of the polymer; numbers of bacteria in all other soils did not differ from those in unamended soils.

Numbers of actinomycetes increased over the chitin concentration range of 0–0.8% during the first 2 weeks of the experiment (Fig. 4C). At the 8- and 10-week samplings maximal numbers of actinomycetes were observed in soils treated with 0.8–1.0% chitin. Numbers of actinomycetes in soils with 4.0% chitin were not significantly different from those in control soils. Also, actinomycete numbers in

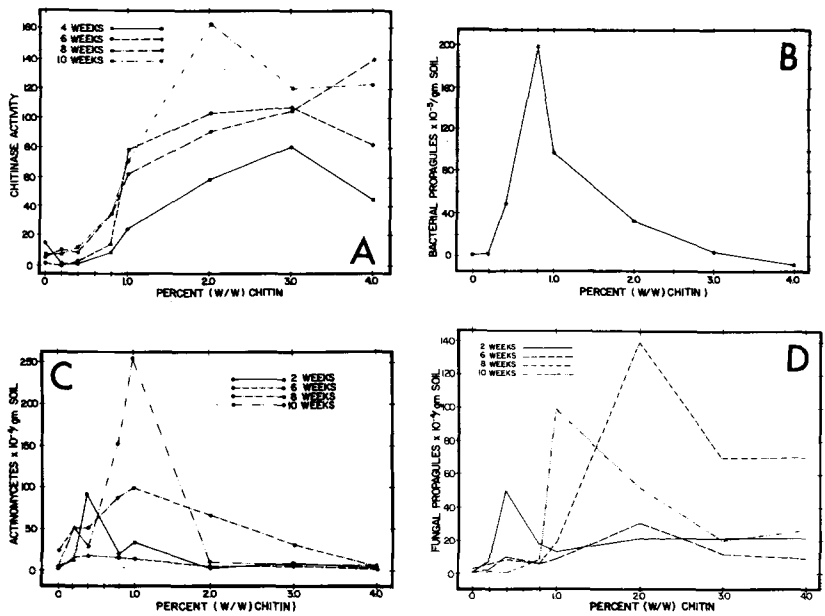

Fig. 4. Effect of chitin addition to soil on chitinase activity (**A**), and numbers of bacteria (**B**), actinomycetes (**C**), and fungi (**D**) in the soil.

soils with 2.0% or 3.0% of the polymer were higher than in control soils only at the 8-week sampling.

There were more fungi in soils treated with 1.0% or more of chitin than in control soils at all samplings (Fig. 4D). At each sampling time maximal numbers were observed in response to individual chitin rates, the rate depending on sampling time. Thus, at the 2-week sampling maximal numbers were observed in soils with 0.4% chitin and in soils with 2.0, 2,0, and 1.0% for the 6-, 8-, and 10-week samplings, respectively.

Numbers of actinomycetes or bacteria were not correlated with soil chitinase activity; however, numbers of fungal propagules (Yf) were significantly ($P=0{\cdot}01$) correlated with chitinase activity (Xc). The relation between the two variables could be described ($R^2=0.560$) by $Yf=0.895e^{0.0237Xc}$.

Discussion

Results obtained from variation of the conditions for the assay of soil chitinase activity followed the patterns expected from classical theory of enzyme kinetics[3]. Thus the relation between amount of soil (enzyme concentration) and chitinase activity was linear. It showed that for the 18-h incubation period and the amount of substrate chosen for the standard assay, the substrate was not limiting when 1 to 10 g of soil were used. Treatment of the data on chitinase activity and substrate concentration with the Michaelis-Menten equation indicated that the 1% (w/w) chitin suspension chosen for the standard assay resulted in chitinase activity that was approximately 48.29% of the maximal velocity and that concentrations of well over 10% chitin would be required to attain maximal velocity. However, concentrations above 1.5% resulted in considerable viscosity of the reaction mixture impairing substrate-soil interaction. In a static assay procedure, such as described in this paper, this could result in impairment of diffusion of the end-product throughout the reaction mixture and consequent reduction in reaction velocity. Indeed, we observed a slight, although statistically insignificant, decline in reaction velocity when substrate concentrations were above 2% (w/w). We chose 1% chitin as substrate since viscosity at this concentration is not sufficiently high to interfere with reaction velocity, is easy to handle, and resulted in approximately 50% of maximal velocity, a fact convenient for kinetic calculations. Our data indicate that for 10 g of soil and up to 72 h incubation at 37°C this substrate concentration is not reaction-rate limiting.

Analysis of results from the study of the effect of incubation temperature on reaction velocity showed that over the range of 16.5–45.5°C, chitinase activity increased continuously in response to temperature. The data fit well ($r=-0.984$) the Arrhenius equation. The log of the reciprocal of chitinase activity (Yc) was related to $T^{-1}\times 1000$ by:

$$\log 1/Yc=-1.9254T^{-1}\times 10^3+7.0453$$

From this equation we estimated that reaction velocity at the standard assay temperature (37°C) was 1.46 times slower than the velocity at 45.4°C, the temperature at which maximal activity was observed. We chose 37°C for the standard assay procedure to conform with the temperature used in most other published procedures for determination of soil-enzyme activity.

Chitinase activity was not affected by temperatures over the range of 0–50°C. However, heating of soil at 70°C resulted in a progressive decline in activity with time. This phenomenon emphasized the biological nature of the activity, and agreed with what is known about the stability of other enzymatic activities of soils[15]. Soil chitinase activity is, apparently, more stable than the activity of chitinase from other sources. Kimura[5] found that chitinase activity of digestive juices of the land snail *Helix peliomphala* was maximal at 37°C but was rapidly deactivated at 60°C. Deactivation of soil chitinase at 70°C followed an asymptotic function, according to which rapid initial losses were followed by continuously decreasing rate of loss in activity. This suggests the presence in soil of a chitinase component which is easily deactivated by heat and other chitinase fractions more resistant to heat. This type of behavior has been noted for other enzymatic activities in soil[2,16]. It is possible that the 'heat resistant' fractions could correspond to enzyme bound to soil organic matter or clay particles.

Results of studies of the effect of pH on reaction velocity indicated a definite optimum at pH's 5.0–5.5. This agrees with reported optima for many soil carbohydrases[6] and other types of enzymatic activities of soil[7,15].

Results from the soil particle size study agree with those of other enzymatic activities of soil. There is generally an increase in soil enzymatic activity with decreasing soil particle size[7,15]. This is probably a reflection of the 'binding' of enzymes to clay and organic matter in soil. For some enzymes this binding can result in enzyme-humus complexes of varying molecular weights[16]. Our results suggest that chitinase may form similar complexes with the organic and mineral fractions of soil.

Other soil enzymatic activities have been shown to decline with increasing depth in the soil profile [2,7]. These results for chitinase activity follow this same general pattern. They also suggest that the magnitude of the decrease in chitinase activity with increasing soil depth is related to the type of fertilization regime used on the soil.Thus, the absolute values of the slopes for the linear regression equations relating chitinase activity and soil depth were highest in soil with NPK+ and lowest in those that received no fertilization. We interpret this finding as the result of greater biological activity, hence more enzyme production in soils with a complete fertilization regime than in those lacking plant nutrients.

Results of the rotation study demonstrated a relation between crop cover, fertilization regime, and chitinase activity. Highest chitinase activity was observed in soils with complete fertilization regimes and corn or soybean and lowest in those with cotton or deficient in any major nutritional element; previous studies on xylanase and catalase activities of soils in this rotation

evidenced similar patterns for those enzymatic activities also[13,14]. The response to crop cover was attributed to differences in root densities between the crops. Corn and soybeans produced the highest root densities in soil while cotton produced the lowest[13]. Root densities can be expected to be directly related to microbial activity, hence enzyme production, through the 'rhizosphere effect'[6].

The effect of winter legume on chitinase activity also agreed with previous findings on soil xylanase and catalase[13,14]. Soil chitinase decreased significantly when winter legumes were removed from a complete fertilization regime in comparison with activity in soils with NPK+, despite the lack of yield differences between the two treatments. It is probably because of these differences between chitinase activity and yields that the relation between the two variables was only significant at the 5% level of probability.

Chitin amendments to soil stimulated chitinase activity. This phenomenon has also been observed for other soil carbohydrases when soil was amended with the appropriate substrate[6,7]. Stimulation by the amendments reflects the development of an adaptive microflora. Our results showed that stimulation of bacterial populations was short lived and unrelated to soil chitinase activity. Although numbers of actinomycetes in soil were also stimulated by chitin additions to soil and the effect of the treatments on actinomycetes lasted longer than for bacteria, numbers of actinomycetes were unrelated to soil chitinase activity. These results were unexpected in that previous reports suggested a possible relation between chitinase activity and numbers of bacteria and actinomycetes[8,11,18]; many species of these organisms are known to produce chitinases[12]. Our results showed that chitinase activity was correlated with increased numbers of fungal propagules in response to chitin amendments. Fungal populations increased in reponse to all levels of chitin added to soil in contrast to populations of bacteria and actinomycetes. We therefore interpret the observed increase in soil chitinase activity in response to chitin amendments as due primarily to production of chitinase by fungi.

Although some species of fungi isolated from chitin-treated soil belong to genera commonly found in soil (*e.g. Fusarium, Trichoderma, Rhizopus, Aspergillus*) many others belonged to genera not frequently encountered in soils. This agrees with previous studies[10] suggesting that a specialized mycoflora is associated with the decomposition of chitin in soil.

In conclusion, our results indicate that chitinase activity is of common occurrence in soil and that its assay can be performed with a procedure that conforms to classical theory of enzyme kinetics. Results also suggest that fungi are the primary producers of chitinase in the soils studied.

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