Enhanced phosphatase activity in earthworm casts is more of microbial origin

Soil enzymes produced by plants, animals and micro-organisms play a crucial role in soil fertility. Soil wormcasts have been shown to have enhanced microbial and enzyme activities and micro- and macro-nutrients[1]. Vermicompost has been shown to enhance the fertility of soil and the yield of many agricultural produce[2]. Higher activities of cellulase, amylase, invertase, protease, peroxidase, urease, phosphatase and dehydrogenase in the wormcasts have been reported[2,3].

Recently, enhanced micro- and macro-nutrients, microbial and enzyme activities in the pressmud vermicasts of *Lampito mauritii* and *Eudrilus eugeniae* have been recorded. The increased microbial activities in pressmud vermicasts were related to higher enzyme activities[4]. However, the origin of gut enzymes and existence of truly indigenous microflora in worms is still an unanswered question[5]. In order to answer the question whether the increased enzyme activities in the vermicasts are contributed by the gut epithelium of the worm or by the microbes in the feed that is transmitted through the gut, an attempt was made in this study with reference to phosphatase in the casts of worms fed with both sterile and non-sterile clay loam soil and pressmud which is known to be a 'hot spot' of microbes[6].

Clay loam soil (S) collected from the Agricultural Experimental Farm of the Annamalai University and two-month-old cured pressmud (PM) obtained from E.I.D. Parry's sugar mill at Nellikuppam, Tamil Nadu, were used as feed substrates for *Eudrilus eugeniae* (Kinberg). Fifteen gut-cleared worms (by feeding with sterile, wet blotting paper strips) were kept in sterile glass containers (20 × 15 × 10 cm), each containing 2 kg of non-sterilized S and PM, sprinkled with water. These were maintained at 60–70% moisture, 29 ± 1°C temperature and 70–75% humidity. To get rid of the microbes from S and PM, these were sterilized in autoclave for 4–5 h. Two kg of sterilized S and PM was sprinkled with sterile water and maintained under conditions mentioned above. After regular feeding on both sterilized and non-sterilized S and PM, freshly deposited casts were collected using sterile spatula and forceps for experiments.

Total microbial population (fungi, bacteria and actinomycetes) from the substrates (both sterile and non-sterile S, PM, S casts and PM casts) was determined by dilution plate techniques. One gram of each substrate was suspended in 1 ml sterile saline in a sterile test tube, shaken thoroughly in a vortex mixer and was used as inoculum. Using standard loop, 0.01 ml of each inoculum was inoculated on blood, nutrient and Mac Conkey agar plates for bacterial growth, Sabouraud's dextrose agar plate for fungal growth and actinomycetes agar plate for actinomycetes growth and incubated at 37°C for 18–24 h for bacteria, 5–7 days for fungi and 11–12 days for actinomycetes. The number of colony-forming units (CFUs) was expressed as CFU × 10^3 g^{-1}. The dehydrogenase and phosphatase activities were determined according to the methods of Stevenson[7] and Jonnossy[8] and the activities were expressed in μl H/5 g of substrates and mg phenol/g of oven-dried substrates for 24 h of incubation, respectively. The statistical significance of difference was tested at 1% level using Student's *t* test.

Analysis of results (Table 1) shows that there were no microbial and enzyme activities in the sterilized S and PM and their respective casts. On the contrary, there was an enhanced microbial activity and a concomitant enhanced phosphatase activity in the non-sterilized S and PM and their respective casts. Microbial population, microbial and phosphatase activities in the PM compared to S was more than 2.7, 2.6 and 2 times, respectively. PM casts compared to S casts had more than 1.5, 3.5 and 2.3 times, respectively, more of microbial population, microbial and phosphatase activities. Concomitant with the increase of microbial population (87%) and activity (68%) in the S casts compared to S, there was a corresponding increase of phosphatase activity (79%). Similarly, concomitant with the increase of microbial population (76%) and activity (77%) in the PM casts compared to PM, there was a corresponding increase of phosphatase (82%) activity.

The digestive epithelium of the simple, straight and tubular gut of worms is known to secrete cellulase, amylase, invertase, protease, phosphatase, urease, acid and alkaline phosphatase[9]. Earthworm being a soil-dwelling organism feeds on soil, litter and other organic matter[2]. Earthworms inevitably consume the soil microbes during ingestion of litter and soil. It has been recently established that earthworms necessarily have to feed on microbes, particularly fungi for their protein/nitrogen requirement for growth and reproduction[10]. These microbes contribute enzymes to the digestive processes of the earthworm. However, it is difficult to ascertain earthworm-derived enzymes from those of microbially-derived enzymes.

A significant positive correlation between organic matter and phosphatase activity has been reported[11]. Bonmati *et al.*[12] observed that soil phosphatase

Table 1. Microbial and enzyme activities in the casts of *E. eugeniae*

Substrate	Total microbial population (CFU × 10^3 g^{-1})	Phosphatase (mg phenol/g oven-dried substrates for 24 h)	Dehydrogenase (μl H/5 g substrates)
Sterilized S	–	#	#
Casts of sterilized S	–	#	#
Sterilized PM	–	#	#
Casts of sterilized PM	–	#	#
Non-sterilized S	188.13 ± 0.13	1.12 ± 0.01	3.13 ± 0.04
Casts of non-sterilized S	1451.32 ± 0.38 (+ 87.00)*	5.25 ± 0.03 (+ 78.66)*	9.91 ± 0.06 (+ 68.41)*
Non-sterilized PM	514.25 ± 0.19	2.27 ± 0.05	8.14 ± 0.02
Casts of non-sterilized PM	2169.76 ± 0.65 (+ 76.29)*	12.29 ± 0.07 (+ 81.52)*	34.89 ± 0.03 (+ 76.66)*

Values are mean ± SE of six observations; (+) indicates the per cent increase over S/PM; *indicates the statistical significance at 1% level; '–' denotes no growth; '#' denotes no activity.

activities were more marked, probably reflecting substantial greater microbial group due to the presence of easily decomposable organic compounds. Pressmud has more organic matter (53%) than clay loam soil (23.26%)[13] and the present finding that there was a significantly enhanced phosphatase activity in the PM casts compared to S casts of *E. eugeniae*, was due to the decomposition of the rich organic matter in PM while passing through the gut and also enhanced microbial activity in the PM casts.

A great variety of enzymes are produced by soil micro-organisms, during their metabolism[14]. Soil phosphatases hydrolyse phosphate and make it available to plants. Thus, phosphatase activity measurement provides an index of potential availability of phosphatase in soil[15]. The increased amount of inorganic P released during cast deposition was related to and preceded by increased microbial and phosphatase activity[3]. High P_2O_5 content in casts supports the phosphatase availability which is required for growth of root, microbial enhancement and in turn, may help drive biological nitrogen fixation[16]. Recently, enhanced phosphate content in the soil and pressmud casts of *L. mauritii* and *E. eugeniae* have been reported[4]. Satchell and Martin[17] have found direct correlation between microbial population and enzyme activity. Microbes like *Pseudomonas* spp., *Bacillus* spp. and *Aspergillus* spp. are known to mineralize phosphate[18]. These microbes were found to be rich in the gut content of worm fed on S/PM, S and PM casts of *L. mauritii* and *E. eugeniae*[6]. Since there is no phosphatase activity in the cast of sterilized S and/or PM, it is evident that gut epithelium of worm or even the indigenous microbes of gut does not contribute phosphatase. Hence enhanced phosphatase activity in the casts with more microbial population is microbial rather than by the epithelium of the gut.

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ACKNOWLEDGEMENT. We are thankful to authorities of Annamalai University for providing facilities.

Received 12 August 2000; accepted 16 September 2000

S. P. VINOTHA
K. PARTHASARATHI
L. S. RANGANATHAN *

Department of Zoology,
Annamalai University,
Annamalainagar 608 002, India
**For correspondence*
e-mail: aulib@hotmail.com

Presence of a possible retinoblastoma protein binding motif in the AC2 protein of subgroups II and III geminiviruses

Geminiviruses are plant DNA viruses with small genomes, comprising one or two components of circular, single-stranded DNA which are less than 3 kb in size. The limited size of the genomes makes geminiviruses heavily dependent on host factors for their proliferation. Members of *Geminiviridae* can infect terminally differentiated cells[1]. Therefore the viruses must control the cellular environment of these cells to produce conditions suitable to support viral DNA replication. In support of this hypothesis, the Rep proteins of some members of *Geminiviridae* are reported to bind to mammalian and plant retinoblastoma (Rb) proteins[2,3]. Rb is a cellular protein that sequesters the transcription factors required for cell progression from G1 to S phase[4]. The viral Rep protein (C1 ORF) binds with its LXCXE motif[2]. The present analysis finds a similar conserved motif (LXCXC) in the AC2 ORF of subgroups II and III of *Geminiviridae.*

Subgroup I geminiviruses are those members of *Geminiviridae* that have a monopartite genome and infect monocots. Typical members of this subgroup, like wheat dwarf virus and maize streak virus, have been shown to bind to human and maize retinoblastoma proteins through one of their complementary sense (C1) gene products[2,3]. The motif in the viral protein implicated in the binding is LXCXE[2]. However, subgroups II and III geminiviruses have not been shown to have such a motif, although the Rep protein (an AC1 ORF) of tomato golden mosaic virus, a member of subgroup III, has been found to bind to Rb[3]. So far, the second complementary sense gene product (AC2) has not been analysed for its Rb binding capacity, although it has been reported to be involved in transactivation of viral gene products[5]. The AC2 ORF