

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-nutrients¹. Vermicompost has been shown to enhance the fertility of soil and the yield of many agricultural produce². 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 vermicast of *Lampito mauritii* and *Eudrilus eugeniae* have been recorded. The increased microbial activities in pressmud vermicast were related to higher enzyme activities⁴. However, the origin of gut enzymes and existence of truly indigenous microflora in worms is still an unanswered question⁵. In order to answer the question whether the increased enzyme activities in the vermicast 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 microbes⁶.

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 × 10³ g⁻¹. The dehydrogenase and phosphatase activities were determined according to the methods of Stevenson⁷ and Jonnosy⁸ 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 phosphatase⁹. Earthworm being a soil-dwelling organism feeds on soil, litter and other organic matter². 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 reproduction¹⁰. 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 reported¹¹. 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 metabolism¹⁴. Soil phosphatases hydrolyse phosphate and make it available to plants. Thus, phosphatase activity measurement provides an index of potential availability of phosphatase in soil¹⁵. The increased amount of inorganic P released during cast deposition was related to and preceded by increased microbial and phosphatase activity³. High P₂O₅ content in casts supports the phosphatase availability which is required for growth of root, microbial enhancement and in turn, may help drive biological nitrogen fixation¹⁶. Recently, enhanced phosphate content in the soil and pressmud casts of *L. mauritii* and *E. eugeniae* have been reported⁴. Satchell and Martin¹⁷ have found direct correlation between microbial population and enzyme activity. Microbes like *Pseudomonas* spp.,

Bacillus spp. and *Aspergillus* spp. are known to mineralize phosphate¹⁸. 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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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 cells¹. 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 phase⁴. The viral Rep protein (C1 ORF) binds with its LXCXE motif². 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 LXCXE². 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 Rb³. 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 products⁵. The AC2 ORF

Table 1. LXCXC motif in the AC2 protein of subgroup II and III geminiviruses

Accession no.	Virus	Sequence	Starting position in the AC2 ORF
U88692	Tomato leaf curl virus	L P C G C	35
Y11097	Sida golden mosaic virus	L E C G C	31
AF012300	Taino tomato mottle virus	L G C G C	31
AJ223191	Chayote mosaic virus	L E C G C	35
U51137	Abutilon mosaic virus	L G C G C	31
AF058015	Tomato mottle virus	L G C G C	31
X70418	Pepper hausteco virus	L A C G C	40
M88179	Bean dwarf mosaic virus	L E C G C	31
U57457	Texas pepper virus	L N C G C	31
U65529	Cabbage leaf curl virus	L N C G C	31
X74516	Ageratum yellow vein virus	L T C G C	35
Z24758	Indian cassava mosaic virus	L N C G C	35
K02029	Tomato golden mosaic virus	L N C G C	31
D00940	Potato yellow mosaic virus	L D C G C	31
AB027465	Squash leaf curl virus	L D C G C	31
X17095	African cassava mosaic virus	L V C G C	35
X76319	Tomato yellow leaf curl virus	L D C G C	35
AJ132575	<i>V. mungo</i> yellow mosaic virus	L S C G C	35
M10070	Bean golden mosaic virus	L N C G C	74
U56975	Beet curly top virus (sub grp II)	L P C K C	34

Table 2. Multiple alignment of the AC2 protein of subgroup II and III geminiviruses created by MACAW program. Region showing the LXCXC motif

U88692	mrnsspspshsthpikvqhkiakkrp-----	IRRRVDLPCGCSYYLGINCashgfshr	55
Y11097	mrsspspshpsiktahrqakkr-----	IRRRIDLECGCSIYFHIGctghgfthr	51
AF012300	mrcsspsqpsikiahrgqkkra-----	IRRRVDLQCGCSIYFHLNCaghgfthr	51
AJ223191	mppsarspsrstqvpikvqhrigkkka-----	IRRRIDLECGCSFYLHIDCalngfahr	55
U51137	mrsspspshpsikkahrqakrra-----	IRRRIDLQCGCSIYFHIDctghgfthr	51
AF058015	mrsspsqpsikrahrgqkkra-----	IRRRVDLQCGCSIYFHLGCaghgfthr	51
X70418	mtgskktpstpskllsppvevklrhrfakrq	IRRRIDLACGCSIYIHINcVnngfphr	60
M88179	mqssslstppsikkahrqakrra-----	IRRRIDLECGCSIYIHIGctghgfthr	51
U57457	mlnssstlpsikaqhriakkrp-----	IRRRIDLNCGCSIFLHINCanngfthr	51
U65529	mqnssllkppsikaqhkiakrra-----	VRRRIDLNCGCSIFLHINCadngfthr	51
X74516	mrnsspsrghctqvpikvqhriakrrp-----	VRRRVLDTCGCSYFYGIDCanhgfshr	55
Z24758	mrpspskdhytqvpikvqhraakrr-----	IRRRVDLNCGCSYYVHINChnngfthr	55
K02029	mrnssstppsikaqhraakrra-----	IRRRIDLNCGCSIYIHIDCrnngfthr	51
D00940	mrsspsqpsikkahrqakrra-----	IRRRIDLDCGCSIYFHIDCaghgfthr	51
AB027465	mpnssskvpsikaqhriakrra-----	VRRRIDLDCGCSIYIHINcKadnggft	51
X17095	mqsspspnhstqvpikvshrqfkkra-----	IRRRVDLVCGCSYYLHINcshngfthr	55
X76319	mqpspstshcsqvsikvqhkiakkkp-----	IRRRVDLDCGCSYYLHLNcshngfthr	55
AJ132575	mrnstpsknhfspssikaqhkvakrra-----	IRSRIDLSCGCSYYIHINCrnygfshr	55
M10070	mrsspsqpsikaqhriakrra-----	IRRRIDLNCGCSFYHIKCadhgfthr	51
U56795	mkplspglyriqpslnspalslikiq-----	KRPRKVNLPCKCHFTHHDCchgfshgt	54

sequence is known for nineteen subgroup III viruses and one subgroup II virus. Here we report the presence of a conserved LXCXC motif in the AC2 protein of all these twenty viruses (Table 1). The plant virus sequences were fetched from Genbank, EMBL and Swissprot databases. The sequence patterns were identified using FindPatterns of GCG⁶. The initial multiple alignment was made with CLUSTALW⁷. The final block searching and alignment (Table 2) were carried out using MACAW⁸. The statistical significance of the block having high-scoring

sequence segments (LXCXC) was shown to be significant ($P = 0.0$) using Karlin–Altschul statistics⁹. The significance of the Glu (E) residue of the LXCXE motif in the RepA protein of bean yellow dwarf virus was shown by mutating it to Gln (Q)¹⁰ and Lys (K)¹¹, which almost completely abolished the binding. However, there are no data available on the effect of a Glu (E) to Cys (C) mutation on Rb binding. The identification of the LXCXC motif suggests the need for experiments to check the Rb binding of the AC2 protein of subgroups III and II gemini-

viruses. The neighbourhood of the motif is also conserved in the subgroup III viruses (it is RRRRID or RRRRVD). In beet curly top virus (Tables 1 and 2), this region is RPRKVN. However, the LXCXC motif is present in the same relative position in the AC2 protein of beet curly top virus also. Our search shows that such a motif is not found in any other group of plant viruses.

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Is there really a ‘quantum-no-deleting principle’?

A recent issue of *Nature*¹ contains a letter entitled ‘Impossibility of deleting an unknown quantum state’ by Pati and Braunstein (PB). The main contention of PB is that it is impossible to delete an ‘unknown’ quantum state. Further, they claim intrinsic security to files in a quantum computer as a corollary. What they have actually considered is uncopying, which means deleting against a copy. To claim intrinsic security, irreversible deleting must also be considered. We find that their claim is not true even for the (restricted) act of uncopying. To uncopy a state, it is necessary to have at least an additional copy. An uncopying device accepts two identical inputs – the original and a copy – and switches the copy to a standard state while keeping the original intact. This is strictly, called conditional uncopying.

Yuen² has defined copying of a quantum state $|y\rangle$ (of two-state system or a q-bit) using the transformation T_c :

$$T_c|y\rangle| \rangle|A\rangle = |y\rangle|y\rangle|A_y\rangle,$$

where $|\acute{O}\rangle$ is the standard state onto which the copy is made, and $|A\rangle$ and $|A_y\rangle$ are, respectively, the initial and final states of the copying device (or ancilla). PB define an uncopying transformation, T , that is analogous to T_c . The unitary operator T , which represents a Schrödinger evolution, transforms a composite state $|y\rangle|y\rangle|A\rangle$ as

$$T|y\rangle|y\rangle|A\rangle = |y\rangle|\acute{O}\rangle|A_y\rangle. \quad (1)$$

PB attempt to show that if T exists for the orthogonal basis states, then, linearity of quantum mechanics will prevent it from working for any superposed state. We show that PB’s arguments are untenable, and hence, there is nothing like a ‘quantum no-deleting principle’.

The operational part of PB’s ‘derivation’ is very simple: They assume that the operator T exists for two orthogonal states $|H\rangle$ and $|V\rangle$. Thus

$$T|H\rangle|H\rangle|A\rangle = |H\rangle|\acute{O}\rangle|A_H\rangle, \quad (2)$$

$$T|V\rangle|V\rangle|A\rangle = |V\rangle|\acute{O}\rangle|A_V\rangle, \quad (3)$$

where $T|A_H\rangle$ and $|A_V\rangle$ are the final states of the device. Now, the question is whether the same T can uncopy a state obtained as an arbitrary linear superposition, $|y\rangle = a|H\rangle + b|V\rangle$, with $|a|^2 + |b|^2 = 1$. (It may be noted that eqs (2) and (3) by themselves do not define T for the complete Hilbert space.) After a few simple steps, one finds that this is indeed possible with an appropriate $|A_y\rangle = (a|A_H\rangle + b|A_V\rangle)$. Also the entangled state, $(|H\rangle|V\rangle + |V\rangle|H\rangle)|A\rangle$, transforms to $(|H\rangle|\Sigma\rangle|A_V\rangle + |V\rangle|\Sigma\rangle|A_H\rangle)$. The conclusion should have been that the sought after transformation is indeed possible – though it has not been constructed explicitly. However, at this point PB take a different view. They observe that $|A_H\rangle$

and $|A_V\rangle$ are orthogonal and $|A_y\rangle$ is a linear superposition of them, and then claim, ‘The transformation is therefore not uncopying at all, but merely swapping onto a two-dimensional sub-space of the ancilla. It appears that there is no option but to move the information around without deleting it’ (emphasis is ours).

We state a consequence directly following from the definition, eq. (1). The orthogonality of $|A_H\rangle$ and $|A_V\rangle$ follows from unitary property of operator T . For instance let $|A_{\phi_1}\rangle$ and $|A_{\phi_2}\rangle$ be the ancilla states corresponding to two states $|y_1\rangle$ and $|y_2\rangle$, respectively, in eq. (1). Then one may argue using unitarity of T and continuity of scalar product $\langle y_1|y_2\rangle$ that orthogonality of $|y_1\rangle$ and $|y_2\rangle$ implies that of $|A_{\phi_1}\rangle$ and $|A_{\phi_2}\rangle$. (Since this is true for every unitary operator T , the mere orthogonality of $|A_H\rangle$ and $|A_V\rangle$ cannot be the deciding factor as to whether T represents uncopying or swapping.)

Also, one might wonder why there is a sudden concern about swapping. (A swapping operator on the state $|y_1\rangle|y_2\rangle$ of two q-bits, transforms it to $|y_2\rangle|y_1\rangle$.) PB had advanced the following argument for not considering swapping as uncopying. According to them, ‘The standard erasure of $|y\rangle$ does not use the original (i.e. first $|y\rangle$), and so is the case, if T swaps the copy (i.e. second) $|y\rangle$ and $|A\rangle$ ’. They thereby imply the equivalence of swapping and erasure. But, erasure is not reversible, while swapping of two states

is a unitary operation which can be considered as legitimate uncopied (*albeit* unconditional).

Finally, while it is indeed true that $|A_H\rangle$ and $|A_V\rangle$ are orthogonal for swapping transformation; the converse is not true. For, consider a trivial case where the device is a single q-bit, and \mathbf{T} be defined as

$$\mathbf{T} |H\rangle |H\rangle |\hat{O}\rangle = |H\rangle |\hat{O}\rangle |V\rangle, \quad (4)$$

$$\mathbf{T} |V\rangle |V\rangle |\hat{O}\rangle = |V\rangle |\hat{O}\rangle |H\rangle, \quad (5)$$

Here, $|A_H\rangle = |V\rangle$ and $|A_V\rangle = |H\rangle$ are orthogonal, but \mathbf{T} does not swap the second and third states. We thus find that PB's argument (which uses the converse) to infer the 'quantum-no-deleting principle' is not correct. Explicit construction, of conditional quantum deleting machines, using a cascade of quantum logic gates is also possible³. In any case, finding $|A_y\rangle = a|A_H\rangle + b|A_V\rangle$ should not be of any concern once the primary objective of uncopied $|y\rangle$, as defined in eq. (1), has been realized. (Rather one would worry about resetting the device in its final state $|A_y\rangle$ back to $|A\rangle$ for the next uncopied operation!)

PB remark that the quantum-no-deleting principle has been 'proved' for

reversible as well as irreversible operations, in spite of their restricting to uncopied through Schrödinger evolution!

They even forget that their concern was limited to only uncopied; nevertheless, PB proceed to make several claims: 'We emphasize that copying and deleting of information in a classical computer are inevitable operations, whereas similar operations cannot be realized perfectly in quantum computers. This may have potential applications in information processing because it provides intrinsic security to quantum files in a quantum computer. No one can obliterate a copy of an unknown file from a collection of several copies in a quantum computer,.... Nevertheless, nature seems to put another limitation on quantum information imposed by the linearity of quantum mechanics.' These high-sounding claims lie well outside the premise of the matter of their discussion.

In summary, reversible uncopied as well as irreversible deleting of a known or unknown quantum state should always be possible. More importantly, there is no case for a quantum no-deleting principle.

For an interested reader, a more detailed account of our analysis, which

also touches upon quantum controlled-NOT operator⁴ for copying and uncopied, is available in a preprint⁵.

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