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AN ENZYME FROM *MYROTHECIUM VERRUCARIA* THAT DEGRADES INSECT CUTICLES FOR BIOCONTROL OF *AEDES AEGYPTI* MOSQUITO

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SUMMARY

Myrothecium verrucaria produced a high activity of extracellular insect cuticle degrading enzymes, chitinases, proteinases and lipases. Both first (I) instar and fourth (IV) instar larvae of a mosquito, *Aedes aegypti*, a vector of yellow fever and dengue, were susceptible to crude culture filtrate (100% mortality within 48 h at 170 mg/l). The supplementation of purified *M. verrucaria* endo-chitinase with commercial lipase decreased the lethal time (LT_{50}) from 48 h to 24 h for I instar and 120 h to 96 h for IV instar. Our results of the larvicidal activity indicate that the cuticle degrading enzyme complex of *M. verrucaria* has a good potential for the control of mosquitoes.

INTRODUCTION

Myrothecium verrucaria produces high activities of an extracellular chitinase complex when grown in a medium containing chitin as the sole carbon source (Vyas & Deshpande, 1989). Lipase and proteinase activities also occur simultaneously. All the three activities, collectively known as cuticle degrading enzymes play an important role in the biocontrol of insect pests (Cohen, 1993; Shaikh & Deshpande, 1993). The world-wide medical and economical aspects of diseases spread by mosquito render these insects as potential targets for various insecticides (Venkateswara Rao et al, 1995). In the present communication, the potential of the *M. verrucaria* crude chitinase complex as well as purified endo-chitinase is evaluated for mosquito biocontrol using the first (I) and fourth (IV) instar larvae of the *Aedes aegypti* mosquito, the vector of yellow fever and dengue fever.

MATERIALS AND METHODS

Microorganism and culture conditions. *M. verrucaria* NCIM 903, was grown for 7 days in a medium which contained (g/l): KH_2PO_4, 3.0; K_2HPO_4, 1.0; $MgSO_4$, 0.7; $(NH_4)_2SO_4$, 1.4; NaCl, 0.5; $CaCl_2$, 0.5; yeast extract, 0.5; bacto-peptone, 0.5 and chitin, 5.0 and pH 5.5.(Vyas & Deshpande, 1989). The purified chitinase preparation for the studies was obtained

by ultrafiltration (Amicon PM-10 membrane) of *M. verrucaria* culture filtrate and preparative polyacrylamide gel electrophoresis (7.5%) as described earlier (Vyas & Deshpande, 1993). The enzyme concentrate obtained after ultrafiltration while carrying out purification was also used in the present studies.

Enzyme assays. The chitinase, chitobiase and proteinase activities were estimated using the substrates, acid swollen chitin, carboxymethyl chitin, ethylene glycol chitin and p-nitrophenyl-β-D-N-acetylglucosaminide (pNP-GlcNAc) for chitinase and chitobiase and casein for proteinase as described earlier (Vyas & Deshpande, 1989). One international unit of chitinase was defined as the amount of enzyme which produced 1 μmol N-acetylglucosamine or p-nitrophenol per min under assay conditions. One unit of proteinase activity was defined as the amount of enzyme required to release 1 μmol of tyrosine from casein per min. The lipase assay was carried out as described in the Worthington Manual(1977). One unit of lipase activity was defined as the amount which released one μmol of fatty acid per min from emulsified olive oil at 28°C and pH 8.2. Protein was determined according Bradford (1976) with bovine serum albumin as a standard.

Bioassay. The bioassay was carried out by suspending ten I or IV instar larvae of *A. aegypti* in 25 ml tap water with different concentrations of enzyme (Busvine, 1971). Experiments were carried out at 28 °C and 80 % RH. In case of crude enzyme, the range of 90-170 mg/l on protein basis was used for bioassay.

RESULTS AND DISCUSSION

The 7 day old culture filtrate of *M. verrucaria* contained the enzyme activities (IU/mg protein) as : Acid swollen chitin degrading activity, 0.88; endo-chitinase measured on ethylene glycol chitin, 0.44 and carboxymethyl chitin, 2.11; chitobiase measured on pNP-GlcNAc, 0.22; alkaline proteinase, 0.33 and lipase, 1611. The enzyme activities used to evaluate its larvicidal potential are tabulated in Table 1. Both, the I and IV instar larvae of the *A. aegypti* were susceptible to the crude cuticle degrading enzyme complex of *M. verrucaria*. The 100% mortality was observed in both the cases within 48 h at 170 mg/l level (Fig. 1a). With 90 mg/l concentration, more than 50% mortality for I instar larvae was noted within 48 h. However, in case of IV instar, the required concentration was 150 mg/l and the exposure time was 72 h (Fig.1b).

To evaluate the role of chitinase in the larvicidal potential of *M. verrucaria* crude culture filtrate, the enzyme concentrate and purified endo-chitinase were used on the equal activity basis for the bioassay. As indicated in Table 2, the lethal time for 50% mortaliity (LT_{50}) of IV instar larvae for concentrated enzyme and purified endo-

chitinase were 24 and 48 h, respectively. The lesser time required for killing with concentrated crude enzyme than the purified preparation can be attributed to the contribution of other enzymes of culture filtrate. The supplementation of purified endo-chitinase with commercial lipase decreased LT_{50} significantly (Table 2). However, the conmbination with alkaline proteinase didnot show any appreciable effect on the larvicidal activity of chitinase alone (Data not shown).

In conclusion, it can be suggested that, the non-commercial preparation of *M. verrucaria* has high potential for use in mosquito control. In view of the capability of *M.verrucaria* to produce high chitinase activity, perhaps it can be inexpensive if used for commercial production of cuticle degrading enzymes.

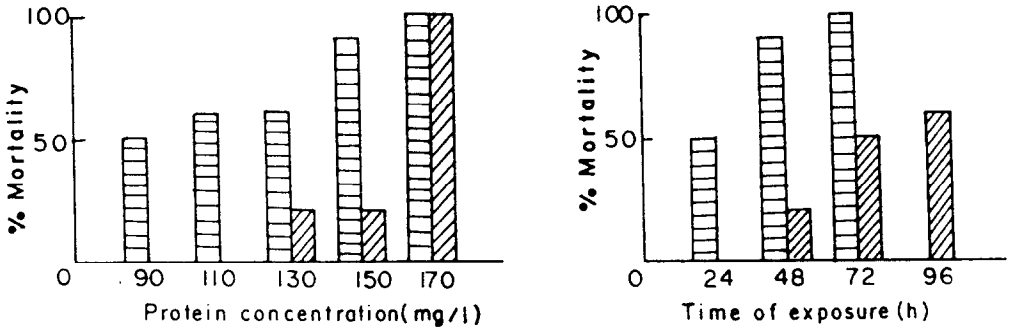

Figure 1. The larvicidal effect of *M. verrucaria* crude culture filtrate. The bioassay was carried out as described under Materials and Methods. a) Exposure for 48 h; b) Crude culture filtrate, 150 mg/l. Symbols : ▤ , I instar larvae; ▨ , IV instar larvae.

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Table 1. Quantitative correlation between protein and enzyme activities of *M. verrucaria* culture filtrate used for bioassay.

Protein	Chitinase activity* on				Proteinase activity on casein	Lipase activity on olive oil
	ASC	EGC	CMC	pNP-GlcNAc		
mg/l	IU/l				IU/l	IU/l
90	79	40	190	20	30	145000
110	97	48	232	24	36	177000
130	114	57	274	29	43	209000
150	132	66	317	33	50	242000
170	150	75	360	37	56	274000

* ASC, acid swollen chitin; EGC, ethylene glycol chitin; CMC, carboxy methyl chitin and pNP-GlcNAc, p-nitrophenyl-β-D-N-acetylglucosaminide.

Table 2. LT_{50} for *A. aegypti* larvae with enzyme preparations of *M. verrucaria*, singly or in combination with commercial lipase.

Enzyme	LT_{50} (h)	
	I instar	IV instar
Crude culture filtrate, 150 mg/l	24	72
Concentrated enzyme, 500 mg/l	24	48
Purified endo-chitinase*	48	120
Commercial lipase preparation*	48	ND
Endo-chitinase plus lipase*	24	96

ND, Not detected upto 120 h.

*Activities used in bioassay (IU): Acid swollen chitin degrading activity, 7.0; lipase, 175.0.