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Heterogeneity in Cellulases of Some of the Local Agricultural Insect Pests

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Abstract.- Although **c**ellulases have generally been reported to be produced by microbes including bacteria and fungi, their activity has been detected in the whole body saline extracts of some insects. In this paper, the cellulase activity is being reported from the locally collected insects *viz.*, tiger beetle (*Cicindela scutellaris*), red cotton bug (*Dysdercus koenigii*), blue pumpkin beetle (*Aulacophora atripennis*), red pumpkin beetles (*Aulacophora foveicollis* and *Aulacophora hilaris*) and grass hopper (*Chrotogonus trachypterus trachypterus*). The whole body extracts of these insects were able to hydrolyze carboxy-methylcellulose and crystalline cellulose. Carboxy-methylcellulase activity produced by the insects was studied for the heterogeneity using PAGE and zymogram technique, which showed that there were at least 2 fractions for each insect enzyme activity. The phenomenon of heterogeneity was comparable to bacterial cellulase enzymes.

Key words: Cellulase, enzyme heterogeneity, cellulase in insects, insect pests.

INTRODUCTION

Cellulases are a group of enzymes capable of converting cellulose molecule, a natural polymer of glucose joined through β-linkage, into its basic units. They are one of the 15 members of the Glycoside Hydrolases family classified on the basis of similarities among amino acid sequences and hydrophobic cluster analysis (*Cazy website).* Cellulases include endo-β, 1-4-glucanase (3.2.1.4), exo-β, 1-4-glucanase (3.2.1.74) and cellobiohydrolase (3.2.1.91) (Mandels and Reese, 1964). Cellulases are reported to be produced by microbes (Wood and Phillips, 1969*;* Almin *et al.,* 1975; Gomes *et al.,* 1992; Ghose and Kostick, 1970), but in recent years it has been shown that insects are also able to produce cellulase activity, apart from the microbes and unicellular flagellates residing in their digestive tract (Tokuda *et al.,* 2005). Watanabe *et al.* (1998*)* reported the isolation of cellulase genes from termite, *Reticulitermes sepratus*. In another report Tokuda *et al.* (1997) reported β-endoglucanase activity in the mid-gut of wood eating termite, *Nasutitermes takasagoensis*. Khademi *et al.* (2002) described structure of an endoglucanase from a termite. Wei *et al.* (2005) have cloned and sequenced endoglucanase from a mulberry longnicorn beetle, *Apriona germari.*

Multiplicity is one of the phenomena that has been reported for cellulases in microbes, by separating proteins through polyacrylamide electrophoresis and then identifying the cellulase activity on substrate agar plate by replica plating (Langsford *et al.,* 1984*;* Sami and Akhtar, 1989, 1993*)*. Four or more fractions of endoglucanases or carboxy methylcellulase activity have been reported, due to proteolysis degradation or different levels of glycosylation of the protein. Here we report for the first time a study on the screening of different common local insect pests cellulase activity and heterogeneous forms of carboxy methyl cellulase activity after separating the enzyme fraction.

MATERIALS AND METHODS

Chemicals

All the reagents used were of analytical grade and were purchased from E. Merck, Darmstadt, Germany.

Insect collection

Insects were collected from agricultural fields of Punjab University, Lahore, and Shahpur, District Sargodha during the months of May and June in the afternoon. All the insects were collected in separate sterilized bottles and then stored at -20°C.

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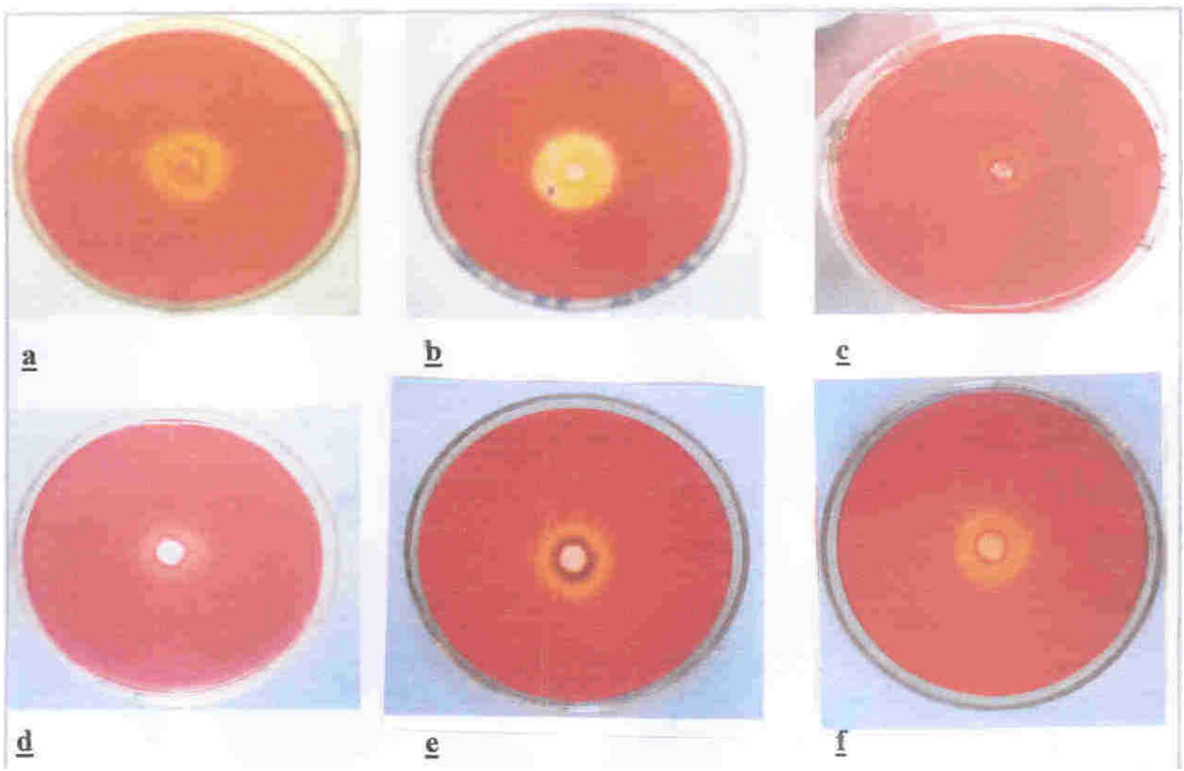

Fig. 1. Screening of insect total cellular proteins for cellulase activity. 0.1mg of total cellular protein was loaded on cellulose agar plate and after incubation at 50°C overnight, plates were stained with Congo red. Substrate hydrolyzed by endoglucanase appeared as clear zone in the centre.
a, pumpkin beetle, *Aulacophora hilaris*; b, grass hopper, *Chrotogonus trachypterus trachypterus*; c, tiger beetle, *Cicindela scutellaris*; d, red cotton bug, *Dysdercus koenigii*; e, blue pumpkin beetle, *Aulacophora atripennis*; f, red pumpkin beetles, *Aulacophora foveicollis.*

Protein extraction

The insects, weighing approximately 5g, were homogenized in 100ml of 0.5M Tris-HCl buffer pH 8.5 and centrifuged at 10,000Xg for 10 minutes at 4°C. The supernatant was stored in 1ml aliquot in eppendorf tubes and used as a source of enzymes.

Screening procedure

The screening procedure was based on the method described previously (Sami *et al.,* 1988) with some modifications, as follows. A solution of 1% agar and 0.5% carboxymethylcellulose in 0.5M Tris-HCl pH 7.1 was poured in petri plates and kept at 50°C for 15 minutes. A hole was then punched in the centre of the plate in which 0.1ml of the enzyme extract was loaded and incubated at 50°C overnight. The plates were stained with 0.1% Congo red for 15 minutes and then washed with 0.1M NaCl several times. Enzyme activity appeared as lighter area around the hole, against the red background.

Determination of heterogeneity

PAGE and zymogram technique were used for determination of heterogeneity.

PAGE was carried out according to the method of Laemmli (1970). For this I mg of total protein extract was loaded on 10% polyacrylamide gel. After electrophoresis, zymogram was prepared as described previously (Sami *et al.,* 1988). Enzyme activity bands were identified by using Congo red dye.

RESULTS AND DISCUSSION

Screening of cellulase activity

A number of insects species were collected from the local flora, which were identified as tiger beetle (*Cicindela scutellaris*), red cotton bug (*Dysdercus koenigii*), blue pumpkin beetle (*Aulacophora atripennis*), red pumkin beetles (*Aulacophora foveicollis* and *Aulacophora hilaris*) and grasshopper (*Chrotogonus trachypterus trachypterus*). Among the identified insects *C. scutellaris* is a known predator, while the rest of the insects are considered as agricultural pests.

The whole body extract of all these insects demonstrated cellulase activity to varying extent (Fig.1). Maximum activity was recorded in *C. trachypterus trachypterus* and minimum hydrolyzed area was represented by *C. scutellaris (*Fig. 1b, c). This could be related to the eating habits of the insects, whereas *C. trachypterus* commonly known as grass hopper, mainly lives on green vegetation as *C. scutellaris* also lives on small insects, aphids and mites. The total protein content of all the insects correspond to the total cellulase activity present in the insects, some of it could correspond to the microbial cellulase activity present in the digestive track of the insects, as described by other workers (Watanabe and Tokuda, 2001). In *Dysdercus koenigii* and *Aulacophora foveicollis* (Fig. 1d,f), the colour of the dye changed to blue, which could be because of enzymatic conversion of glucose to gluconic acid.

Heterogeneity of carboxymethylcellulase activity

Total cellular protein from *A. hilaris* and *C. chrotogonus* separated on 10% PAGE gel and stained with Congo red in zymogram showed presence of two fractions in *A. hilaris,* and three protein bands active against cellulose in *C. chrotogonus* (Fig. 2 a,b). Heterogeneity has previously been reported for bacterial cellulases (Sami *et al.,* 1988; Langsford *et al.,* 1984). One of us has reported a comparison of multiple forms of insect cellulases with bacteria (Sami and Akhtar, 1989; Sami *et al.,* 2005). Heterogeneous forms of cellulases in insects could be firstly because of proteolysis of enzymatic protein by proteases produced either by the insect digestive tract or by the symbiotic microbes. Secondly, it could be because of glycosylation of the enzyme, as glycosylation is required for the activity of cellulases of beetle, *Apriona germari* (Wei *et al.*, 2005*).* Different levels of glycosylations may be responsible for appearance of more than one forms of cellulase activities. Thirdly, it could be because of gene duplication. However, another possibility of cellulases of microbial origin produced by the microbes residing in the digestive tract of insects cannot be ruled out unless further work is carried out to investigate the reason for the multiplicity of cellulases among insects.

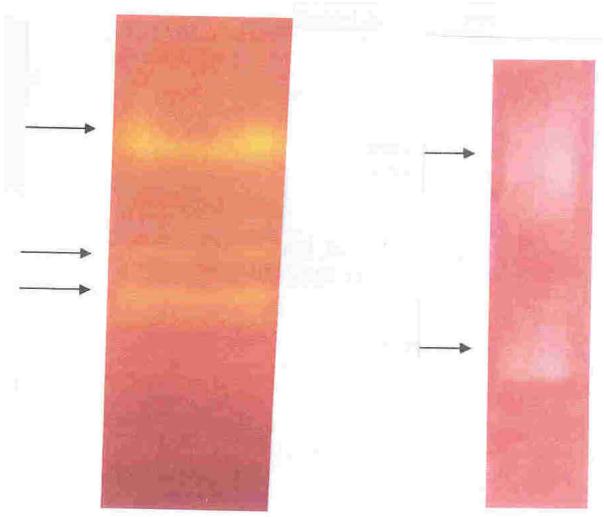

Fig. 2. Heterogeneous forms of endoglucanases appeared on zymogram after separation on 10% PAGE on substrate agar plate. Endo-β 1,4–glucanase activity of grass hopper, *Chrotogonus trachypterus trachypterus* (left), and pumpkin beetle, *Aulacophora hilarais* (right).

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