Enzyme Activities of G-6-Phosphate Dehydrogenase in Relation to Infection with *Bacillus thuringiensis* to the Larvae of the Wax Moth, *Galleria mellonella* L.

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ABSTRACT

Glucose-6-phosphate dehydrogenase activity in third larval instar of *Galleria mellonella* had been determined after their subjection to LC_{50} of *Bacillus thuringiensis kurstaki* (Dipel 2X). The electrophoretic patterns of the enzyme activity using PAGE, showed the presence of 19 peptides. Ten of these isozymes were found only in the treated larvae, *i.e.*, P1, P3, P4, P5, P6, P16, P18, P19 and P20 with molecular weights of 212, 197, 196, 195,194, 102, 97, 95, and 30 kda, respectively. Meanwhile, another 9 peptides were detected only in the control larvae, *i.e.*, P7, P8, P9, P10, P11, P12, P13, P15, and P21 showing molecular weights of 192, 184, 183, 179, 178, 117, 116, 104, and 21 kda, respectively. The P14 (107 kda) was found in both larval groups. The new peptides that appeared in the treated larvae are due to the action of delta endotoxin on the activity of the enzyme that was obvious in the dendrogram showing the similarity levels of the resulted peptides.

Key Words: *Bacillus thuringiensis kurstaki, Galleria mellonella,* G-6-phosphate dehydrogenase.

INTRODUCTION

G-6-phosphate dehydrogenase is an important enzyme found in all living organisms as described by Eichhorn and Corbus (1988). Its multiple isoenzymes and their activity patterns or levels are widely used as a physiological parameter for variations among different species, varieties, genetic alterations, and metabolic changes due to nutritional, physical or pathological factors. For example, the G-6-ph-dehydrogenase deficiency was related to pathogenic origins in humans as reported by Gili *et al.* (2000), Verle *et al.* (2000), and May *et al.*(2000); or to the race as in pigs (Liu, 1999), jumping horses (Almosny *et al.*, 2000) and in fish (Amcoff *et al.*, 2000). It is commonly used in agricultural crops in relation to many morphological and physiological characteristics (Reisch *et al.*, 1993; Nemoto and Sasakuma, 2000; Wedt *et al.*, 2000 and Esposito *et al.*, 2001).

In insects, the activity levels of different enzymes were mostly studied in normal (healthy) individuals, *e.g.*, estrases in larval haemolymph of *G. mellonellaI* L. (Semyanov *et al.*, 1981; Nemic and Zenka, 1996); the peroxidase (Shen and Qian, 1994a); the trypsin in the spurce budworm, *Choristoneura fumiferana* (Milne and Kaplan, 1993); the phosphatases in *G. mellonella* (Shen and Qian, 1994b); and the *Galleria* digestive enzymes amylase, proteinase and trypsin (Shen and Qian, 1995). Studying the available literature, G-6-ph-dehydrogenase as far was not studied before neither in healthy nor in the *B. thuringiensis* treated larvae of *G. mellonella.* Thus, the present study is dealing with this subject.

MATERIALS AND METHODS

Larval infection

G. mellonella was reared in the laboratory on the diet described by Ibrahim *et al.* (1984), but bee honey was excluded from diet prepared for present experiments because of its known antimicrobial effect (Omar, 2004). The LC_{50} of the *B. t. kurstaki* formulation Dipel 2X (4.784g/100g diet) was used for infecting the larvae *per os.* to allow obtaining about 50% survived diseased larvae among a period of at least 5 days post treatment (Omar, 2004). About 1500 larvae (L_3) were fed on the treated diet, while another 700 ones were fed on untreated diet to serve as control. Daily samples (each of 100 larvae) were collected from both treated and untreated larvae and processed for isozyme determination.

Enzyme activity of G-6-ph dehydrogenase

The activity patterns of glucose-6-phosphate-dehydrogenase were investigated by electrophpresis technique according to Shaw and Koen (1968). For extraction of the protein, a sample of 250 mg crushed larval body in the extraction enzyme buffer (tris-HCl, pH 6.8) was well ground in a mortar and pestle. The clear supernatant containing the soluble proteins was recovered after centrifugation at 12,000 rpm for 20 min. to remove cell debris (Khalil, 1981).

An amount of 30 µl from each sample was electrophorised using a constant current of 2 mA for 4 hrs until marker location was 0.5 cm before the end of gel.

Detection of isozyme resulted patterns were stained after Sammons *et al.* (1981) and Khalil (1981) in a solution composed of 0.1 M tris-HCl buffer (pH 8.8), 7.5ml Glucose–6-phosphate (disodium salt), 20.0mg NADP, 10.0mg MTT, 10.0mg PMS, and 0.2M MgI_2. The gel was incubated in a staining solution in the dark at 37°C until dark blue band appear. Developed gel was washed in water and fixed in 50% ethanol.

Densitometric scanning of the dried gel was done through a computerized program. The scanning included all major and minor bands in each isozyme. From values for rate of flow (RF)

which were obtained by densitometer scanning (Gottlieb and Hepden, 1966), the highest and the lowest RF values were determined (from 0.01–0.99) and presence of a band at a particular Rf was designated as (+), and its absence at the same Rf was designated as (-). Data were subjected to cluster analysis.

Cluster analysis (Dendrogram)

Grouping of the daily detected peptides by SDS-PAGE in sampled untreated (control) and *B.t. kurstaki* treated larvae of *G. mellonella,* as well as a sample of the microbe vegetative cells were computerized using the 1-D Advanced–[Dendrogram Window Program]. They were clustered by the average linked technique (unweighed pair-group method) (Joseph *et al.*, 1992) to study the similarity levels (degrees %) among the resulted peptides. The results were expressed as Phenogram. In this analysis, clustering began with fusion of the two most similar fractions and proceeded until all fractions were used. The clustering process was presented in the form of a Phenogram (tree or dendrogram) in which the top branch indicated the highest fusion level, and so on for reference purposes, the fusion levels were designated 1, 2, from top to bottom, respectively.

RESULTS AND DISCUSSION

Effect of sub lethal concentration (LC_{50}) of *B.t. kurstaki* on activities of the enzyme G-6-phosphate dehydrogenase was studied in larvae of *G. mellonella* based on the isoenzyme activity patterns fractionated and detected by PAGE (Fig. 1) for their presence in treated and untreated larvae (Table 1). Results revealed 6 peptide fractions (P1-P6) of low mobility (194-212kda) appeared only in the treated larvae at different times post treatment. P1 (212kda) appeared on the 4th day, P2 (204 kda) on the 1st day, P3 (197kda) on the 6th day, P4 (196 kda) on the 3rd day, P5 (195kda) on the 2nd day, and P6 (194kda) on the 6th day post treatment.

Other13 peptides of medium mobility (95-192kda) were detected, from which 4 peptides were found only in the treated larvae, *i.e.,* P16 (102kda) on the 5th day; P18 (97kda) on the 6th day; and P19 (95kda) on 1st day post treatment. Besides, a fraction with fast mobility, *i.e.*, P20 (30kda) was detected only in the treated larvae on the 2nd day post treatment. On the other hand, another fast mobile peptide (P21) of 21 kda was detected only in the control larvae on the 1st and 2nd day of the test. Accordingly, and in comparison with results of the control larvae, 10 new isoenzyme patterns were detected in larvae of *G. mellonella* fed on diet mixed with spore-endotoxin material of *B .t. kurstaki.*

With the exception of the peptide P14 (107kda) which appeared in treated larvae on the 3rd and 4th days and in the control larvae on the 5th day of the test, 9 fractions were detected only in the control larvae including 8 of medium mobility and one with fast mobility that appeared at different developmental times. Peptide No. 7 (192kda) appeared with P14 on the 5th day. Meanwhile, P11 (178kda) appeared in association of the fast mobility P21 (21kda) on the 1st day. P8 (184kda) appeared twice, on the 2nd day with P21, and on the 3rd day with P12 (117kda). On the 4th day, P9 (183kda) and P13 (116kda) were detected, followed by P7 (192kda) and P14 on the 5th day as mentioned previously, and followed by P10 (169kda) and p15 (104kda) on the 6th and last day of the test.

Hence the isoenzyme patterns of G-6-phosphate dehydrogenase in vegetative cells of *B. t. kurstaki* showed only three peptides of 26, 232 and 285kda that were not detected in *B. t.* diseased and healthy larvae of *G. mellonella* (Table 1), the present results revealed 10 specific patterns of peptides with molecular masses of different mobility for the treated and 9 for the untreated larvae. Treated larvae showed three pattern groups of low (P1-P6), medium (P14, P16, P18, and P19), and fast mobility (P20), while larvae of the control showed two pattern groups of medium (P7-P15) and fast mobility (P21), as extracted from Table (1).

It is well known that glucose-6-phosphate dehydrogenase is an oxidative enzyme since it converts the glucose-6-phosphste acid in the presence of coenzyme II resulting in energy that needed for the organism. The dimorphic pattern of isozymes detected in the untreated larvae (P_7-P_{15}) and P_{21} suggest that the pathogen (*B.t.*) converted the G-6-ph dehydrogenase isoenzyme patterns to the trimorphic shape in the diseased *G. mellonella* larvae (P_1-P_6, P_{14}, P_{16}, and P_{18}-P_{20}). The formation of the new G-6-ph dehydrogenase isoenzymes especially those of low mobility (P_1-P_6) may be due to the action of delta endotoxins on the enzyme in the treated larvae. This action could be seen by clustering more sub enzyme units to form these low mobile enzymes. Anyhow, this point needs further studies for kinetic and activities of these isoenzymes.

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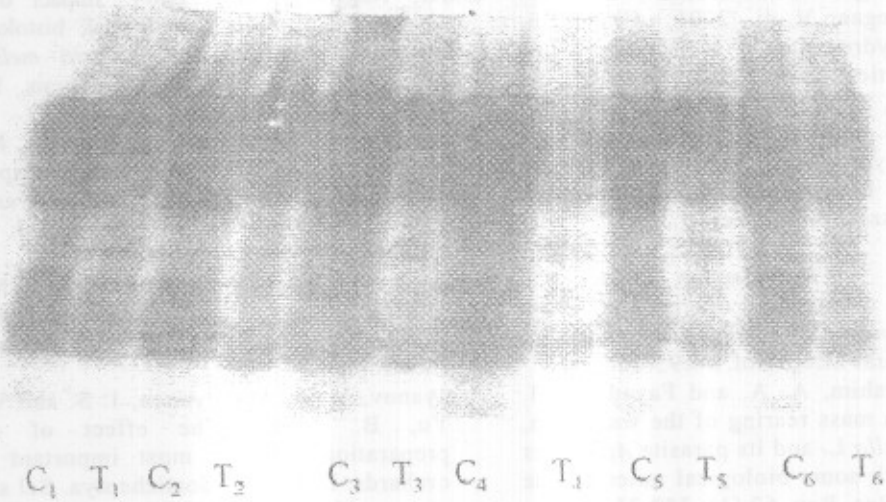

Fig. (1): PAGE for activities of G-6-ph dehydrogenase in control (C) and treated larvae (T) of *G. mellonella*.
S=standard proteins; C_1-C_6=daily sampled control larvae; T_1-T_2=daily sampled treated larvae.

Table (1): Presence (+) or absence (-) of isoenzyme peptide patterns (P) for G-6-phphosphate dehydrogenase in *B.t.* var. *kurstaki* treated (T) and untreated (C) larvae of the greater wax moth, *G. mellonella* detected by PAGE.

Peptide Code	kda	Days after treatment											
		1		2		3		4		5		6	
		T	C	T	C	T	C	T	C	T	C	T	C
P1	212	-	-	-	-	-	-	+	-	-	-	-	-
P2	204	+	-	-	-	-	-	-	-	-	-	-	-
P3	197	-	-	-	-	-	-	-	-	-	-	+	-
P4	196	-	-	-	-	+	-	-	-	-	-	-	-
P5	195	-	-	+	-	-	-	-	-	-	-	-	-
P6	194	-	-	-	-	-	-	-	-	+	-	-	-
P7	192	-	-	-	-	-	-	-	-	-	+	-	-
P8	184	-	-	-	+	-	+	-	-	-	-	-	-
P9	183	-	-	-	-	-	-	-	+	-	-	-	-
P10	179	-	-	-	-	-	-	-	-	-	-	-	+
P11	178	-	+	-	-	-	-	-	-	-	-	-	-
P12	117	-	-	-	-	-	+	-	-	-	-	-	-
P13	116	-	-	-	-	-	-	-	+	-	-	-	-
P14	107	-	-	-	-	+	-	+	-	-	+	-	-
P15	104	-	-	-	-	-	-	-	-	-	-	-	+
P16	102	-	-	-	-	-	-	-	-	+	-	-	-
P17	100	-	-	-	-	-	-	-	-	-	-	-	-
P18	97	-	-	-	-	-	-	-	-	-	-	+	-
P19	95	+	-	-	-	-	-	-	-	-	-	-	-
P20	30	-	-	+	-	-	-	-	-	-	-	-	-
P21	21	-	+	-	+	-	-	-	-	-	-	-	-

Note: *B. t.* isoenzyme patterns: 285, 232 and 26kda.

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