IDENTIFICATION AND CHARACTERIZATION OF ALPHA-AMYLASE IN THE ROSE APHID, *MACROSIPHUM ROSAE* (LINNAEUS, 1758) (HEMIPTERA: APHIDIDAE)

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[Darvishzadeh, A. & Bandani, A. R. 2012. Identification and characterization of alpha-amylase in the rose aphid, *Macrosiphum rosae* (Linnaeus, 1758) (Hemiptera: Aphididae). Munis Entomology & Zoology, 7 (2): 1089-1096**]**

ABSTRACT: Rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae), is a cosmopolitan pest that can cause severe damage to various members of the Rosaceae family. This pest have various hosts during the year, however, it is most commonly found feeding on roses (Mound & Teulon, 1995).The present study for the first time showed that α-amylase is present in the *Macrosiphum rosae* L. or rose aphid (Hemiptera: Aphididae). α-Amylases have been found to be active in different insect species (Takagi et al., 1971; Baker, 1991; Kazzazi et al., 2005; Zibaee et al., 2008). Rose aphid α-amylase activity pH was determined to be around 7. Generally, optimal pH is corresponding to the pH prevailing in the midgut from which the enzyme has been extracted so these discrepancies seen in midgut pH are related to the different feeding habits and feeding sources. The optimal temperature for Rose aphid α-amylase activity was 45°C, the enzyme was active over a broad temperature range from 20 to 60°C. Analysis of enzyme homogenates by electrophoresis indicated a band in polyacrylamide gel.

KEY WORDS: α-Amylases, Rose aphid, gut, *Macrosiphum rosae* L.

Aphids are considered serious agricultural and horticultural pests (Hill, 1997). They cause severe damage to crops by their feeding. They also transmit various plant viruses that are pathogenic to their hosts (Schepers, 1987). In addition, honeydew excreted by the aphids attracts saprophytic fungi which cover the leaves leading to reduction of photosynthetic capacity of the host plant (Schepers, 1987).

Rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae), is a cosmopolitan pest that can cause severe damage to various members of the Rosaceae (Hill, 1997). This pest chooses various plant species as host. However, it is most commonly found feeding on roses (Mound & Teulon, 1995). It is a significant pest of rose plants and can become very abundant on roses thus reducing their decorative value. In the case of a high infestation, the aphid causes significant damage, e.g. bent stems, weak foliage and early leaf fall. The most significant damage is to the inflorescences, especially at bud burst (Jaskiewicz, 1997). *M. rosae* feeds mainly on the young leaves and developing flower buds. The honeydew excreted by the aphid promotes the growth of sooty mould on flowers and surfaces of leaves. All of these factors together cause significant economic damage to rose crops by decreasing their beauty and the value of cut flowers (Alford, 1991). This aphid is a heteroecious holocyclic species in temperate regions but may be completely anholocyclic on roses in warmer climates (Blackman & Eastop, 2000).

Amylases are main insect enzymes that digest starch and glycogen present in the hosts. In insects α-amylases are synthesized and secreted by midgut epithelial cells, along with other digestive enzymes (Baker, 1983; Terra & Ferreira, 2005).

Amylases have been investigated and found to be active in Coleoptera, Hymenoptera, Diptera, Lepidoptera and Heteroptera by several researchers (Hori, 1970, 1972; Buonocore et al., 1976; Kanekatsu, 1978; Terra & Ferreira, 1983; Baker & Woo, 1985; Colepicolo-Neto et al., 1986; Santos & Terra, 1986; Baker, 1987, 1989, 1991; Terra et al., 1988; Ferreira & Terra, 1989; Schumaker et al., 1993; Ferreira et al., 1994). The aim of this study was extraction and characterization of α-amylases from rose aphid to reach a better understanding of the digestive physiology and to find a winning procedure to use amylase specific inhibitors for control of this pest.

Aphids have specialized mouthparts (piercing and sucking stylets), extracting plant sap from vascular tissue. Plant sap is usually rich in sugar and deficient in nitrogen (Douglas, 2006; Miles, 1987). Thus, the aphids to meet their requirements, they need to obtain a lot of plant sap and extract their needs and the remaining are excreted as honeydew. As a result heavy yield loss can occur especially when infestation is high. Damage to plants occurs as a result of direct feeding and excretion of its honeydew which is rich in hydrocarbons and free amino acids, resulting in pathogenic and saprophytic fungal growth (Klingauf, 1987). Aphids make use of symbiotic bacteria such as *Buchnera aphidicola* thus exploiting them to synthesise amino acids lacking in their diet (Gunduz & Douglas, 2009). There is an old view that aphids as plant sap feeders do not carry out digestion of ingested materials which this view has been questioned because of existence of both biochemistry and molecular biology evidence suggesting these insect also use hydrolytic enzyme to digest ingested materials (Foissac et al., 2002; Pyati et al., 2011). Also, there are reports that phloem sap in addition to free amino acids contains a verity of polymers including lectins, proteins, and petides (Kehr, 2006).

An understanding of biochemistry and physiology of gut digestion is essential when developing methods of insect pest control using enzyme inhibitors. Thus, the aim of the current study was to extract α-amylase from digestive system of rose aphid, *M. rosae*, and determine its characterization using specific substrate for the enzyme. The knowledge thus achieved will lead to better understanding of digestive physiology of the aphid species which could be used to devise new management strategies for its control.

MATERIALS AND METHODS

Insect

Adult aphids were collected from the rose plant and transferred to the laboratory.

Sample preparation and enzyme assays

For sample preparation 20 adult aphids were homogenized in distilled water. The samples were put in the 1.5 ml tubes and centrifuged at 15000 rpm for 15 min at 4 °C. Supernatant was separated from pellet and kept at -20 °C as an enzyme source for subsequent analysis.

α-Amylase assay

α-Amylase activity was assayed by the dinitrosalicylic acid (DNS) procedure (Bernfeld, 1955), using 1 % soluble starch (Merck, Darmstadt, Germany) as the substrate (Bandani et al., 2009). Assay mixture composed of ten microliter of the enzyme, 10 microliter substrate (soluble starch) and 80 microliter universal buffer (0.02 M) containing succinate, glycine and 2-morpholinoethanesulfonic acid (pH 7). Then the mixture was incubated at 35 °C for 30 min. After addition of

100 microliter DNS and heating in the boiling water for 10 min, absorbance was read at 540 nm. As a blank, instead of enzyme, distilled water was used. All assays were repeated at least three times.

Effect of PH and temperature on enzyme activity
The effects of temperature and pH on α-amylase activity were examined. Optimal pH was determined using universal buffer with pH set at 3, 4, 5, 6, 7, 8, 9 and 10. The effect of temperature on α-amylase activity was determined by incubating the reaction mixture at 20, 30, 35, 40, 45, 50, 60 and 70 °C for 30 min followed by measurement of activity as described by Kazzazi et al. (2005).

Electrophoresis
Native SDS- polyacrylamide gel electrophoresis (PAGE) was carried out as described by Laemmli (1970) and Campos et al. (1989). SDS–PAGE was performed in a 10 % (w/v) separating gel and a 5 % stacking gel, both with 0.05 % SDS. The electrode buffer was prepared based on the method of Laemmli (1970), but SDS was not used. The sample buffer contained 25 % stacking buffer (0.5 mol/ L Tris–HCl [pH 6.8]), 20 % glycerol, 2 % SDS, 0.005 % (w/v) bromophenol blue, but no mercaptoethanol, and it was not heated. Electrophoresis was conducted at room temperature at 120 V until the blue dye reached the bottom of the slab gel. To prepare gels for α–amylase assay, the gel was rinsed with water and washed by shaking gently with 1 % (v/v) Triton X-100 in phosphate buffer [pH 7] containing 2 mM CaCl2 and 10 mM NaCl for 1.5 h. Staining of the gel was done with 0.05% KI and 0.05% I2 solution. The visualized α-amylase activity was seen as light bands in dark background.

Protein determination
Protein concentration was measured based on the method of Bradford (1976), with bovine serum albumin (Bio-Rad, München, Germany), as a standard.

RESULTS

α-amylase activity
In the current study using starch as a substrate, it was found that α-amylase activity was present in the gut of the rose aphid. The activity of enzyme was 1.59 U (unit activity/ml) (Fig. 1).

Effect of pH and temperature on enzyme activity
The α-amylases activity in rose aphid showed an optimal pH of 7.0 (Fig. 2). The enzyme activity increased from pH 4.0 to 7.0, and then decreased with increasing pH. Thus, as can be seen (Fig. 2) the enzyme was active over a wide range of pH from pH 4.0 to pH 8.0. The enzyme activity in pHs 7.0 and 8.0 was not different. Sharp decrease in the enzyme activity was seen after pH 8.0. The enzyme activity between pHs 6.0 and 7.0 or 8.0 was different. Also, there were not significant differences in the enzyme activity at pHs 4.0, 5.0, and 6.0. Amylase was active over a broad range of temperatures from 30°C to 50°C. The optimal temperature for α-amylase activity was 45°C (Fig. 3). However, there were not differences between the enzyme activity at 35, 40 and 45°C.

Electrophoresis
Native SDS- polyacrylamide gel electrophoresis (PAGE) has shown a clear α-amylase band in the gel when using starch as substrate (Fig. 4).

DISCUSSION

The present study for the first time showed that α-amylase is present in the rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae) showing that the insect needs this enzyme in the some stage of its life. It has been long established that aphids because of the feeding habit does not need enzyme in their gut for the digestion of the food. However, current study showed that α-amylase activity present in the aphid.

Regarding protein digestion it has been reported that aphids use proteinases especially of cysteine proteinase for protein digestion. Cystein proteinase (Cathepsin L) is the major protease in the gut of *Aphis gossypii*, *Acyrthosiphon pisum* and *Sitobion avenae* (Cristofoletti et al., 2003; Deraison et al., 2004; Pyati et al., 2011). In addition to Cathepsin L, Cathepsin B has been reported to be present in the aphids gut (Rispe et al., 2007). However, regarding carbohydrate digestion in the aphid gut no studies have been undertaken to show that this enzyme is present in the gut aphids, so far.

Further characterization of the insect α-amylase was done by determination of the pH and temperature effects on the enzyme activity. It has been reported that optimal pH correspond to the pH prevailing in the midgut of the insect which amylase has been isolated (Terra et al., 1996). High pH gut has been reported for the Lepidoptera thus optimal pH for the α-amylase activity in these insects has been shown to be high (> 7.0).There are reports that pH values of 9.0 for Chilo suppressalis (Lepidoptera: Crambidae), 9.2 for A. mylitta, 12.0 for Acherontia atropos (Lepidoptera: Sphingidae), 10.8 for Lasiocampa quercus (Lepidoptera: Lasiocampidae), 11.3 for Manduca sexta (Lepidoptera: Sphingidae) and 10.8 Lichnoptera felina (Lepidoptera: Noctuidae) (Dow 1984; Zibaee et al,2009). It has been reported that high values of gut pH in insects is adaptation to feed on plant materials rich in tannins (Chapman 1998), which at lower pH values bind to proteins thus decreasing the digestion efficiency (Dow 1986). In the current study it was found that α-amylase was active at pH 7.0. So these discrepancies in the enzyme activity among different insect species could be attributed to the different feeding habits and feeding sources.

Optimum temperature for α-amylase activity was shown to be at 45°C which can be attributed to the environment that the insect live and feed. Gel electrophoresis showed that only one enzyme was present in the insect gut. However, a mixture of the α-amylase isoenzyme has been reported to exist in some insects. A mixture of different α-amylase isoenzymes has been reported for other insects such as *Sitophilus oryzae*, *Tribolium castaneum*, *Anthonomus grandis*, *C. maculates*, *R. dominica*, *S. granarium*, *E. intergriceps* (Terra et al, 1977; Chen et al, 1992; Oliveira-Neto et al, 2003; Kazzazi et al, 2005; Mehrabadi et al, 2011). Presence of different α-amylase isoenzymes could be related to importance of this enzyme in the insect food digestion.

In conclusion it should be mentioned that although the rose aphid feed on plant sap, it needs α-amylase for the digestion of some carbohydrate the insect encounter during feeding. However, because the insect does not rely on carbohydrate digestion for the growth and development it does not have a mixture of isoenzymes.

ACKNOWLEDGEMENTS

This project was founded by University of Tehran.

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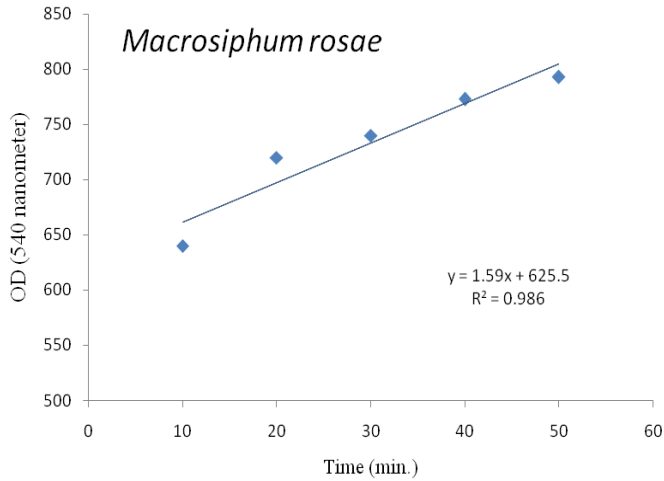

Figure 1. α-Amylase activity of *M. rosae*.

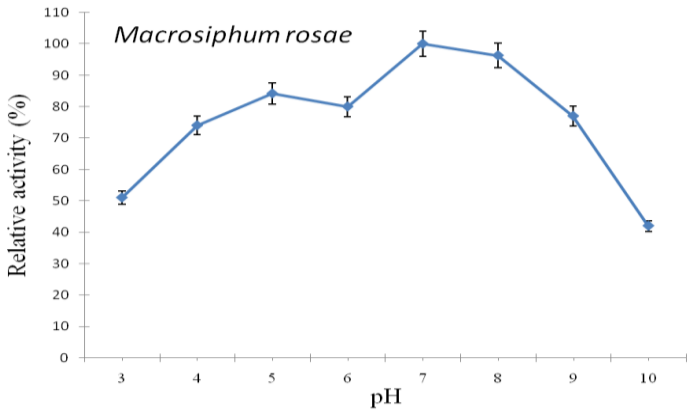

Figure 2. The effect of pH on α-Amylase activity of *M. rosae*.

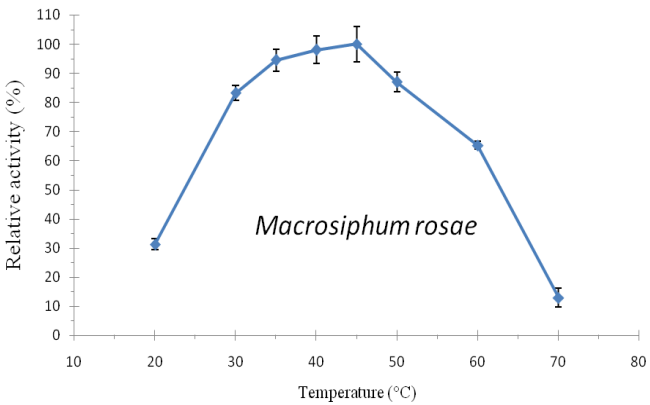

Figure 3. The effect of Temperature on the activity of *M. rosae* α-amylase activity.

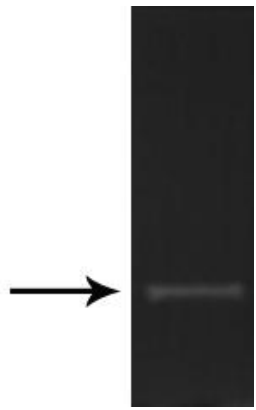

Figure 4. Native gel electrophoresis showing α-amylase activity.