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Characterization of alkaline phosphatase activity in genotype of whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae)

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Abstract
Whitefly, *Bemisia tabaci* (G) causes severe damage to crops directly by sucking and indirectly as vector of several plant viral diseases. It was hypothesized that physiological superiority of B-biotype in *B. tabaci* could partly be attributed to its improved ability to utilize the alkaline phosphatase pathway. Alkaline phosphatases (ALP) are considered as key metabolically active enzyme ubiquitously present in diverse group of insects and are functionally implicated in several physiological processes. The present study we have characterize the alkaline phosphatase (ALP) in Asia I and Asia II-1, the two predominent genotype of *B. tabaci* collected from different agroclimatic zone of India. The study was conducted in the year 2013-2015 in controled environment of insect proof climatic control chamber, Division of entomology-IARI, New Delhi. From the result it was found that at pH-7, Asia II-1 has shown significantly higher specific activity (0.99±0.02 nmol/µl/µg), as compare to Asia I, 0.93±0.02nmol/µl/µg which has implicated with higher Vmax- i.e. 1.338±0.141 and Km- 0.394±0.1247 nmol whereas Asia I have shown lower Vmax- 1.021±0.044 and Km- 0.1826±0.036. In this experiment we also tested the effect of temprature and pH on enzyme activity in both the genotype at optimum substrate consentration of 0.75 mM.

Keywords: alkaline phosphatase (ALP), Asia I, Asia-II, genotype, enzyme kinetics

1. Introduction
Whitefly, *B. tabaci* believed to be originated from Asia has drawn global attention in recent past with its wide distribution across the world, owing to its polyphagous pest status [20]. It has broad host range of over 700 different plant species with an ability to transmit more than 200 viruses specifically those belonging to the genus *Begomovirus*, leading to severe economic loss [12, 22]. *B. tabaci* is regarded as a global invasive species with the emergence of B biotype and its rapid spread across the continents. The genetic complexity of *B. tabaci* was first recognized during 1950s, which has led to the development of biotype concept with the description of a series of biotypes [4]. It is well known that there is a genetic structure which breaks up *B. tabaci* into a series of well-defined subgroups [5]. Our present understanding of *B. tabaci* is that, it is a species complex with over 36 morphologically indistinguishable genetic groups [11]. The largest diversity of *B. tabaci* genetic groups is present in Asia with the distribution of about 16 out of 36 genetic groups reported so far in Asian countries and nine from Indian condition [1].
Alkaline phosphatase (ALP) is a ubiquitous metabolic enzyme found in bacteria, insects and mammals. This enzyme hydrolyses the phosphates group from nucleotides and proteins and maintains ionic balance [9, 10]. ALP enzymes are distributed in the tissues of head, salivary gland, intestine, malpighian tubules, cuticle, fat bodies, and reproductive organs of the insects and they play an important role in several physiological processes like conduction of impulses, synthesis of hormones, metabolism, diapauses and caste differentiation in social insects [3]. Two silkworm gut isozymes, a membrane-bound form and a soluble form were associated with the transport of glucose and fatty acids across gut membranes [21]. A membrane bound - ALP was associated with the digestion and absorption of nutrients, while soluble form was reported to involve in the regulation of ionic balance [9, 10].
ALP was the first enzyme to be characterized from the salivary glands of whitefly [25]. It has been identified from several others species of insects namely silkworm, Fruit fly, Mosquitoes

and grasshopper [14, 23, 10]. Comparison of ALP activities in two whitefly species *B. tabaci* and *Trialeorodes vaporariorum* revealed that B-biotype of *B. tabaci* had higher enzyme activity than *T. vaporariorum*. The kinetic of ALP from B biotype indicated that the higher metabolic capability by the ALP provided better host plant adaptation and as such improved competitiveness to utilize a wider host plant range to the B biotype in comparision to *T. vaporarium* [25]. From this studies we also tried to find out the level of substrate utilization capacity by ALP in both the Asian genotype *i.e* Asia I and Asia II-1 which may serve the purpose to support the previous study to correlate the potential competitiveness to utilize the host as well as other physiological superiority within the species and among genotype based on the substrate utilization capacity and affinity towards the substrate. Further studies may throw light in the involvement of the ALP in physiological process and metabolic pathways in insects which can provide novel strategies for pest management.

2. Materials and Methods

The present investigation was carried out under controlled conditions of Insect Proof Climate Control Chamber (IPCC) at temperature 25±2 °C and relative Humidity 60±5% in the Division of Entomology, Indian Agricultural Research Institute, New Delhi, during the years 2013-2015.

2.1 Collection and Rearing of Whitefly Population

The *B. tabaci* genotype evaluated in the study were originally collected from cotton fields of Guntur (Andhra Pradesh, India: 16.3008° N, 80.4428° E) and from and New Delhi, India (28° 38' 4.790" N, 77° 10' 1.590" E). Whitefly cultures were maintained in IPCC, Division of Entomology, IARI, New Delhi, India at favorable temperature 25±2 °C and relative Humidity 60±5% on cotton plant (variety- Sujatha).

2.2 Development of isofemale Lines of *B. tabaci* Population

The purity of genotype was maintained by developing population from a single isofemale line by using clip cages. One adult pair of male and female whitefly was transferred into a clip cages soon after emergence and allowed to proliferate for further generation. Population generated from single clip case is multiplied and used for further experiment.

2.3 Authentication of Whitefly Genotype

Species authentication of *B. tabaci* was done by using distinct taxonomic characters (Martin, 2007) and genetic group status of *B. tabaci* populations was ascertained by partial sequencing of *Mitocondrial cytochrome oxidase 1 (mtCO1) gene* [8]. From the result it was revealed that population collected from Guntur and Delhi belongs to Asia I and Asia II-1 genotype respectively. Further homogeneous populations of females from both the genotype were used for enzyme assay.

2.4 Biochemical Characterization of Alkaline Phosphatase (ALP)

Alkaline phosphatase activity was estimated by microplate assay technique, using 100mM TAPS (pH-7.8) and 0.75 mM of *para*-Nitrophenyl Phosphate (pNPP) as substrate [24]. Ten adult female are drawn from Asia I and Asia II-1 populations and homogenized using a hand held homogenizer (Pellet pestle motor, Sigma-Aldrich) in 50µL of ice cold 10mM sodium acetate-magnesium acetate buffer solution (pH-7) and makeup volume up to 100 µL. The homogenates were centrifuged at 10000g and 4 ºC for 15 min and supernatant was utilized for enzyme assay. ALP activity was measured by adding 20 µl of enzyme source to well of micro titer plate containing 200µl of substrate buffer and allowed to incubate at 37 ºC for 30 min. The reaction was stopped by keeping the plate on ice bath for 5 min and absorbance was measured at 405 nm in Microplate reader (Molecular Device). The enzyme unit (U/mg protein) was defined as the production of 1 µmol of p-nitrophenol by a reaction between the substrate and enzyme per min at 37 °C. A linear standard curve of *Para*-nitrophenol was prepared using different concentrations in 10mM sodium acetate-magnesium acetate solution (pH-7). The total protein content of all the enzyme sources was determined by the *coomassie* brilliant blue method using BSA as standard [2]. All reaction was taken as in three replicate.

2.5 Determination of Vmax and Km for ALP

To determine the kinetics of alkaline phosphatase, five different concentrations (1.75, 1, 0.75, 0.5, 0.25 mMol) of substrate (*para*-nitrophenylphosphate) was prepared in 100mM TAPS (pH-7.8) buffer. The observed parameters of enzyme kinetics viz., Vmax and Km were calculated by using non-linear regression analysis in GraphPad Prism 6.0 programme.

2.6 Effect of Temperature on ALP Activity

The effect of temperature on ALP activity was estimated by incubating the reaction mixture at range of temperature varies from 15 ºC, 20 ºC, 25 ºC, 30 ºC, 35 ºC, 45 ºC for 30 min

2.7 Effect of pH on ALP Activity

To see the effect of pH on enzyme activity, buffer solution of different pH concentration *i.e.* 3, 4, 5, 6, 7, 8, and 9 was prepared and enzyme activity was measured at respective pH concentration.

2.8 Statistical Analysis

ALP activity in both the genotype were analyzed by Students *t*-test and tukey test. Km and Vmax was determined through non-linear regression by using software GraphPad Prism 6.0 programme. Data were analyzed with SAS (Statistical analysis software) through GLM (Generalized linear model) procedure.

3 Results

3.1 Biochemical Characterization of Alkaline Phosphatase (ALP)

Biochemical characterization of alkaline phosphatase was done by measuring alkaline phosphatase activity at optimum substrate, temperature and pH concentration. Enzyme activity was significantly higher at optimum condition for both the genotype as compare to suboptimal condition.

From the study it was found that at 0.75mMol substrate, pH-7 and temperature 25 ºC, both the genotype has shown highest ALP activity, so this parameter were considered as optimal requirement for ALP activity in *B. tabaci*.

3.2 Determination of Vmax and Km for ALP

During **s**tudy with five different substrate concentrations (1.75, 1, 0.75, 0.5, 0.25 mMol), it was found that at 0.75 mMol concentration, the rate of reaction for ALP activity was high for both the genetic groups. At this substrate concentration, Vmax of Asia II -1 was found higher *i.e* 1.338±0.141 as compare to Asia I 1.021±0.044. The measured Km of Asian genetic group was obtained 0.394±0.1247 nmol and 0.1826±0.036 nmol for Asia II -1 and Asia I respectively (Fig- 1 and 2).

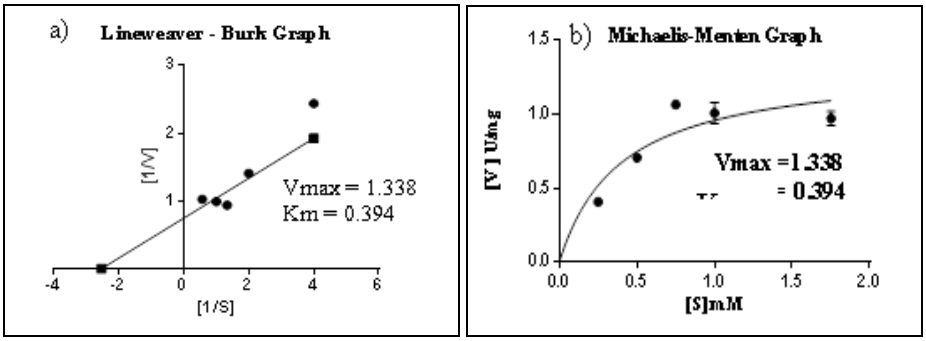

Fig 1: Representation of ALP enzyme kinetics through Lineweaver- Burk (a) and Michaelish-Menton (b) graph for Asia II-1 genotype.

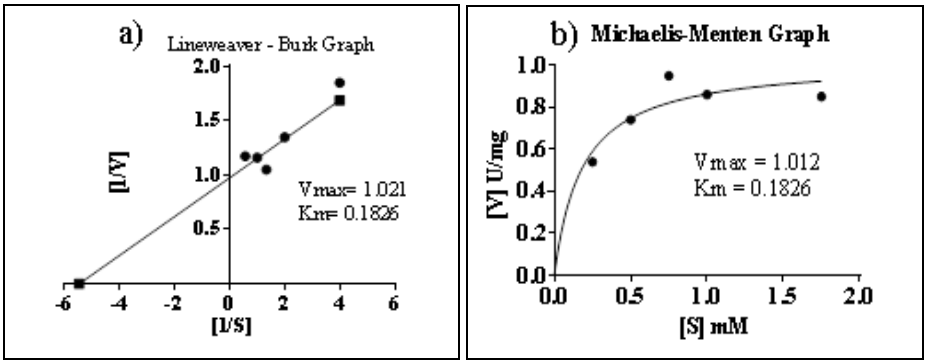

Fig 2: Representation of ALP enzyme kinetics through Lineweaver- Burk (a) and Michaelish-Menton (b) graph for Asia Igenotype

3.3 Effect of pH on ALP Activity

To determine the optimum pH, buffers of different pH concentration ranging from 3 to 9 were used and it was observed that ALP activity was increasing from 3 -7 pH and after that there was a marked decrease in activity. The highest enzyme activity and specific activity were recorded at pH 7 in both the genetic groups. At this pH, Asia II-1 has shown significantly higher specific activity (0.99±0.02 nmol/µl/µg), as compare to Asia I, 0.93±0.02nmol/µl/µg (Table -1) where as the enzyme activity were found 0.45±0.007nmol/µl/min and 0.267±0.007 nmol/µl/min respectively for Asia II-1 and Asia I genotype (Fig-3).

Table 1: Specific activity of ALP at different pH

Treatment	Specific activity nmol/µl/mg	
	Guntur (Asia I)	Delhi (Asia II 1)
pH	Mean±SE	Mean±SE
3	$0.38±0.02_{E}$	$0.34±0.02_{E}$
4	$0.47±0.02_{D}$	$0.46±0.02_{DE}$
5	$0.59±0.02_{D}$	$0.75±0.02_{DC}$
6	$0.70±0.02_{BC}$	$0.84±0.02_{BC}$
7	$0.93±0.02_{B}$	$0.99±0.02_{A}$
8	$0.78±0.02_{AB}$	$0.72±0.02_{AB}$
9	$0.70±0.02_{AB}$	$0.71±0.02_{BC}$
Treatment mean	$0.654±0.009_{B}$	$0.683±0.009_{A}$

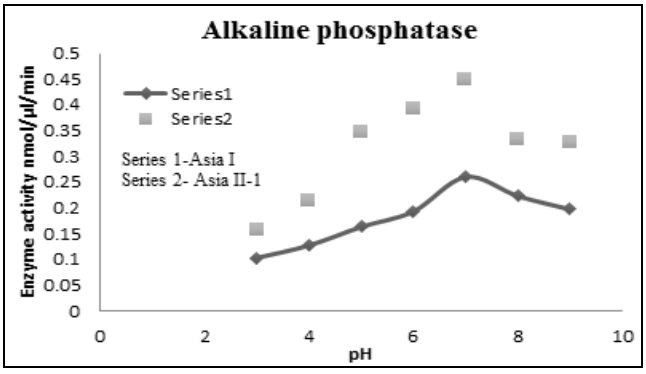

Fig 3: Enzyme activity of ALP at different pH

3.4 Effect of temperature on ALP activity

Estimation of ALP activity at different temperatures ranging from 15 to 45 ºC revealed that the maximum enzyme activity was recorded at 25ºC for both the genotype. At this optimum temperature, Asia II-1 has shown the highest enzyme activity, 0.393±0.010 nmol/µl/min in comparison to that of Asia 1 (0.267±0.010 nmol/µl/min) (Fig-4) whereas at 25ºC recorded specific activity were 0.97±0.02 nmol/µl/mg and 0.84±0.02 nmol/µl/mg for Asia I and Asia II-1 respectively. (Table- 2)

Table 2: Specific activity of ALP at different temperature

Treatment	Specific activity nmol/µl/mg	
	Guntur (Asia I)	Delhi (Asia II 1)
Temprature	Mean±SE	Mean±SE
15	$0.66±0.02_{E}$	$0.66±0.02_{E}$
20	$0.79±0.02_{ABC}$	$0.68±0.02_{DE}$
25	$0.97±0.02_{A}$	$0.84±0.02_{ABC}$
30	$0.91±0.02_{AB}$	$0.77±0.02_{BCD}$
35	$0.85±0.02_{ABC}$	$0.72±0.02_{CDE}$
45	$0.82±0.02_{BCD}$	$0.66±0.02_{E}$
Population mean±SE*	$0.228±0.004_{B}$	$0.337±0.004_{A}$

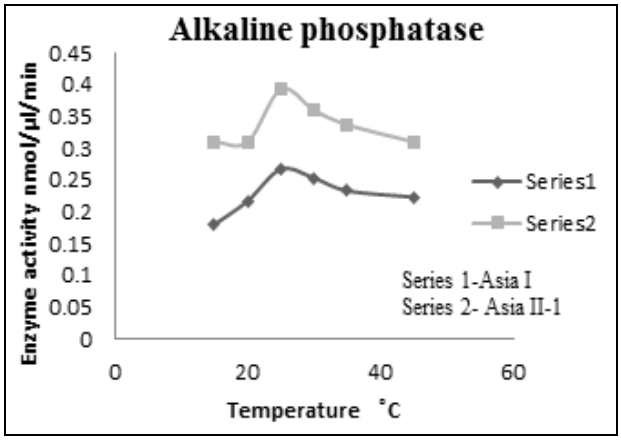

Fig 4: Enzyme activity of ALP at different temperature

4. Discussion

The functional role of orthologous mammal ALP gives indication that insects ALP may also be involved in sucrose metabolism [7, 17, 23]. Considering the role of ALP in sucrose metabolism and metabolic capability of substrate utilization may facilitate the insect to have wider host adaptation [3, 7, 17]. It was observed in an earlier study that swift in host adaptation of B biotype of *B. tabaci* could be attributed to its better utilization of the ALP [24]. Along with variation in substrate utilization and tissue localizations, a property of ALP also varies with insect to insect and within the insect species [14, 9, 15]. Understanding the metabolic capabilities *vis-a-vis* enzyme kinetics will help to resolve the physical fitness traits of insects with in the *B. tabaci* genotype. The kinetics of ALP from B biotype indicated that the efficient metabolic capability of alkaline phosphatase enzyme provides a better host plant adaptation and improved competitiveness to utilize a wider host range as compare to *T. Vaporarium* [25].

The kinetics of ALP in this study has shown that Asia II-1 was found to have significantly higher rate of reaction with the Vmax of 1.338±0.141 as compared to Asia I where Vmax is found to be 1.021±0.044. The differential ALP activities recorded in Asia I and Asia II-1 populations of *B. tabaci* in this study is supported by similar studies showing significant differences in ALP activity in developmental stages of two whitefly species, *B. tabaci* and *T. vaporariorum* [24].

However, Asia I was found to have higher affinity for substrate, as exemplified by lesser Km value of 0.1826±.036 nmol as compared to the Km of 0.394±0.1247 nmol in Asia II-1. The higher affinity for substrate for ALP in Asia I indicates that it may possibly confer physiological superiority in utilizing the enzyme for metabolism as like B. biotype as compare to *T. Vaporarium* shown in previous study [25]. Recent studies on survey of *B. tabaci* populations across the country have shown that Asia I is the most widely distributed genetic group in India [11] and it is also reported to be a dominant genetic group of *B. tabaci* across the Asian countries with broad host range [16]. However, further studies are required to confirm the correlation of ALP activity with the sucrose metabolism in *B. tabaci* and its increased activity with the host plant utilization pattern in Asia I genetic group.

5. Conclusions

It may be concluded that Asia I genotypes is more efficient than Asia II-1 due to its improved substrate affinity for alkaline phosphatases. That may be reason which might have facilitated it wider adaptation to host and dominance with its wide distribution across the country. Further studies are needed to elaborate the intricacies and variation in function and behavior of enzyme among different genetic group and its interaction in efficient host utilization and prevalent distribution of *B. tabaci* genetic groups

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