International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 4, Issue 10 - 2017

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.10.022

Molecular analysis of heat shock protein with different stress and lipase production from the gut associated microorganisms of *Bombyx mori*. L

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Abstract

The silkworm, *Bombyx mori* L (Lepidotera: *Bombycidae*), as a model for Lepidoptera, is an agriculturally important insect for silk production. It is very heat sensitive organism. It mainly feeds on mulberry leaves, although tricuspid cudrania leaves can also act as the forage of the larvae. In the present study to isolate the microorganism from mid gut of silk worms. Screening the lipase producing bacteria in Rhodamine B agar. Characterize the efficient lipase producing isolate by morphological and biochemical characterization methods. To study the growth curve assay for lipase producing bacteria. To perform lipase assay using olive oil substrate, the factors influencing lipase enzyme production. To estimate the weight of protein quantitatively by Lowry's et al method. The role of heat shock protein with thermal and UV stress and the protein profiling by SDS-PAGE. Molecular characterization of the isolate by 16Sr RNA sequencing.

Keywords: Silkworm, *Bombyx mori*. *L*, Lepidoptera, lipase enzyme and SDS-PAGE

Introduction

Silk worm fibers are naturally extruded from two silk worm glands s a pair of primary filaments (brin), which are stuck together, with sericin proteins acting like glue to form a bave. Silk is made up of the aminoacids and forms β-pleated sheets. Silk protein is an essential constituent of cocoon filament (Komatsu 1975). Mulberry leaves were mainly composed of pectin, xylem, cellulose and starch. Cellulose is the plant cell wall. Nutrition plays an important role in improving the growth and development of the silk worm *Bombyx mori*. L. (Elebari, 2002).

The mid gut is the major organ for food digestion, nutrient absorption and also a barrier for foreign substance. The fifth in star larval stage of silkworm is very important for larval growth, development and silk production (Saizhang *et al;* 2011).

Enzymes have a great industrial potential and are widely found in various sources, like plants, animals and microbes. Microbes have determined plants and animals as sources of enzyme due to their broad biochemical diversity, mass culture and also due to the ease with which they can be genetically modified.

Microbial enzymes are more useful than enzymes from plants or animals because of the great variety of catalytic activities available, possible high yields, genetic manipulation, continuous supply due to absence of seasonal fluctuations and rapid growth on inexpensive media. (Higaki, 1968).

Lipolytic enzymes and Lipases belong to a group of enzymes whose biological function as to catalyze the hydrolysis of triglycerols into diacylglycerols, monoacylglycerols, free fatty acids and glycerol. Microbial lipases are the most important group of biocatalysts used for a variety of different biotechnological applications. Heat shock is nothing but a thermal injury caused by sudden increase in temperature in biological molecules like DNA, RNA, lipids, etc., of the cell which are vulnerable to heat stress. Another most important effect of temperature (or stress of any kind) is on cellular proteins by unfolding them (Feder, 1996).

The heat shock proteins (HSP) are presumed to ensure survival under stressful conditions by involvement in damage protection or damage repair due to their action as molecular chaperons (Hightower, 1991). In the present study in order to make this progress more effective it is necessary to study that the microorganisms which produce lipases were isolated from midgut of *Bombyx mori*. The bacteria isolates *Bacillus* sp and *Staphylococcus sp.* These microorganisms secrete lipase enzymes which are important for the digestion of mulberry leaves. The effect of temperature stress and UV stress on *Bombyx mori* larvae. The heat shock protein were presumed to ensure survival under stressful conditions by involvement in damage production due to their action as molecular chaperons. The protein profile of different proteins at different stress. These stress leads to the expression of heat shock proteins. Which has a great economic importance in silk production.

Materials and Methods

Sample Collection

Rearing of Silkworm *Bombyx mori*

The fifth instar larvae of *Bombyx mori* were collected from the Central Sericulture Research and Training Institute, CSR and TI Samayanallur, Madurai, South India. The fifth instar larvae, was fed on young tender leaves which are rich in protein and water content. The mature instar larvae fed on mature leaves that are rich in carbohydrate with lower amounts of protein and water content (Aruga, 1994). The larvae were reared in sterile cages at room temperature 28-24$\pm 1^0$C at humidity of 85 – 65. Larvae were fed with mulberry leaves that had been sterilized by exposure to UV light.

Isolation, Identification and characterization of Bacteria from the gut of *Bombyx mori*

The gut of Lepidoptera worm contain bacteria that produce digestive enzyme that help digestion of mulberry leaf constituents such as cellulose, xylan, pectin and starch. The entire digestive tract was aseptically isolated in a UV laminar flow hood. The isolated digestive tract was washed, chopped with a sterile blade and homogenized. Then the supernatant was serially diluted and plated on nutrient agar plates and incubated for 24 hours at room temperature. After incubation, the total number of colonies was counted. The colonies with different morphological characteristics were selected and streaked on the nutrients agar slants separately and incubated. After incubation the slant were stored in refrigerator for further use.

Screening of lipase producing microorganisms

A sensitive and specific plate assay for detection of lipase producing bacteria makes use of rhodamine-olive oil-agar medium. The growth medium contained (g/L): nutrient broth, 8.0; NaCl, 4.0 and agar-agar 20. The medium was adjusted to p^H 7.0, autoclaved and cooled to about 60^0C. Then, 31.25 ml of olive oil and 10 ml of Rhodamine B solution was added with vigorous stirring. It was then poured into petriplates under aseptic conditions and allowed to solidify. The bacterial culture was inoculated onto the medium in these plates. Lipase producing strains were identified on rhodamine agar plates for 48 hours at 37^0C.

Characterization of the Bacterial Isolates

Morphological and Biochemical characterization

Morphological characteristics such as abundance of growth, pigmentation, optical characteristics, size and shape were studied on nutrient agar plate. Biochemical characterization such as Indole, Methyl red, VP-test, citrate utilization, Triple sugar iron, starch hydrolysis, Nitrate reduction, Gelatin hydrolysis test.

Growth Curve Assay of Lipase-Producing Bacteria

Each lipase – producing strain was inoculated into a 250 ml Erlenmeyer flask containing 25 ml of medium,

consisting of 0.5% beef extract, 0.5% peptone, 0.5% sucrose, 0.3% NaCl, 0.2% K_2HPO_4, p^H =9.2 – 9.8, and incubated at 30^0C for 24 hours on a rotary shaker at 200 rpm. Subsequently, the inoculum were transferred into production medium (2.0% peptone, 0.5% sucrose, 0.1% $(NH_4)_2SO_4$, 0.1% $MgSO_4$, 0.2% K_2HPO_4, 1.0% olive oil, p^H = 9.2-9.8) at a rate of 2.0%, followed by incubation at 30^0C on a rotary shaker at 200 rpm. The samples were taken after every 12 hours until 72 hours. Cell density was measured by taking the optical density at 600 nm against the cell - free control.

Determination of Protein

Protein concentration of the sample was determined by the method of Lowry *et al* (1951), using bovine serum albumin as the standard.

Enzyme Production

The production medium for lipase comprised of (w/v) 0.01% $MgSO_4$, 0.1% KH_2PO_4, 1.0% tween 20, 0.2% glucose, 0.5% peptone (p^H 7.0). Fermentation was carried out with 100ml medium inoculated with 5% overnight culture and incubated on a rotary shaker (180rpm) for desired period.

Enzyme Assay

1 ml of 24hrs old *Bacillus* and *Staphylococcus* culture was inoculated in 100 ml of mineral media and incubated on rotary shaker adjusted at 30^0C and 120 rpm. After every 24hrs protein concentration and lipase activity was determined. 10% olive oil emulsion in 2% gum acacia was used as substrate .

Reaction mixture composed of 0.5ml substrate emulsion, 0.4ml o.1M Tris HCl buffer (pH 7.2) and 0.1 ml lipase solution. In blank lipase solution was replaced with equal amount of distilled water. Reaction was carried out at 30^0C for30 min. Reaction was stopped by adding 2ml acetone. Liberated fatty acids were titrated with 0.05N NaOH using phenophthalene indicator. Amount of NaOH required to achieve end point (colorless to pink) was recorded. From this enzyme units and specific activity was calculated as follow.

1 Unit of lipase activity is calculated by the formula

Lipase activity =

$$\frac{5.61 \text{ Volume of NaOH Normality of NaOH}}{\text{Volume of enzyme taken}}$$

Where 5.61 = unit constant for identifying acid value.

Unit Definition

1 Unit lipase activity = Amount of enzyme required to release 1 micro mole of fatty acid per ml per minute under above assay conditions.

Specific Activity

Enzyme unit / Protein concentration

Enzyme assay

The enzyme assays were performed with the cell free supernatant of the fermented broth as the crude enzyme source.

Effect of Different Carbon Source on Lipase Activity:

The carbon sources (glucose, sucrose, galactose, lactose and starch) were used at 1% production medium and inoculated with lipolytic strain-1 and strain-2 for lipase production.

Effect of Different Nitrogen Source on Lipase Activity:

Organic and inorganic nitrogen sources like Yeast extract, Peptone, Ammonium nitrate, Ammonium chloride and Ammonium orthophosphate were amended to the culture medium at 1% with the lipolytic strain-1 and strain-2 for lipase production.

Effect of p^H on Lipase Activity:

The optimum p^H for enzyme production was selected by varying the p^H of the lipase production medium from5 to 9 and inoculated with the lipolytic strain-1 and strain-2.

Effect of Temperature on Lipase Activity:

For selection of optimum temperature for the production of lipases, the temperatures varying from 20 to 40^0C were selected and the lipase activity was assayed.

Heat Shock Protein

For heat shock treatment, 20 larvae were used at one time and each experiment was repeated at least 3 times. The silkworm larvae (IV and V instars) were placed in thin – walled test tubes/beakers and exposed

to heat shock temperatures of 38 and 42^{0}C in water bath for 3hr. Then the larvae were transferred to room temperature for recovery lasting 3hr. Thereafter heat shocked and control larvae were reared in four replications in controlled environmental conditions.

Extraction and analysis of heat shock proteins

Haemolymph of silkworm larvae recovering from heat shock were collected by rapid centrifugation method of Nathon and Thomas (1965). To inhibit the tyrosine's activity of the haemolymph, phenhylthiourea was added.

Molecular Weight Determination in SDS – PAGE

The molecular weight of the haemolymph of heat shock protein and normal protein treated worms was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), (Laemmli, 1970). Polyacrylamide gel electrophoresis was done to separate proteins. The haemolymph was separated using SDS – PAGE.

Results

Isolation of Bacteria from gut of *Bombyx mori*

The fifth instars larvae of *Bombyx mori* were collected from central sericulture Research Institute, Samayanallur, Madurai, South India. The collected worms were dissected out to extricate the gut tissues. (Plate: 1). The tissues along with microorganisms present in gut epithelium were grounded finely and serially diluted and spread plate on nutrient agar plates and were incubated at 28^{0}C for 24 hours. After incubation morphologically distinct strains were observed on the surface of nutrient agar which was depicted in Plate: 2. The isolated colonies were pure cultured and stored at 4^{0}C for future use.

Screening of Lipase Producing Bacteria

The screening of the isolated gut microorganisms were screened for lipase production in Rhodamine B agar medium. of the three microorganisms screened for lipase production , only two microorganisms revealed Pink zone with 20mm and 28mm in diameter indicated the lipase producing strains were denoted as Strain-1 and Strain-2. The results were depicted in Plate: 3.

Identification of the Effective Lipase Producing Bacterial Isolates

The selected potential lipase producing isolates Strain-1 and Strain-2 were identified by morphological and biochemical characterization methods. From the obtain results the Strain-1 was conformed as *Bacillus cereus* and Strain-2 was identified as *Staphylococcus* species. The results were depicted in (Table: 1).

Table: 1.Biochemical Characterization of Lipase Producing Strain-1 and Strain 2

Biochemical Test	Strain-1	Strain-2
Gram staining	Positive rods	Positive cocci
Spore Staining	Present	Present
Indole Test	Negative	Negative
Methyl Red Test	Positive	Negative
Voges – Proskauer Test	Negative	Negative
Citrate utilization Test	Positive	Positive
Triple Sugar Iron Test	Alkaline Slant	Alkaline Slant
Gelatin Hydrolysis	Positive	Positive
Nitrate Reduction	Positive	Positive
Starch Hydrolysis	Negative	Negative

Identification of dominant bacteria

Further characterization of the dominant intestinal bacteria at molecular level, strain identification was performed using 16S rRNA sequencing. The results revealed that the dominant isolate Strain-1 was found to be *Bacillus sp* and *Staphylococcus* species. with base pairs of DNA fragments analyzed by PCR amplification (Thermo cycler). All the sequence was deposited in the gen bank database. To understand the phylogenetic position of these dominant bacteria a phylogenetic tree was constructed based on 16S rRNA gene sequence homology. These both microorganisms are the highest homology with *Bacillus* sp *Staphylococcus* species respectively.

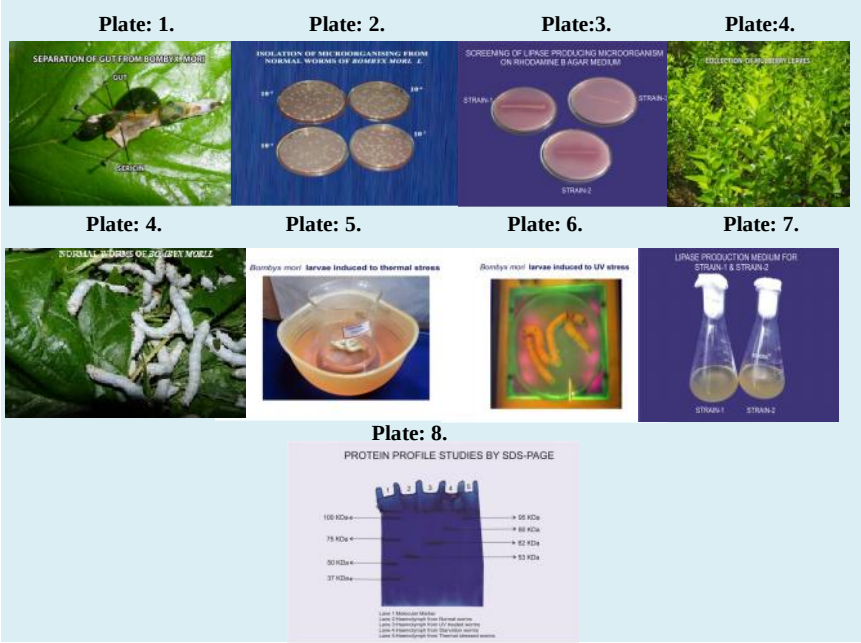
Growth curve assay of lipase producing bacteria

The bacterial density was examined after 12 hours until 72 hours the maximum cell density of both the lipase producing bacteria was observed at ˜48 hours of fermentation. After that the bacterial density reduced probably due to the consumption of nutrients. The maximum bacteria density showed a significant difference, Strain-1 (*Bacillus cereus*) had the maximum yield where as Strain -2 (*Staphylococcus* sp) had the minimum yield. (Figure: 1).

Assay of Lipase activity

Lipase activity was determined using UV spectrophotometer. Results revealed that, maximum total lipase activity was observed after 48 hours of Incubation. At their maximum bacteria density Strain-1 showed the highest lipase activity 32.4 (unit/ml) while Strain-2 showed the lowest activity 29.6 (unit/ml). (Plate:7).

Strain1 - *Bacillus cereus*, Strain 2 - *Staphylococcus* sp



Protein Estimation by Lowry's Method

The total protein present in the sample of silkworm larvae was quantitatively estimated by Lowry's et al method. The amount of protein content of sample was found to be 0.96 mg/ml for Strain-1 and 0.61 mg/ml for Strain-2.

Optimization studies

Effect of pH on Lipase Activity

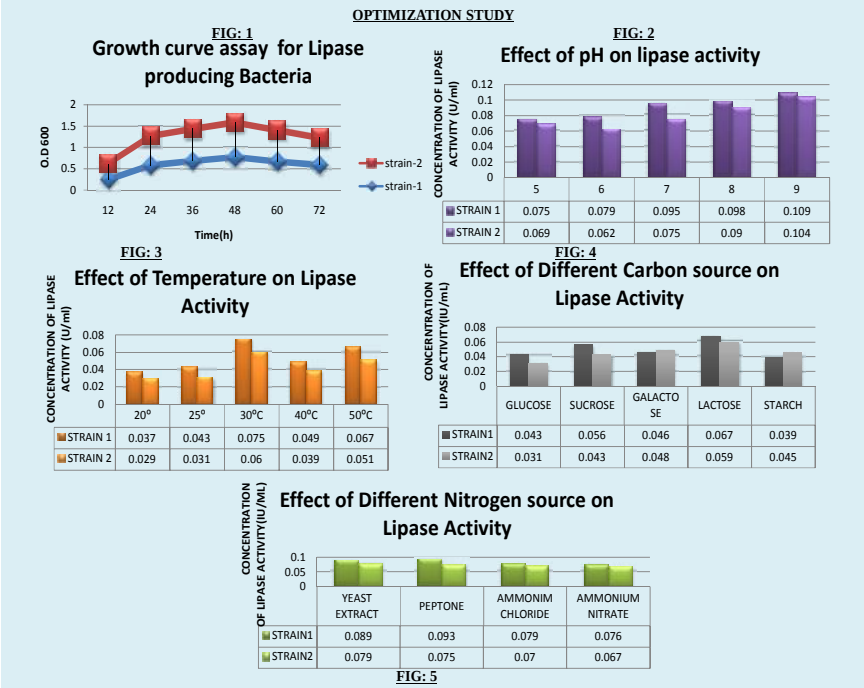
Figure: 2 denoted the effect of pH on lipase activity. The effect of pH on lipase production was determined by *Bacillus sp* and *Staphylococcus* sp (Strain-1 and Strain-2) in the production medium with different pH values (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0). The enzyme production was maximum when pH was 9.0. Above that pH, production decreased significantly. Among the strains of *Bacillus* sp strain-1 was found to yield maximum enzyme.

Effect of Incubation temperature on lipase activity

In order to determine, the optimum incubation temperature on lipase production by *Bacillus* sp and *Staphylococcus* sp (Strain-1 and Strain-2). The isolate was grown in production medium at various temperatures 20,25,30,40 and 50^0C. It showed the ability to grow at respective temperature especially at 30^0C. (Figure: 3).

Effect of Carbon source on Lipase Activity

Figure: 4 exhibited the basal production medium for lipase has Sucrose Lactose, Galactose, Glucose and Starch. Lactose was found to be an effective carbon source (0.067 unit/ml and 0.059 unit/ml) for *Bacillus* and *Staphylococcus* strains. Different carbon sources were tested for their ability to support lipase production.



Effect of Nitrogen source on Lipase Activity

Figure: 5 illustrates the effect of Nitrogen source on lipase activity. Organic nitrogen sources were used efficiently by *Bacillus* and *staphylococcus* for production of lipase. Peptone in general was the nitrogen source in production medium for lipase. But it was replaced by Yeast extract, Ammonium chloride and Ammonium nitrate on test media. Peptone as Nitrogen source yielded 0.093 unit/ml and 0.079 unit/ml for *Bacillus* sp and *Staphylococcus* sp Strain-1 and Strain-2 respectively.

Determination of molecular weight of protein from normal and heat shock protein

Total protein extracted on Haemolymph from body of V instars larvae of *Bombyx mori* was analyzed by considering the recent concept of larvae to cellular protein and stress physiology, about role of heat shock protein were treated in different stress like thermal stress and UV stress and normal treated *Bombyx mori* worms were exhibiting proteolytic bands appeared in SDS – PAGE. (Plate: 4, 5 and 6) Protein profile studies for larval haemolymph proteins in silkworm were analyzed the electrophoresis variation among the normal worm and heat shock protein were investigated. A 53KDa haemolymph protein band was observed which is absolutely near to 50KDa of the marker Lane1. A 53KDa haemolymph protein for *Bacillus* sp.

The appearance of 53KDa of protein band of normal worm was recorded. The term heat shock protein referred to stress protein in the present study differential expressions of HSP in silkworm strains performed with UV treated worms, it was found to be 62 KDa. The starved worms to express its protein pattern recorded 80KDa. Similarly thermal stress were given to worms as to express Heat Shock Protein, revealed 95 KDa of Heat Shock Protein were recorded.

Discussion

In the present study the isolation of microorganisms from the gut of *Bombyx mori* were performed to isolate microorganisms producing lipase. In order to ascertain the lipase producing bacteria screening were performed for three strains out of which 2 isolate Strain-1 and Strain-2 were found to be promising producers of lipase. The organisms Strain-1 was found to produce increased lipase activity on Rhodamine-B agar medium. Our results are total confirmatory with (Feng, et al., 2011), wherein he reported that *Bacillus* and *Staphylococcus* were involved in lipase production. The lipase producing bacterial community revealed change in response to food source. These result provide evidence that diet has the significant impact on gut microbial community (Broderisk, et al., 2004).

It is important to perform morphological and Biochemical characterization for the isolates Strain-1 and Strain-2. One isolate was identified on *Bacillus* species and another isolate-2 was identified on *Staphylococcus* sp. Further characterization of dominant strain a molecular level identification was performed using 16S rRNA sequencing. Promising results of the isolates Strain-1 found to be *Bacillus* and *Staphylococcus* species with base pairs of DNA fragments analyzed by PCR amplification. Similarly maximum lipase activity was found 48 hours for Strain-1 followed by Strain-2 similar results were obtained by (Feng, et al., 2011). The ability of (Strain1 and Strain2) to grow in production medium, were optimized, revealed that 30^0C was found to produce good yield. Our results are in total agreement with (Gupta, et al., 2004), reported that *Bacillus* sp has ability to grow over wide range of temperature ranging from 35-50^0C.

The initial pH of the medium need to be controlled pH is another important factor in enzyme synthesis. The effect of pH on lipase production determined by *Bacillus sp* and *Styphylococcus* sp (Strain-1 and Strain-2) revealed a alkaline pH of 9.0 and further increase in pH reduced enzyme production our findings coincide with the reports of (Gupta, 2004) and Patel, et al., 2005, has reported a pH of 9.0 to be optimum for protease production by *Bacillus* sp.

Organic nitrogen sources were efficiently utilized by *Bacillus* sp for production lipase. Among the various nitrogen source tested, Peptone, Ammonium chloride and Yeast extract were found to produce pronounced production. High specific activity of lipase was reported with Peptone for lipase production by *Bacillus* sp by (Limponbora, 2007) which coincides with our findings.

In tune with above discussion, a supportive result was reported by (Mabraic, et al., 1999), found out that addition of SDS. From the forgiving discussion, crude substrates were tested for enzyme production. Olive oil was found to produce excellent yield of lipase followed by coconut oil. Similar findings were reported by (Thomas, et al., 2003).

Lipase was produced almost throughout the course of fermentation. Maximum production was recorded at 48 hours of incubation. In accordance with above result, (Mehootra, et al., 1998) has observed maximum production of lipase during late exponential phase of growth of *Bacillus* sp. Lipase production from *Bacillus* was found maximum at 48 hours.

In order to ascertain the lipase activity of Strain-1 total protein content of the bacterial isolates presents in the gut epithelium *Bombyx mori* were established. In tune with above discussion Steinheu, 1989 reported nearly 69 proteins were present in the midgut of silkworm because *Bombyx mori* is an important economic insect which has the capacity to convert leaf protein into silk.

Total protein extracted as haemolymph from whole body of *Bombyx mori* was analyzed for SDS-PAGE to understand the molecular basis of protein to study molecular weight by protein under shocks such as heat shock. The role of HSP was studied. In present study it was adopted a new strategy to evaluate lipase acting as influenced by heat shocks. Our results are in total confirming with (Rajesh, et al., 2011).

The haemolymph, the carrier of an nutrient substance distributes the each and every part of the body for cellular metabolism, where in the carbohydrates (Tazima, 1978). Having assessed the difference of normal and heat shock protein profile of haemolymph of *Bombyx mori* were analyzed the comparative basis with market. In haemolymph the appearance of 53 KDa consequent to heat shock was evident bivoltine races of silkworm analyzed. Our results are in total agreement with (Omanajoy, 1950. In this contest presence of heat shock protein in the haemolymph of *Bombyx mori* can be considered as desirable feature in conferring thermo tolerance to the larva. The larva haemolymph proteins in silkworm were analyzed on PAGE on polyacyrlamide gel electrophoresis variation among normal and HSP protein were investigated. A 50 KDa haemolymph protein having strong of affinity to the cell wall of gram (+) bacteria was purified from the haemolymph of the silk worm, *Bombyx mori*. The tools of molecular biology offered novel techniques to characteristic hitherto uncultivable gut micro flora. PCR probes based on 16S rRNA genes are widely used for detection and characterization of microbes.

Conclusion

The present study in order to make progress more effective to perform experiment with commercialist silkworm which a more economic importance the bacterial isolates from gut epithelium were studies lipase production. Two different isolates *Bacillus* sp and *Staphylococcus* sp were isolated among which *Bacillus species* noted for screening Lipase enzyme for digestion. In addition, the role of HSP with different stresses such as UV exposure, Starvation and Thermal stress were conducted to study the expression

of HSP. The presence study is attempt to address The Role of HSP and characterization of lipase producing in *Bombyx mori* which has commercial important in the field of Seri-biotechnology.

References

Etebari, K and Matindoost L, 2002. Application of multi – vitamins as nutrients on biological and economical characteristics of silkworm *Bombyx mori* L. J.Aaia-pacefic Entomol, 8; 1-6

Feder, J.H., Rossi, J.M., Solomon, J., Solomon, N. and Lindquist, S.(1996) The consequences of expressing hsp 70 in Drosophila cells at normal temperatures. Genes Den. 6, 1402-1413.

Feng H, Wang L, Liu Y, He L, Li M, Lu W, Xue C. Molecular characterization and expression of a heat shock protein gene (HSP90) from the carmine spider mite, *Tetranychus cinnabarinus* (Boisduval). J Insect Sci. 2010;10:112. doi: 10.1673/031.010.11201. [PMC free article] [PubMed] [Cross Ref].

Gupta R, Gupta N and Rathi P, 2004, Bacterial Lipases : an overview of production, purification and biochemical properties, Appl. Microbiol, Biotechnol, 64 : 763-781.

Higaki S, 1968, Estimation of protein by the biuret method, Biotechnology procedure and experiments handbook. Infinity science press LLC, New Delhi India, Pp 67-78.

Hightower L E 1991 Heat shock stress protein, chaperones and proteotoxicity; Cell 66 191-197.

Komatsu, K. (1975) Studies on dissolution behaviors and structural characteristic of silk Sericin. Bull. Sericult. Exp. Sta.26, 135-256.

Laemmli U K 1970 Cleavage of structural proteins of the head of bacteriopage T4; Nature (London) 227 680-685.

Limpon Bora, Minakshi Bora. Optimization of extracellular thermophilic highly alkaline lipase from Thermophilic Bacillus sp isolated from hot spring of arunachal pradesh, India. Brazilian Journal of Microbiology (2012): 30-42.

Nathan, C. F., and J. B. Hibbs. 1991. Role of nitric oxide synthesis in macrophage antimicrobial activity. Curr. Opin. Immunol. *3:65.*

Patel, A., Van De Poll, M. C., Greve, J. W. et al. Early stress protein gene expression in a human model of ischemic preconditioning. Transplantation, 2004, 78: 1479–1487.

Rajesh Pati, Geetika Pant, Ugam Kumari Chauhan, Sudhakar Malla, S. Banupriya. Heat Shock Proteins in *Cassia* Species. Biotechnology Journal International. 17(4): 1-10, 2017; Article no. BJI. 31006.

Saizhang x, Qi X, Hao B, Qu Y(2011) studies of substrate specifities of lipases from different sources. Eur. J Lipid Sci Technol 110; 1095-1101.

Tazima, Y., Ohta, N., and Harada, C. 1978. On the sex determinating method by colouring genes of silkworm eggs. I. Induction of translocation between the W and the tent chromosomes. Jpn J Breed 1: 47-50.

Thomous. M, Munetsuna H, Sato T, Adachi T, Hino R, Hayashi M, et al. Transgenic silkworm produce recombinant human type III procollagen in cocoons. Nat. Biotechnol. 21:52-56, 2003.

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	Website: www.ijarbs.com
Quick Response Code	Subject: Biotechnology
DOI:10.22192/ijarbs.2017.04.10.022	

How to cite this article:
Soma Prabha, A. and Prabakaran, V. (2017). Molecular analysis of heat shock protein with different stress and lipase production from the gut associated microorganisms of *Bombyx mori*. L . Int. J. Adv. Res. Biol. Sci. 4(10): 171-178.
DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.10.022