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Changes in the Activity of Digestive Enzymes Produced from the Gut Microflora of Silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae) in Response to Fortification of Mulberry Leaves

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A B S T R A C T

Gut micro-organisms have the ability to adapt themselves to changes in insect diet and the enzyme activity fluctuates in response to dietary changes. A total of 72 dominant isolates was successfully collected from the intestine of the fourth and fifth instar silkworm larvae fed with fortified mulberry leaves. The isolates represented 12 phenotypical forms on nutrient agar plates. Eleven phenotypes existed in the intestine of the larvae fed with mulberry leaves supplemented with *Aloe vera* and *Tinospora cordifolia* and ten found in larvae fed with only mulberry leaves. Ten of them were common in the intestine of three groups. The total highest cultivable bacterial count found to be $4.82 \pm 0.446 \times 10^6$ and $6.32 \pm 0.269 \times 10^6$ CFU/ml of *B. mori* larval digestive tract suspension of fourth and fifth instar respectively. The results showed that the clearing zone and hydrolysis capacity value of cellulose, starch and lipid degrading bacteria were found to be highest in treated groups compared to control. The enzyme assay for cellulase, amylase and lipase activity was found to be highest for BMGB42 isolate with 0.428, 1.02 and 11.33 (U/ml/min) respectively. The results indicate that the diet had significant impact on the gut bacterial community and enzyme activity. It was also observed that there was a proportional increase in the total bacterial count as well as percent dominance of cellulase, amylase and lipase with respect to the growth of *B. mori*.

Introduction

The *Bombyx mori* L. is an important economic sericigenous insect which feeds mainly on mulberry leaf and convert leaf protein into silk protein (Babu *et al.,* 2009). In silkworm, majority of the characters that contribute to the yield of silk are under the control of polygenic nature (Seshagiri *et al.,* 2009; Konate *et al.,* 2011). The factors responsible to influence the growth, development and subsequent physiology of insect body include: nutritional qualities of

food, biochemical status of nutrients in the food, hormonal level in the body and environmental conditions (Murugan and George, 1992). The leaves of mulberry are the sole source of food for larval instars of silkworm, *Bombyx mori* (L), biochemically constituted with proteins, lipids, carbohydrates and minerals (Subramanyam, 1992). Therefore, the corresponding diversity of enzymes capable of hydrolyzing the biocompounds of mulberry is exhibited by gut

of larval instars of silkworm, *Bombyx mori* (L). Enzymes play a vital role in the metabolism of dietary food in the body of an organism. In silkworm, the digestive enzymes in the gut breakdown the complex form of nutrients present in the food into simpler forms. These simpler forms are easily absorbed into the body through the semi permeable membrane of alimentary canal.

Gut micro-organisms have the ability to adapt themselves to changes in insect diet, by induction of enzymes or by population changes in the microbial community, where the enzyme activity fluctuates in response to dietary changes (Kaufman and Klug, 1991; Santo Domingo *et al.,* 1998). Scientists have tried alternative food for the rearing of silkworms which are of cost effective. The activity of the amylase, sucrase, and protease were increased on *Dolichos lablab* supplementation to silkworm and it is presumed that the bean might have activated the enzyme molecules to act on their substrates (Manjula *et al.,* 2010). The fibroin content of the cocoon shell significantly increased in response to the dietary supplementation of botanicals. Javed and Gondal have also reported that silkworm fed with nitrogen and ascorbic acid supplemented mulberry leaves showed higher growth and lower mortality.

Treating the mulberry leaves with herbal drug: kho-go and feeding them to fifth instar larvae was found reflected into significant improvement in the levels of proteins and velocities of biochemical reactions catalyzed by protease and amylase (Khyade Vitthalrao and Doshi Sucheta, 2012).

The efforts towards the qualitative silk production through the improvement in the efficiency of consumption and utilization of food by larval instars of silkworm, *Bombyx mori* (L) include: improvement in the quality of mulberry leaves and fortify the leaves with nutrients, antibiotics, juvenile hormones, dusting with botanicals or extrafoliation of mulberry leaf with sugars, vertebrate hormones, feed additives, protein rich flours etc. The present study was aimed to understand the changes in the digestive physiology by analyzing the activities of the digestive enzymes when supplemented with the mulberry leaves fortified with plant extracts.

Materials and Methods

The experiments were conducted in the Microbiology laboratory, Forest College and Research Institute, Mettupalayam which is located at an altitude of 300 meters MSL, 77.56' E latitude and 11.19' N longitude.

Rearing of silkworm with the mulberry leaves fortified with plant extracts

The disease free laying (DFLs) of double hybrid race (CSR6 x CSR26) x (CSR2 x CSR27) were procured from Silkworm Seed Production Centre, Coimbatore. The larvae were reared from first to fifth instar under hygienic conditions with optimum temperature (25-28ºC) and relative humidity (75-85%) in rearing room (Krishnaswami, 1978).

The mulberry leaves of V1 variety were fed 3-4 times a day from first to third instar and were divided into three groups; one group reared with mulberry leaves fortified with *Tinospora cordifloia* at 2 per cent concentration, the second group on mulberry leaves fortified with *Aloe vera* 100% pure gel extract and the third group reared with only mulberry leaves and served as control. The treatment was given for the first feed of 4th and 5th instar and once during fifth mid instar (4th day). The remaining feed was given only with mulberry leaves.

Isolation and enumeration of gut microflora

The 4th instar larvae prior to change to the next instar and 3 days old 5th instar larvae were used in the experiment and subjected to starvation for 24 hours. Subsequently, the larvae were anesthetized with chloroform soaked cotton pads and surface sterilized by submersion into 75% ethanol for one minute and rinsed three times using sterile distilled water prior to dissection. The larvae were dissected in a UV laminar flow hood using dissection scissors and the entire alimentary canal of individual larva was aseptically separated using sterilized fine-tipped forceps. The isolated digestive tract was washed with sterile ice-cold saline (0.9 per cent NaCl) solution, chopped with a sterile blade, homogenized and incubated for 30 minutes at 37º C. The supernatant was taken and serially diluted 1000 to 10,000 times.

The pour plate method was used to estimate total bacterial count on nutrient agar plates. The total viable count of cultivable total bacteria was expressed as the number of CFU in 1ml of sample.

Isolation and screening of cellulase producing bacteria

The isolated gut bacterial colonies where inoculated on Berg's minimal agar (Berg's *et al.,* 1972) plate with 0.1% carboxy methyl cellulose (CMC) as substrate and incubated at 30ºC for 24 to 48 hours. Colonies showing degradation capacity was identified using Congo red overlay method according to Bergey's manual of systemic bacteriology (Sneath *et al.,* 1984). For the Congo red method, plates were flooded with 0.1% aqueous Congo red for 30 minutes with intermittent shaking and destain with 1M NaCl solution. A clearing zone around the colony indicates the hydrolysis of cellulose

(Wood 1980). Cellulose-degrading potential of the positive isolates was also qualitatively estimated by calculating hydrolysis capacity (HC), that is, the ratio of diameter of clearing zone and colony (Hendricks *et al.,* 1995)

Isolation and screening of amylase producing bacteria

The gut bacterial isolates were screened for producing amylolytic activity by starch hydrolysis test on Berg's minimal agar (Berg's *et al.,*1972) plate with 1% starch as substrate and incubated at 30º C for 24 hours. Colonies showing degradation capacity was assayed by plate screening using the iodine method according to Bergey's manual of systemic Bacteriology (Sneath *et al.,* 1984). For the iodine method starch plates were flooded with iodine solution resulting in dark blue plates with uncoloured zones where the starch had been degraded (Hols *et al.,* 1994). Starch degrading potential of the positive isolates was also qualitatively estimated as reported by Hendricks *et al.,* 1995.

Isolation and screening of lipase producing bacteria

The isolated bacterial colonies were inoculated on Rhodamine B agar plate containing 12% olive oil emulsion as substrate and incubated at 30º C for 24 to 48 hours (Feng *et al.,* 2011). Lipase production was observed as a pink zone of hydrolysis around the bacterial colony. Lipid degrading potential of the positive isolates was also qualitatively estimated as reported by Hendricks *et al.,* 1995.

Assays for enzyme activity

The gut bacterial isolates which are positive for all three enzyme activities viz., cellulase, amylase and lipase are only considered for assay studies.

Cellulase assay

Endoglucanase (1, 4-β endoglucanase - EC 3.2.1.4) activity was assayed by measuring the amount of reducing sugar from carboxy methyl cellulose using 3, 5- dinitrosalicylic acid. The enzyme activity was determined according to the methods recommended by the International Union of Pure and Applied Chemistry (IUPAC) commission on biotechnology (Ghose, 1987). The One unit (U/mL) of enzymatic activity is defined as the amount of enzyme that releases 1µmol of reducing sugar (measured as glucose) per mL per minute under the assay conditions. All the tests were conducted in triplicates and mean activities were determined.

Amylase activity

The enzyme activity was assayed following the method of Bernfeld (1955) using 3, 5-dinitrosalicylic acid. The substrate used for studying α-amylase (EC 3.2.1.10) was 1% starch. The One unit of enzymatic activity is defined as the amount of enzyme that releases 1µmol of maltose per mL per minute under the assay conditions.

Lipase assay (Titrimetric method)

The lipase activity was assayed using the method of Yadav et al., (1993). One unit (U) of lipase activity was defined as the amount of enzyme which produces 1µmol of fatty acids per minute under assay conditions.

Results and Discussion

Bacterial isolates from the digestive tract of *Bombyx mori* L. with fortified mulberry leaves

Gut micro-organisms have the ability to adapt themselves to changes in insect diet, by induction of enzymes or by population changes in the microbial community. In the present study, a total of 72 dominant isolates was successfully collected from the intestine of the fourth and fifth instar silkworm larvae fed with fortified mulberry leaves. The isolates represented 12 phenotypical forms on nutrient agar plates. Eleven phenotypes existed in the intestine of the larvae fed with mulberry leaves supplemented with *Aloe vera* and *Tinospora cordifolia* and ten found in larvae fed with only mulberry leaves. Ten of them were common in the intestine of three groups. The total highest cultivable bacterial count found to be $4.82 \pm 0.446 \times 10^6$ and $6.32 \pm 0.269 \times 10^6$ CFU/ml of *B. mori* larval digestive tract suspension of fourth and fifth instar respectively (Table 1). *Aloe vera* fed silkworm expressed the presence of highest number of population. The main feature of the *Aloe vera* plant is its high water content, ranging from 99% to 99.5%, while the remaining 0.5–1.0% solid material is reported to contain over 200 different potentially active compounds, including vitamins, minerals, enzymes, simple and complex poly-saccharides, phenolic compounds, and organic acids (Boudreau *et al.,* 2013a; Rodriguez *et al.,* 2010). It was also observed that there is a proportional increase in the total bacterial count with respect to the growth of *B. mori.* Some of the plant products contain insect juvenoids (like engenol) which are known to increase the capability of consumption and utilization of food by insects like silkworm. Administration of botanicals to silkworm help in supplementation of various biochemical constituents (Amino acid, proteins, alkaloids, glucoside, phyllanthine, psoralen, betasitosterol) which have the property of phagostimulants and attractants was reported in mulberry (Manoharan, 1996). In phytophagous insects, the exogeneous compounds through herbal feed mimic the action of natural juvenile hormone, which enhance the synthesis of poly (A) RNA for major silk protein (Khyade Vitthalrao and

Doshi Sucheta, 2012). Feng *et al.,* 2011 reported the diversity of bacteria from the intestine of silkworm fed on tricuspid cudrania leaves and mulberry leaves. The lipase producing bacterial community likely shows great changes in response to the food source. Diet is also known to influence the intestinal microorganism community in cockroaches and crickets, where microbial populations fluctuate in response to dietary changes (Kane and Breznak, 1991; Santo Domingo *et al.,* 1998). This appears to the case in other lepidopteran caterpillars such as the gypsy moth, with 15 phylotypes at its most complex and 7 phylotypes at its simplest (Broderick *et al.,* 2004).

Hydrolytic capacity of bacterial isolates utilizing cellulose, starch and lipid from the digestive tract of *B.mori.*

In insects, the complex food molecules are utilized after they have been processed into simpler molecules through the action of digestive enzymes in the gut of the larva. Thus the enzyme system in the silkworm plays a vital role in determining the performance of the larva in terms of effective transformation of organic food molecules of the leaf into useful biomolecules. So in this study, the 72 isolates obtained from silkworm gut fed with different plant extracts were subjected to screening for enzyme production and their respective hydrolytic activity. The results showed that the clearing zone and hydrolysis capacity value of cellulose degrading bacteria ranged between 11.0 to 39.0 mm and 1.80 to 2.03 for *Aloe vera* treated group, 10.0 to 37.0 mm and 1.33 to 2.05 for *Tinospora cordifolia* treated group and 10.0 to 27.0 mm and 1.56 to 1.98 for mulberry control group (Table 2). The clearing zone and hydrolysis capacity value of starch degrading bacteria ranged between 12.0 to 55.0 mm and 1.20 to 2.14 for *Aloe vera* treated group, 10.0 to 48.0 mm and 1.38 to 2.06 for *Tinospora cordifolia* treated group and 14.0 to 42.0 mm and 1.38 to 2.08 for mulberry control group (Table 3).

The formation of pink zone and hydrolysis capacity value of lipid degrading bacteria ranged between 9.0 to 37.0 mm and 1.01 to 1.59 for *Aloe vera* treated group, 10.0 to 29.0 mm and 1.03 to 1.30 for *Tinospora cordifolia* treated group and 8.0 to 30.0 mm and 1.03 to 1.29 for mulberry control group (Table 4). The hydrolytic value found between 1.38 to 2.33 and 0.15 to 1.37 for cellulolytic aerobic bacterial isolates from farming and forest soil, respectively (Hatami *et al.,* 2008). The clearing zone and HC value ranged between 28.0 to 50.0 mm and 4.3 to 9.0 for cellulose feeding organisms like termite, caterpillar, bookworm, and snail (Gupta *et al.,* 2011). The Suseela *et al.,* (2014) reported the lipase activity of fungal isolates ranged between 25.0 to 40 mm by cup plate method.

Table.1 Population of total cultivable bacteria from the digestive tract of *Bombyx mori* L. with fortified mulberry leaves

Group	Total viable population of bacteria (CFU/ml)	
	IV instar	V instar
Mulberry + *Tinospora cordifolia*	4.34 (± 0.386) [b]	5.12 (±0.303) [b]
Mulberry+ *Aloe vera*	4.82 (± 0.446) [a]	6.32 (±0.269) [a]
Mulberry only (control)	3.78 (± 0.291)[c]	4.48 (±0.333)[c]

Values are means ± S.d. for each group. a-c Values not sharing a common superscript letter within each column differ significantly at P < 0.05.

Table.2 Effect of fortification of mulberry leaves on maximum clearing zone and hydrolytic
capacity (HC) of bacterial isolates from the gut of silkworm for cellulase production

Isolates from Mulberry + *T. Cordiflia*	Maximum clearing zone (mm)	HC value	Isolates from Mulberry + *A. vera*	Maximum clearing zone (mm)	HC value	Isolates from Mulberry control	Maximum clearing zone (mm)	HC value
BMGB1	23	1.93	BMGB25	20	1.96	BMGB49	11	1.86
BMGB2	10	1.78	BMGB26	15	1.87	BMGB50	-	-
BMGB3	28	1.91	BMGB27	19	1.88	BMGB51	-	-
BMGB4	13	1.80	BMGB28	13	1.85	BMGB52	19	1.58
BMGB5	11	1.83	BMGB29	-	-	BMGB53	-	-
BMGB6	20	1.87	BMGB30	28	1.93	BMGB54	20	1.94
BMGB7	-	-	BMGB31	-	-	BMGB55	-	-
BMGB8	-	-	BMGB32	11	1.83	BMGB56	-	-
BMGB9	-	-	BMGB33	14	1.86	BMGB57	-	-
BMGB10	27	1.98	BMGB34	15	1.87	BMGB58	-	-
BMGB11	-	-	BMGB35	21	1.92	BMGB59	15	1.56
BMGB12	21	1.92	BMGB36	-	-	BMGB60	19	1.87
BMGB13	11	1.77	BMGB37	12	1.84	BMGB61	27	1.98
BMGB14	20	1.84	BMGB38	35	2.00	BMGB62	-	-
BMGB15	-	-	BMGB39	-	-	BMGB63	-	-
BMGB16	15	1.76	BMGB40	22	1.91	BMGB64	23	1.82
BMGB17	37	2.05	BMGB41	20	1.80	BMGB65	12	1.73
BMGB18	19	1.91	BMGB42	39	2.03	BMGB66	16	1.79
BMGB19	12	1.33	BMGB43	24	1.93	BMGB67	10	1.70
BMGB20	-	-	BMGB44	-	-	BMGB68	11	1.75
BMGB21	15	1.89	BMGB45	-	-	BMGB69	19	1.85
BMGB22	-	-	BMGB46	16	1.88	BMGB70	-	-
BMGB23	-	-	BMGB47	18	1.93	BMGB71	-	-
BMGB24	-	-	BMGB48	-		BMGB72	-	-

- = Negative for cellulase production

Table.3 Effect of fortification of mulberry leaves with plant extracts on maximum clearing zone and hydrolytic capacity (HC) value of bacterial isolates from the gut of silkworm for amylase production

Isolates from Mulberry + *T. Cordiflia*	Maximum clearing zone (mm)	HC value	Isolates from Mulberry + *A. vera*	Maximum clearing zone (mm)	HC value	Isolates from Mulberry control	Maximum clearing zone (mm)	HC value
BMGB1	27	1.90	BMGB25	41	1.95	BMGB49	39	1.95
BMGB2	12	1.71	BMGB26	-	-	BMGB50	-	-
BMGB3	40	1.88	BMGB27	-	-	BMGB51	14	1.76
BMGB4	10	1.38	BMGB28	38	1.90	BMGB52	40	2.02
BMGB5	-	-	BMGB29	30	2.14	BMGB53	36	2.03
BMGB6	-	-	BMGB30	12	1.33	BMGB54	38	1.92
BMGB7	-	-	BMGB31	-	-	BMGB55	-	-
BMGB8	15	1.87	BMGB32	12	1.20	BMGB56	-	-
BMGB9	-	-	BMGB33	19	1.90	BMGB57	-	-
BMGB10	-	-	BMGB34	15	2.00	BMGB58	-	-
BMGB11	37	2.05	BMGB35	25	1.92	BMGB59	-	-
BMGB12	-	-	BMGB36	-	-	BMGB60	-	-
BMGB13	15	1.89	BMGB37	-	-	BMGB61	-	-
BMGB14	10	1.88	BMGB38	-	-	BMGB62	-	-
BMGB15	11	1.80	BMGB39	13	1.85	BMGB63	15	1.38
BMGB16	19	1.72	BMGB40	-	-	BMGB64	42	1.94
BMGB17	48	1.95	BMGB41	-	-	BMGB65	-	-
BMGB18	16	1.79	BMGB42	55	2.03	BMGB66	19	1.79
BMGB19	38	2.06	BMGB43	20	1.81	BMGB67	-	-
BMGB20	-	-	BMGB44	28	1.93	BMGB68	-	-
BMGB21	-	-	BMGB45	-	-	BMGB69	-	-
BMGB22	-	-	BMGB46	19	1.72	BMGB70	-	-
BMGB23	-	-	BMGB47	-	-	BMGB71	-	-
BMGB24	-	-	BMGB48	27	1.93	BMGB72	-	-

- = Negative for amylase production

Table.4 Effect of fortification of mulberry leaves with plant extracts on maximum clearing zone and hydrolytic capacity (HC) value of bacterial isolates from the gut of silkworm for lipase production

Isolates from Mulberry + *T. Cordiflia*	Maximum clearing zone (mm)	HC value	Isolates from Mulberry + *A. vera*	Maximum clearing zone (mm)	HC value	Isolates from Mulberry control	Maximum clearing zone (mm)	HC value
BMGB1	21	1.30	BMGB25	35	1.59	BMGB49	28	1.04
BMGB2	-	-	BMGB26	11	1.08	BMGB50	-	-
BMGB3	27	1.04	BMGB27	15	1.03	BMGB51	21	1.18
BMGB4	-	-	BMGB28	24	1.04	BMGB52	30	1.29
BMGB5	-	-	BMGB29	-	-	BMGB53	29	1.20
BMGB6	11	1.12	BMGB30	9	1.05	BMGB54	8	1.09
BMGB7	29	1.25	BMGB31	30	1.37	BMGB55	23	1.17
BMGB8	-	-	BMGB32	12	1.10	BMGB56	-	-
BMGB9	10	1.08	BMGB33	27	1.08	BMGB57	20	1.12
BMGB10	-	-	BMGB34	25	1.01	BMGB58	-	-
BMGB11	-	-	BMGB35	28	1.40	BMGB59	-	-
BMGB12	10	1.15	BMGB36	-	-	BMGB60	-	-
BMGB13	20	1.18	BMGB37	-	-	BMGB61	10	1.05
BMGB14	12	1.09	BMGB38	23	1.02	BMGB62	18	1.17
BMGB15	-	-	BMGB39	-	-	BMGB63	25	1.08
BMGB16	19	1.07	BMGB40	21	1.05	BMGB64	29	1.12
BMGB17	20	1.20	BMGB41	29	1.03	BMGB65	-	-
BMGB18	23	1.15	BMGB42	37	1.52	BMGB66	-	-
BMGB19	28	1.27	BMGB43	33	1.31	BMGB67	-	-
BMGB20	14	1.13	BMGB44	30	1.29	BMGB68	-	-
BMGB21	10	1.03	BMGB45	19	1.05	BMGB69	-	-
BMGB22	-	-	BMGB46	25	1.09	BMGB70	-	-
BMGB23	-	-	BMGB47	-	-	BMGB71	20	1.03
BMGB24	15	1.10	BMGB48	-	-	BMGB72	-	-

- = Negative for lipase production

Fig.1 Per cent dominance of digestive enzymes producing bacteria from the digestive tract of *Bombyx mori* L. with fortified mulberry leaves
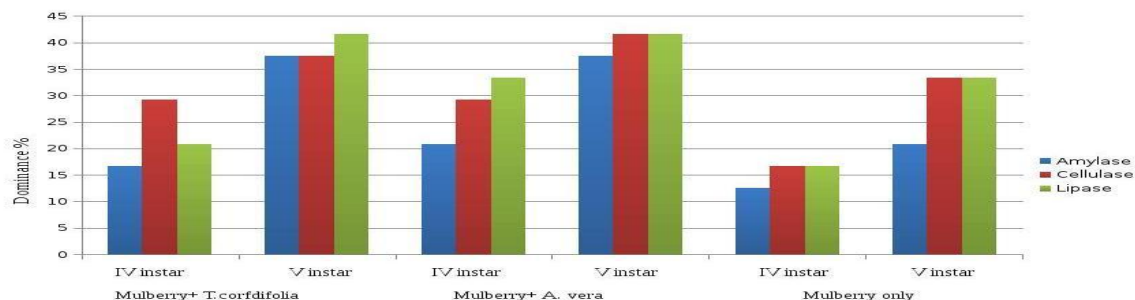
Fig.2 Cellulase and amylase activity of selective bacterial isolates
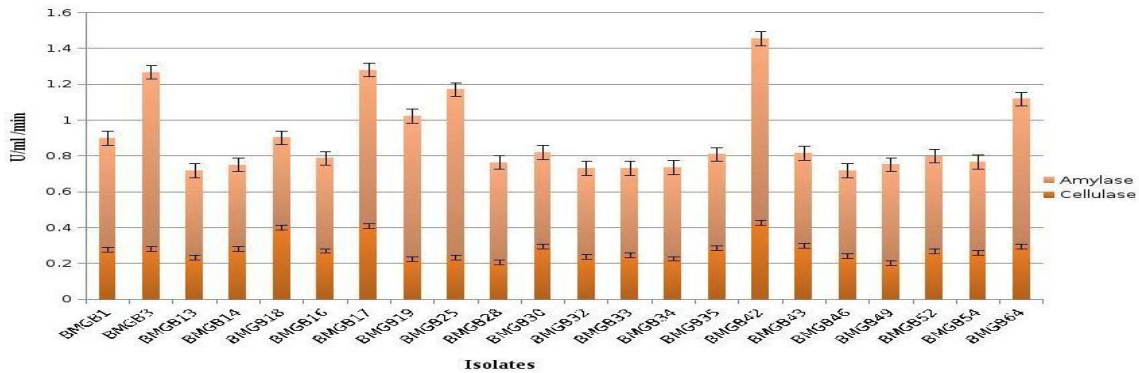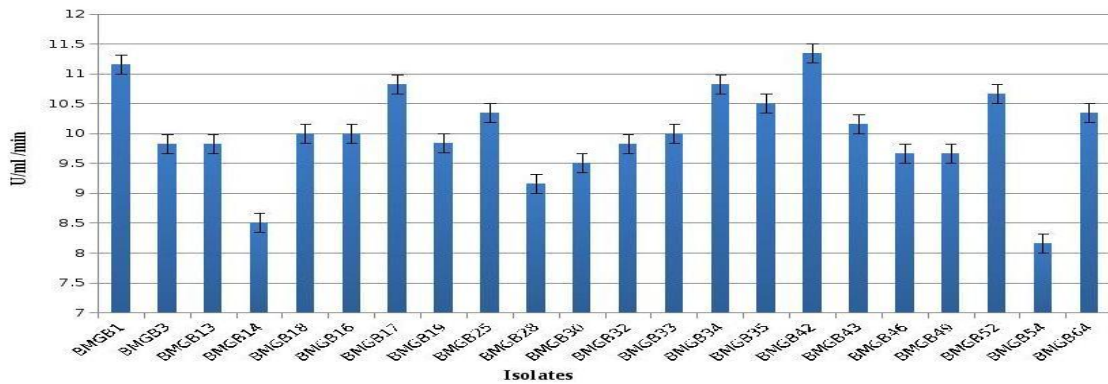

Fig.3 Lipase activity of selective bacterial isolates

Of the isolates tested, 29.17 to 41.67 per cent of isolates were positive for cellulase production, 20.83 to 37.50 per cent of isolates were positive for amylase production and 33.33 to 41.67 per cent of isolates were positive for lipase production in the *Aloe vera* supplemented group (Fig. 1). However in the fifth instar, supplementation with *Tinospora cordifolia* also resulted in increased lipase producing bacteria in the gut and lowest positive isolates for cellulase, amylase and lipase were found in the control group fed only with mulberry leaves. It was found that there was a proportional increase in the percentage of cellulase, amylase and lipase producing bacteria from fourth to fifth instar larval gut system. The increase in these types of bacteria from fourth to fifth instar might be the reason for increased feed consumption.

The consumption of mulberry leaf during final instar accounts for more than 80% of the total consumption during its larval life and the leaves fed for fifth instar larvae are matured leaves rich in carbohydrate content (Kandylis *et al.,* 2009). Food consumed in this stage is effectively utilized for the production of silk proteins as well as to support its metabolism (Lokesh *et al.,* 2006). Thus the energy acquired by the larvae as a consequence of feeding is utilized in the subsequent non-feeding stages. We also found that these cellulase, amylase and lipase producing bacteria are higher in the gut receiving plant products such as *Aloe vera* and *Tinospora cordifolia* along with mulberry leaves. The results are in line with Lokesh *et al.,* (2012) reported that the digestive enzyme activity was found to be higher in fourth and fifth

instar larvae fed with mulberry leaves supplemented with Diethyl Sulfate at 8mM dosage. The amylase, cellulase and β-glucosidase produced by the midgut of *Tenebrio molitor* larvae treated with antibiotics were high compared to non-treated controls (Genta *et al.,* 2006).

Enzyme activity of bacterial isolates from the digestive tract of *Bombyx mori*

The isolates which are found to be positive for all three enzymes *viz.,* cellulase, amylase and lipase production were only considered for enzyme assay studies. A total of 22 positive isolates were selected for enzyme activity. The cellulase and amylase enzyme activity level of the isolates obtained from the gut of silkworm fed with mulberry leaves supplemented with plant extracts at pH 7 ranged from 0.202 to 0.428 (U/ml/min) and 0.46 to 1.02 (U/ml/min) respectively (Fig. 2). The lipase enzyme activity level of the isolates ranged from 8.167 to 11.33 (U/ml/min) at pH 7 (Fig. 3). The enzyme assay for cellulase, amylase and lipase activity was found to be highest for BMGB42 with 0.428, 1.02 and 11.33 (U/ml/min) respectively with *Aloe vera* supplemented through mulberry leaves. Treating the mulberry leaves with herbal drug: kho-go and feeding them to fifth instar larvae was found reflected into significant improvement in the levels of proteins and velocities of biochemical reactions catalyzed by protease and amylase. The activities of mid gut protease and amylase were increased by 21.444 to 83.706 percent and 14.54 to 52.257 percent respectively over control (Khyade Vitthalrao and Doshi Sucheta, 2012). The mulberry leaves supplemented with nitrogenous compounds had a great influence on the amount of glucose in the larval hemolymph (Etebari *et al.,* 2007). The amylase activity increased with increase in age during the fifth instar in EMS treated

batches of silkworm with 1.1 mg/g/min (Mahesha and Honnaiah, 2002).

From the present study it can be concluded that the fortification of mulberry leaves with plant extracts have an impact on improving physiological performances like enzyme activity in silkworms. Gut micro-organisms have the ability to adapt themselves to changes in insect diet, by induction of enzymes or by population changes in the microbial community. We suggest that bacteria from gut of the silkworm fed with mulberry leaves fortified with plant extracts provide digestive enzymes in a synergic manner and contribute to larval growth.

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