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ORIGINAL ARTICLE

Prevalence of *Enterococcus mundtii* in the Fat Body and Its Effect on the Development of Silkworm, *Bombyx mori* L.

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ABSTRACT

The bacteria isolated from fat body of silkworm larvae *Bombyx mori* have been characterized as *Enterococcus mundtii* strain NBRC 100490 and tested for its sensitivity using antibiotic. Effect of the *E. mundtii* NBRC 100490 on the development and silk production has been evaluated. The oral inoculation of bacteria affected the growth of silkworm larvae including silk production, whereas, larvae treated with antibiotic resulted in reduction in mortality and better health, no significant difference in quality and quantity of cocoons, filament length as compared to control. Thus it may be concluded that treatment of erythromycin able to improve the silkworm growth by improving the haemolymph protein and silk cocoon characters. Since bacteria isolated from the fat body is naturally occurring bacteria in tissue may be involved in digestion of fat body tissue when ever is needed especially during starvation to produce energy for maintaining the routine process and act as non pathogenic, whereas turns into the pathogenic for the silk worms, when they are introduced through oral feeding into digestive system.

KEY WORDS: *Bombyx mori,* bacteria, *Enterococcus mundtii,* fat body, silkworm, 16s rRNA

INTRODUCTION

Insects are a large, unexplored and unexploited source of potentially useful compounds for modern medicine (Robert, 1999). Approximately 80% of animal species on earth are insects, 99% are invertebrates. We share a large proportion of our genetic material with all life on earth down to the simplest worms. Silkworm *(Bombyx mori)* is one of the well-known beneficial lepidopteron insects for the production of sleek and sensuous silk fibre, often considered as "Queen of textiles".

Silk is a highly versatile fabric which has proven to be ideal for a variety of uses from formal wear to sleep wear, from parachutes to rugs, from medical sutures to prosthetic arteries. In addition to its economic importance arising from applications in agribusiness, *B. mori* is the main lepidopteron used in scientific research as a genetic resource capable of elucidating a wide range of biological problems (Mandal *et al.,* 2007).

Silkworm, *B. mori,* is domesticated for silk production. All the species of silkworm have four stages in their life cycle egg, larva, pupa and adult. Among these species mulberry silk is the most important and contributes as much as 95% of the total silk production. Therefore the term 'silk' in general refers to the mulberry silkworm, Mulberry silkworm *B. mori* produces cocoons with continuous silk filament and therefore can be industrially reeled to produce raw silk in the form of cocoon. Commercial rearing of silkworms has been in practice for over 5000 years in different parts of the world (Sidhu, 2013) and an estimated 4310 silkworm germplasm strains are being reared worldwide.

In sericulture, apart from silk, there are many other by products and waste products obtained at different stages of silkworm rearing. Eggs, larvae, pupae and feces find their use in pharmaceuticals, cosmetics and the paper and leather industry (Anonymus, 1996). In China, sericulture products are exploited considerably. Silk is made up of mainly two proteins, fibroin and sericin. Fibroin is secreted

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in the posterior part of the silk gland while sericin is from the middle part.

Success of sericulture depends on proper management and protection of silkworm crops from diseases. Four silkworm diseases are very common in India viz., grasserie (viral), flacherie (bacterial), muscardine (fungal) and Pebrine (protozoan). Of these diseases, bacterial flacherie is one of the serious diseases of silkworm causing cocoon crop loss to the tune of 50-70% (Sidhu & Singh 1968); 30-40% (Chitra *et al.,* 1975); 47.9% (Savanurmath *et al.,* 1992). Antibiotics of chemical origin such as erythromycin, Kanamycin, streptomycin, terramycin, etc., have been used to suppress bacterial flacherie especially the bacterial disease of digestive origin. But prolonged exposure to these chemicals may lead to development of resistance in silkworms bacteria in the long run.

Pseudomonas spp., Staphylococcus spp., Streptococcus bombycis, Serratia marcescens, Micrococcus spp., Bacillus spp. and *B. thuringiensis* have been reported associated with significant cocoon crop losses by weakening the immunity of silkworm (Rajendra *et al.,* 2011). Haemolymph plays a key role in the innate immune response triggered by bacterial action. Antibiotics exhibit therapeutic and healing effects in *B. mori* larvae septic with bacterial pathogens. The evolutionary and diversification success of insects into large-scale ecological niches depends on the beneficial microbiome, which is known to promote insect fitness, protect hosts against parasites and pathogens, detoxify insecticidal defence chemicals, and stimulate host immune responses, in addition to its biotechnological applications (Xiang *et al.,* 2007). Liang *et al.* (2018), reported that *E. mundtii,* isolated from the *B. mori* gut, efficiently produces lactic acid under extremely alkaline conditions and is an important metabolite for industrial bioplastic polylactic acid production.

The addition to biochemical responses, insect symbiotic bacteria play key roles in countering plant defences (Hammer & Bowers, 2015). For generalist insects, to some extent, their polyphagous habits rely on several symbiotic bacteria to adapt to phytochemicals from different host plants (Santos-Garcia *et al.,* 2020). However, specialist insects may need specific bacteria to degrade the toxic compounds in their host plants, such as *Enterococcus sp.* From *Hyles euphorbiae* and *Brithys crini,* which have the ability to tolerate alkaloid and latex (Vilanova *et al.,* 2016).

Antibiotics have been used in silkworm rearing for management of diseases, promotion of growth, and improve feed consumption (Mohanta *et al.,* 2013). The antibiotics supplemented mulberry leaves enhance the growth and development of silkworm. Oral supplementation of anti-infection agents along with mulberry leaves to silkworm, helps in the development, fertility and silk contents and decrease the frequencies of diseases (Tayade *et al.,* 1988). To build up the body and spin cocoons, silkworm larvae receive nutrients from mulberry leaves. It has been found that feeding antibiotics with mulberry leaves increased the larval weight and production of silk. The growth, development and their other economic characteristics are also greatly improved (Murthy *et al.,* 1951).

Antibiotics are commonly used as a bed disinfectants and helpful applications against bacterial ailments in the sericulture industry. Antibiotic supplement used to boost the oxygen absorption of the silkworm gut, which has a beneficial effect on the regulation of the larval intestinal flora (Shyamala & Bhatt, 1961).

The fat body is the silkworm's intermediate metabolic organ, playing important physiological roles in nutrient storage and transport (Arrese & Soulages, 2010), metabolic detoxification (Cheng *et al.,* 2006), and immune regulation (Trenczek & Faye, 1988). The insect fat body is an organ that functions in drug metabolism, similar to the liver in mammals where a number of enzymes such as sulfotransferases, involved in drug detoxification, are present in the fat body. Hence Silkworms have been used as infection models to screen and to discover novel therapeutic antimicrobial agents (Hamamoto *et al.,* 2004).

Among 157,424 recognized lepidopteran species (Mitter *et al.,* 2017), <0.1% have been screened for bacterial associates, which reveals that our knowledge on bacterial associates in Lepidoptera is still limited. Hence to explore the prevalence of bacteria in the fat body in the late fifth instar before spinning and evaluate its effect on development of silkworm *B. mori*.

So, the present study focused on the evidence of bacterial presence in the fat body of silkworm, their antibiotic sensitivity and role in development of silkworm *B. mori* L.

MATERIALS AND METHOD

Rearing and Maintenance of silkworm

The disease free egg laying (DFLs) were obtained from Center of Sericulture and Bio-resource Management Research (C.S.B.R) RTM Nagpur University, Nagpur. Silkworm larvae reared in the room well ventilated, clean and thoroughly sterilized and disinfected using disinfectants. The application used for the rearing also disinfected using 2-5% formalin. Temperature and relative humidity of the rearing room was maintained as per the requirement at different stages of development. During winter temperature and humidity maintained using appliances such as water required. During summer cooling

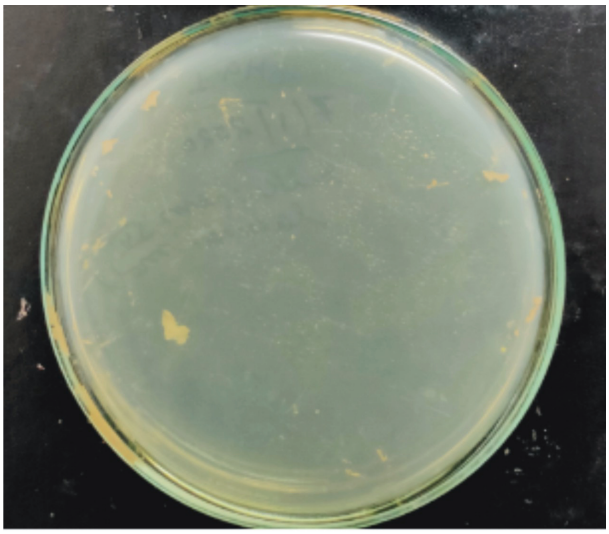


Fig. 1a. Control Agar Plate

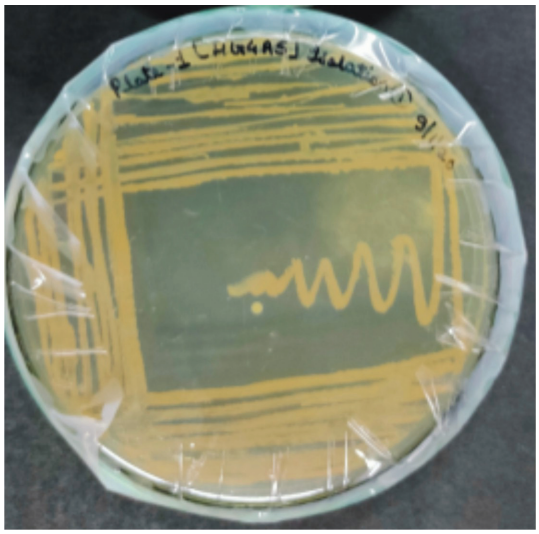


Fig. 1b. Agar plate containing Developed fat body bacteria

Fig. 1c. Bacterial slant for identification

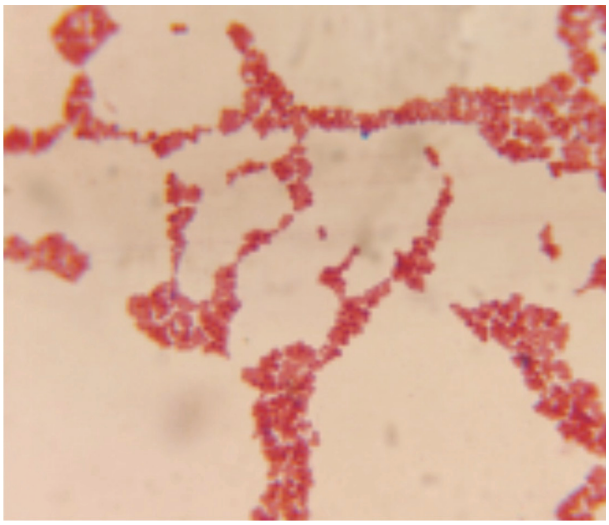


Fig. 2. Gram stained bacterial observed under Microscope

Table 1a: Colony morphology and gram staining result of bacterial isolates.

	Purified isolates	Morphological characters	Colour	Shape	Positive/Negative
Fat Body	Bacterial isolate	Small rounded, dotted colony	Purple	Circular shape	Gram + ve

Table 1b: Bacterial isolates identified using 16S rRNA from Fat body of *B. mori.*

Name of bacteria	Strain of identified bacteria	Fat body
Enterococcus mundtii	Strain NBRC 100490	Gram +ve

Aligned sequence data of sample FB.

GCTGATCCGCGATTACTAGCGATTCCGGCTTCATGTAGGCGAGTTGCACTCCAATCCGAA

CTGAGAGAAGCTTTAAGAGATTAGCTTAGCCTCGCGACTTCGCGACTCGTTGTACTTCCC

ATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACC

TTCCTCCGGTTTGTCACCGGCAGTCTTGCTAGAGTGCCCAACTAAATGATGGCAACTAAC

AATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACA

ACCATGCACCACCTGTCACTTTGCCCCCGAAGGGGAAGCTCTATCTCTAGAGTGGTCAAA

GGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCG

CTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCG

GAGTGCTTAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTCCAACACTTAGCACTC

ATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGAGC

CTCAGCGTCAGTTACAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATATCTA

CGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAGTCTCCCAGTT

TCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAACCGCCTGC

GCTCGCTTTACGCCCAATAAATCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGC

TGGCACGTAGATAGCCGTGGCTTTCTGGTTAGATACCGTCAAGGGGTGAACAGTTACTCT

CACCCTTGATCTTCTCTAACAACAGAGTTTTACGATCCGAAAACCTTCTTCACTCACGCG

GCGTTGCTCGGTCAGACTTTCGTCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAGG

AGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCACCCTCTCAGGTCGGCTATGCATC

GTGGCCTTGGTGAGCCGTTACCTCACCAACTAGCTAATGCACCGCGGGTCCATCCATCAG

CGGCACCGTAAAGCGCCTTTCAAAACGAAACCATGCGGTTTCGATTGTTATACGGTATTA

GCACCTGTTTCCAAGTGTTATCCCCTTCTGATGGGCAGGTTACCCACGTGTTACTCACCC

GTTCGCCACTCCTCTTTCCCGGTGGAGCAAG

RESULT: *Enterococcus mundtii* strain NBRC 100490

Fig. 3: Sequence obtained of *E. mundtii* Strain NBRC 100490 isolated from fat body of silkworm *B. mori.*

equipment were used to maintain the temperature and humidity to avoid leaf drying and desiccation of larvae due to the high temperature. Quality silkworm depends on the management practices *i.e.,* rearing temperature, humidity, nutrition, and photoperiod. The better rearing conditions, environment and nutrition during larval period may leads to higher fecundity of silkworm moths. The rearing condition was maintained as described earlier (Dandin *et al.,* 2003). Bivoltine di-hybrid race of mulberry silkworm, *B. mori* was used for the present study.

Surface sterilization of Fat Body

Late fifth instar larvae before spinning were selected to isolate bacteria from fat body. The larvae surface sterilized and dissected in aseptic condition. Fat body tissue was removed and kept in the sterile vial for further sterilization process to remove surface contaminants. The fat body was further rinsed with 70% ethanol (sterilizing agent) twice within a minute and then washed with sterile distilled water to remove ethanol residue. Immediately, fat body was dipped gently in 0.1 % $HgCl_2$ for a minute and

later rinsed with sterile distilled water. Fat Body tissue was separated to avoid any chance of mixing along with the bacteria.

Isolation of bacteria

Fat body tissue dissected out from silkworm larvae, surface sterilized and washed with sterile water. Sterilized fat body tissue was homogenized in sterile distilled water. A plate having nutrient agar was plated using fat body homogenate and incubated for any bacterial growth. Similarly another agar plate was plated with sterile distilled water used for making homogenate, also incubated at similar condition and considered as control (Fig. 1a). Both homogenate platted in the nutrient agar and control plates were incubated at 37°C for 24 hours, and 36h to assure complete growth of bacterial colonies (Fig.1b).

Identification of bacteria

Gram staining and Morphology

The bacterial colonies deveopled on agar plate were sub cultured, purified and then mass produced on nutrient agar slants for further investigation. Isolated bacterial colonies were gram stained and morphological characters were recorded using binocular microscope (Carl Zeiss Primo).

Identification of bacteria using 16S rRNA

Bacterial cultures isolated from the fat body used for the genomic isolation following modified CTAB protocol, and further through PCR reaction as described below. The amplified 16S rRna gene fragment was purified and sequenced using DNA sequencing services (Eurofins Scientific, Bangalore. About 200 ng of bacterial DNA was used to amplify 16S rRNA gene applying following 16S universal primers:-

16S Forward primer:
5' AGA GTT TGA TCC TGG CTC AG 3'

16S Reverse primer:
5' AAG GAG GTG ATC CAG CCG CA 3'

The sequences were checked against the microbial nucleotide databases using BLASTN search algorithms (Zhang, 2003; Zhang *et al.,* 2013). In requirement online BLAST program available at web link http://blast.ncbi.nlm.nih.gov/ was used. In an output result, the file was retrieved as DND which was used to build the phylogram in MEGA5 software based on the alignment obtained in CLUSTALW analysis.

Antibiotic Sensitivity Test

The Kirby Bauer Method of antibiotic Sensitivity assay was implemented to test sensitivity of bacteria against antibiotics. 10µl bacterium from $1.5x10^8$ bacterial broths (which was prepared using McFarland standard) is spread on the nutrient agar plate for 10 mcg discs of antibiotics. Plates were inoculated with antibiotic discs of Gentamicin (10mcg), Streptomycin (10mcg) and Erythromycin (10mcg) under aseptic conditions and further incubated at 37°C for 24 hrs. The results obtained were recorded after completion of incubation period.

Bacterial inoculation and antibiotic treatment

About 200 fourth instar freshly moulted larvae were selected separated and divided into different groups and coded as;

A. Freshly moulted 50 fourth instar larvae selected and used as control (C),
B. 50 larvae inoculated with low concentration of bacterial isolates about 10ul having conc. of $1.5x10^8$ cfu/ml smeared on pieces of mulberry leaf, air dried and feed to larvae of respective groups are reared (B),
C. 50 larvae treated with erythromycin every alternate days and remaining feeding was using normal leaves (ER).
D. 50 larvae treated with bacteria (B) and Erythromycin (ER) treatment given every alternate days (B+ERY).

The bacterial suspension, separately and also in combinations using antibiotic were used. Larvae of all the groups reared using chowki rearing method. Feeding, cleaning and other rearing schedule was followed as described earlier (Dandin *et al.,* 2003). The following parameters, duration of life cycle, larval weight, survival, haemolymph protein content have been recorded.

Estimation of protein

Haemolymph collected from fifth instars larvae of silkworm from control and inoculated (treated) group and estimated for protein Using Lowry's method (1951). The larvae were weighed, prolegs were amputed and oozing out haemolymph collected in eppendorff coated with phenylthiourea (to avoid coagulation of protein), stored at -20°C until use. Amount of protein estimated using optical density (O.D) at 660 nm to 680 nm Spectrophotometer.

RESULTS

The mulberry silkworm has been reared in the laboratory condition at Centre for Sericulture and Biological Pest Management Research (CSBR), RTM Nagpur University, Nagpur. The rearing condition was maintained having specific temperature and relative humidity and hygienic condition as described earlier.

Prevalence of bacteria in the fat body

The observation made after the incubation of

Table 2: Antibiotics sensitivity *E. mundtii* isolated from fat body of *B. mori*

Treatments	Zone of inhibition in (mm)	
	Horizontal	Vertical
Gentamicin (mcg)	18	17
Streptomycin (mcg)	16	15
Erythromycin (mcg)	19	20

Table 3: Effect of *E. mundtii* and erythromycin treatment on mortality in *B. mori.*

		Mortality			
		Enterococcus mundtii			
Instar	Day	C	E	B	B+E
V	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	1	0
	6	0	0	0	0
	7	0	0	0	0
	Spinning	3	2	6	0

C = Control, B = Bacterial culture, E = Erythromycin, B+E = Bacteria + Erythromycin

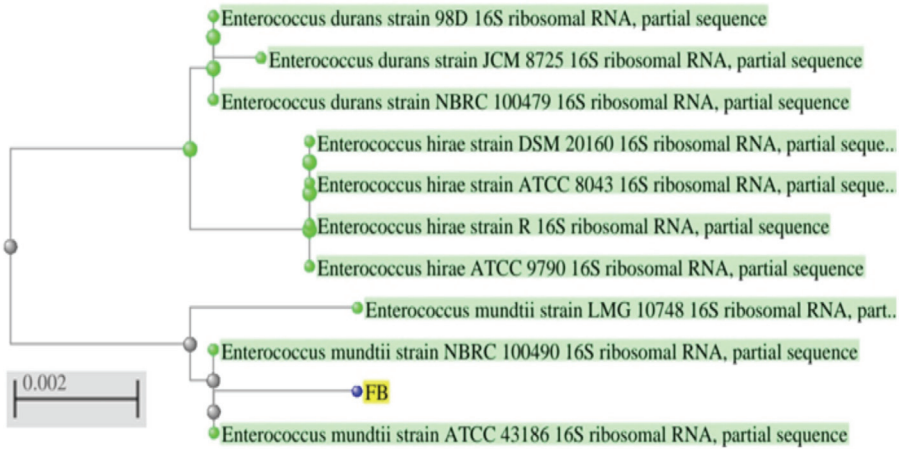
Fig. 4: Sequence producing significant for *E. mundtii* Strain NBRC 100490

Blast Homology

Sl.No.	Organism Name	Accession No.	% Match
1	Enterococcus mundtii strain NBRC 100490 16S ribosomal RNA	NR_113906.1	99.54%
2	Enterococcus mundtii strain ATCC 43186 16S ribosomal RNA	NR_024906.1	99.54%
3	Enterococcus mundtii strain LMG 10748 16S ribosomal RNA	NR_114784.2	99.07%
4	Enterococcus durans strain 98D 16S ribosomal RNA	NR_036922.1	98.84%
5	Enterococcus durans strain NBRC 100479 16S ribosomal RNA	NR_113900.1	98.84%
6	Enterococcus durans strain JCM 8725 16S ribosomal RNA	NR_113257.1	98.76%
7	Enterococcus hirae strain DSM 20160 16S ribosomal RNA	NR_114743.1	98.76%
8	Enterococcus hirae strain ATCC 8043 16S ribosomal RNA	NR_114452.1	98.76%
9	Enterococcus hirae strain R 16S ribosomal RNA	NR_037082.1	98.76%
10	Enterococcus hirae ATCC 9790 16S ribosomal RNA	NR_075022.1	98.76%

Fig. 5: Phylogenic position of *E. mundtii* Strain NBRC 100490

Phylogenetic Tree



inoculated agar plates with distilled water and without distilled water no growth of bacterial colony was observed (Fig. 1a). Observation made to confirm bacterial growth from the smear of fat body (Fig.1b). The result shows that, plates smeared with homogenate of fat body sample contained one colony. In sample the morphology of the colony was rounded in shape. The colonies observed were picked and streaked into slants for further identification and characterization.

Identification of Bacteria

Gram Staining

Based on the morphological characters appearance, colour, shape and their negative or positive characteristics, the strain was categorized and grouped (Fig. 2). The Gram stained sample suggested that, the isolates obtained from the fat body is gram positive cocci and looked purple colour (Table 1a,1b).

Characterization Using 16S rRNA

Genomic DNA was isolated from the sample. The ~1.5 kbp, 16s-rDNA fragment was amplified using high-fidelity PCR polymerase. The PCR product was sequenced Bi-directionally (Fig. 3). The sequence data was aligned and analyzed to identify the Bacteria and its closest neighbors (Fig. 4).

Genomic DNA analysis

The Microbe was found to be *Enterococcus mundtii* strain NBRC 100490 16S r RNA.

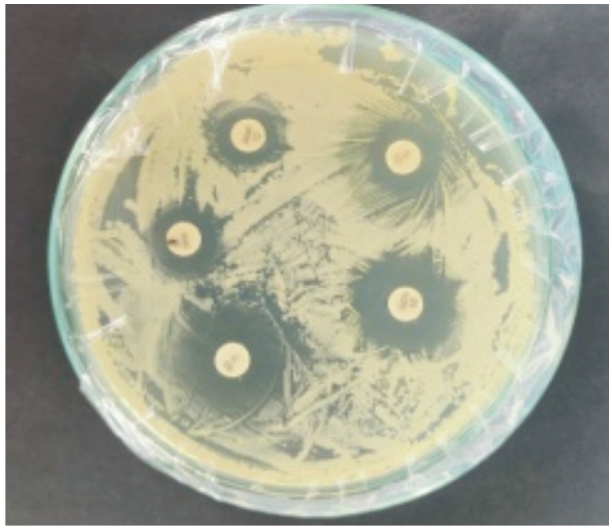
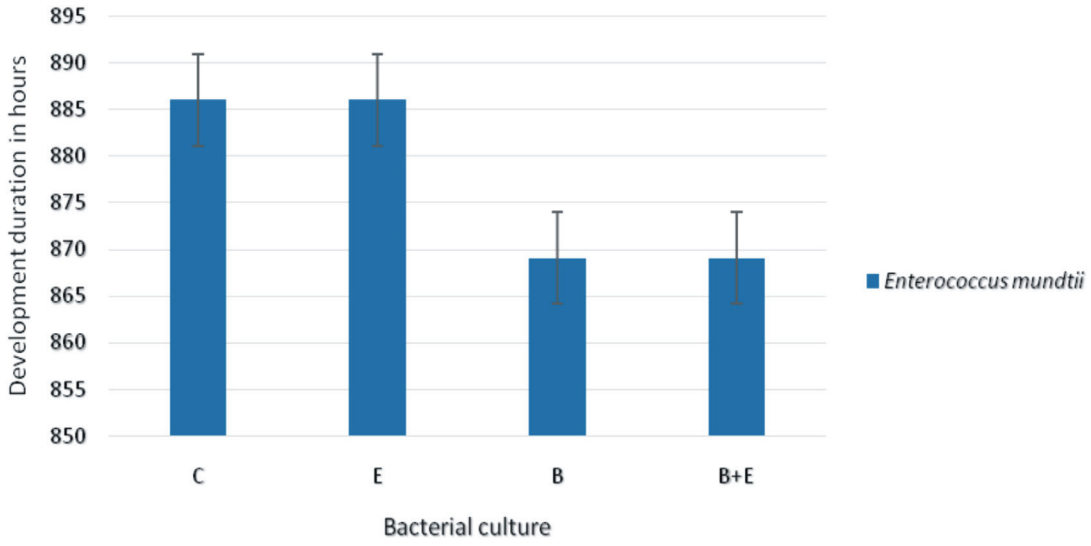


Fig. 6. Zone of inhibition using Antibiotic sensitivity test

Sequence ID: NR_113906.1. The next closest homologue was found to be *Enterococcus mundtii* strain ATCC 43186 16S ribosomal RNA, Sequence ID: NR_ 024906.1 (Fig. 5).

Antibiotic Sensitivity Assay

Antibiotic susceptibility test of the isolates were performed by Kirby-Bauer disk diffusion method in compliance and clinical and laboratory standards institutes (CLSI) guidelines using Nutrient agar media. All aspect of



Graph 7: Effect of *E. mundtii* and antibiotic treatment on development duration in *B. mori.*
C = Control, E = Erythromycin, B = Bacterial Culture, B+E = Bacteria + Erythromycin

the Kirby-Bauer procedure was standardized to ensure reliable results. The plates were held 3 inches above the plates illuminated. End point of inhibition could be even judged with the naked eye at the point of abrupt diminution of growth or the point of 80% inhibition. The zone of inhibition was measured for bacterium shown in the culture (Table 2, Fig. 6). *E. mundtii* was more sensitive to Erythromycin and produce zone of inhibition 19 mm, whereas for Streptomycin 16mm and Gentamicin 18mm.

Development Duration

The larvae of groups inoculated with *E. mundtii*. The total development duration of control larvae was 36 days 22 hours (886 h), whereas larvae after inoculation treated with antibiotic took 36 days 20 hours (864 h), inoculation of bacterial isolates *E. mundtii* required 36 days 5 hours (869 h), and other group treated with antibiotic and bacterial inoculated group development duration was 36 days, 7 hours (871 h) in B+E group. The results obtained from different groups treated with erythromycin and bacteria inoculated then treated with antibiotic showed the shorter developmental period when compared with control and antibiotic treatment group (Fig. 7).

Larval Body Weight

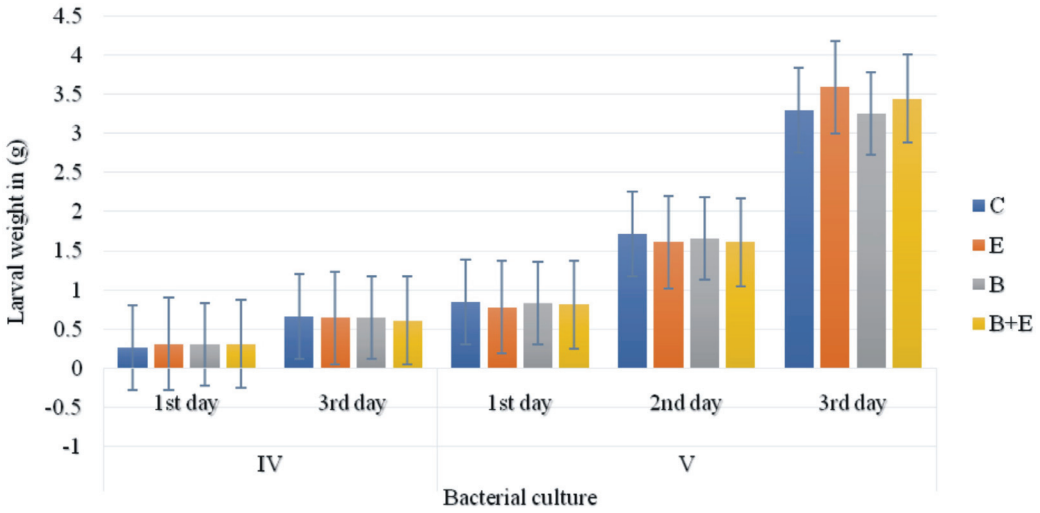
The bacterial isolate shows difference in larval weight of silkworm *B. mori* (Fig. 8). The bacterial inoculation of first was given in fourth instar and doses of bacteria and antibiotic was given alternately up to fifth instar seventh day. The weight gained by the Erythromycin treated and Bacteria inoculated from fourth instar 1st day up to spinning stage show that, the weight of antibiotic treated group was slightly higher on fourth instar 1st day. The other groups, which treated with antibiotic after inoculating bacteria shows increased in weight as compare to bacterial inoculated. The larval weight on fifth instar 1st day the Control, Bacteria and B+E treated group show increase in weight but Antibiotic (Ery) shows slightly lower weight. On the sixth day the weight of Erythromycin treated group is 3.59±0.07g shows higher weight than *E. mundtii* treated group 3.25±0.03g and B+E group 3.44±0.05g. There is no significant change in overall larval weight of all groups (Fig. 8).

Mortality

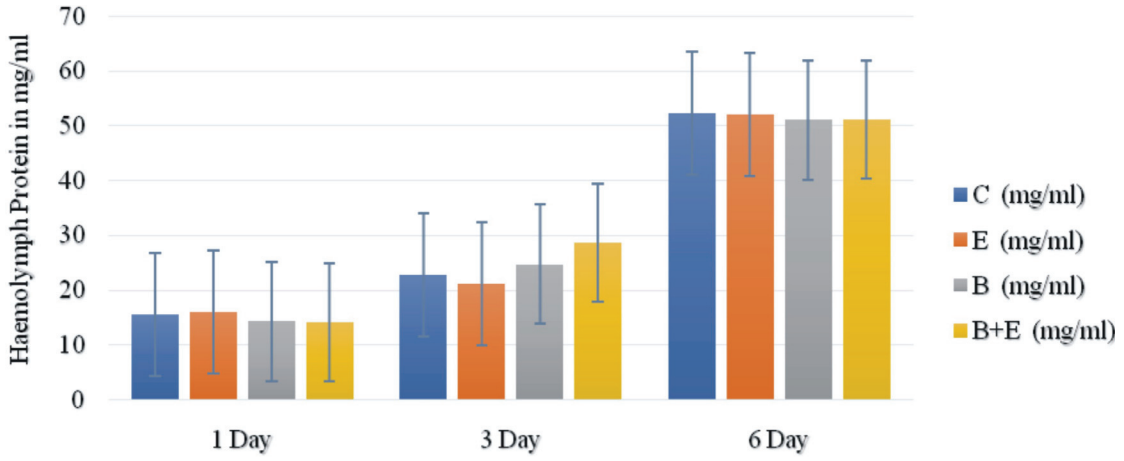
The larvae inoculated with bacteria turned blackish, became sluggish, small liquid from mouth and from anal part oozed out of the body at the later stage of infection (Table 3), Silkworm did not show any mortality till fifth instar 4th day, but it was observed in the fifth instar stage on 5th day. Few larvae appeared dull, blackish mark observed on body and reduced in size. Higher mortality observed during spinning. Larvae unable to spin and were able formed flimsy cocoons (Table 3).

Haemolymbph Protein

Haemolymph protein level fluctuated during larval development. Erythromicin treated larvae has slightly higher level compared to control on day 1 and even much higher than other two groups. On day three, protein level increased significantly in the larvae inoculated with bacteria and inoculated then treated with erythromycin, compared to control and only erythromycin treated larvae. Surprisingly on day 6 protein levels in the larvae of all the groups showed very similar level of protein in the haemolymph which showed that protein level raised



Graph 8: Effect of *E. mundtii* and erythromycin on larval weight during development of *B. mori.*



Graph 9: Effect of *E. mundtii* and erythromycin on haemolymph protein of *B. mori.*

sharply in control and antoibiotic treated larvae (Fig. 9). The result suggests that there is no effect of antibiotic and bacteria either in isolation or in combination.

DISCUSSION

In the present study, mulberry silkworm rearing was maintained at specific temperature and relative humidity and a hygienic condition was maintained as described by (Dandin *et al*., 2003). Temperature and humidity played important role in the development and growth of immature stages of insect. The optimum temperature and relative humidity required for the proper growth of early age larvae ranged from 26 to 28°C and 80-90% respectively and of late age larvae ranged from 24-26°C and 70-75%, respectively.

Silkworm, *B. mori,* feeds only upon mulberry leaves is very sensitive to surrounding environmental conditions. During the summer season there is a need of maintaining temperature and humidity as per requirement so that the larvae are not infected. Flacherie disease is the most common of all diseases. Hence, the larvae can be infected with such diseases easily. Precautions must be taken for prevention of such diseases during rearing.

The insect fat body, mainly containing adipocytes, is the central tissue to store excess nutrition. The fat body participates in synthesis and decomposition of lipid, amino acids, protein, carbohydrate, and glycogen (Arrese & Soulages, 2010). The contribution of *Enterococcus* species to the competitive exclusion of pathogenic bacteria in the host gut microbiota by the synthesis and release of bacteriocins has been reported for a range of insect hosts (Shao *et al.,* 2017). The bacteriocin mundticin was first identified as a bioactive molecule produced by *E. mundtii* NFRI 7393 isolated from grass silage (Kawamoto *et al.,* 2002), and more recently shown to be secreted by *E. mundtii* associated with the larval gut of *Spodoptera littoralis* and proposed to protect the host gut from establishing pathogenic interactions.

In the present study the full-length sequence of 16S rRNA gene of LAB isolates shared similarity to genera *Enterococcus* and *Weissella* with higher than 99%. Among 51 isolates, 35 were identified to be *Enterococcus mundtii,* which represented 68.6% of total isolates, followed by *Entercoccus faecalis* (15.7%), *Weissella cibaria* (7.8%), *Enterococcus hirae* (3.9%), *Enterococcus lactis* (2%), and *Enterococcus faecium* (2%). *Enterococcus* and *Lactobacillus* have also been reported earlier to be the predominant LAB genera in mulberry silkworm, where the relative abundance varied depending on silkworm species and their physiological activities (Yeruva *et al.,* 2020).

Mundcitin is also available in the genome of *E. spodopteracolus n. sp.* sggesting this bacterium can provide additional defensive mechanisms against pathogen colonization of the gut. As lactic acid bacteria, both *E. entomosocium n. sp.* and *E. spodopteracolus n. sp.* May be able to produce organic acids during growth, reducing the medium pH to facilitate their growth in alkaline environments like the gut of lepidopteran larvae (Zhang *et al.,* 2022).

Applying the commercial probiotic formulation was found to improve the larval body weight, effective rate of rearing, cocoon weight, pupal weight, shell ratio, and silk productivity (Anitha *et al.,* 2015, Yadav *et al.,* 2016). Since synthetic antibiotics are widely applied in sericulture for disease control and some previous studies showed the antibiotic potential of LAB from silkworm against a range

of both Gram-positive and gram-negative pathogenic bacteria, Considering the use of laboratory isolated bacteria from silkworm gut with the antimicrobial activity has been proposed to be an alternative way for ecofriendly management of silkworm diseases (Sonnenburg *et al.,* 2006).

The result of the present study suggested that the development period drastically reduced with the bacterial inoculation and remained the same even after the antibiotic treatment. Furthermore, some Enterococci strains showed their ability to produce several bacteriocins (Shao *et al.,* 2017), which confirm a competitive advantage toward the pathogenic microbes, and some *Enterococcus* spp. have been reported to be used as probiotics, such as *E. faecium, E. faecalis, E. lactis, E. hirae,* and *E. durans* (Mala & Vigila, 2018).

In the present study, control larvae show slight increase in body weight, better cocoon formation and also the silk content but not significant when larvae received bacterial inoculation or inoculation and followed by erythromycin treatment, this even not affected the haemolymph protein level in the silkworm. The present results are in accordance with previous experiment of oral supplementation using anti-infection agents along with mulberry leaves to silkworm helped in the development, fertility and silk contents and decrease the frequencies of diseases (Tayade *et al.,* 1988). Antibiotics in silkworm are approved for four different purposes, disease treatment, disease prevention, disease control and for health maintenance or growth promotion (Phillips *et al.,* 2004).

In the present investigation, bacteria isolated from fat body of late fifth instar eighth day larva before spinning considered as indigenous bacteria and might be available throughout the development of *B. mori.* Presence of bacteria in the fat body may have defence against the other pathogenic microorganisms, and might be involved in the improvement autoimmunity and also help in reproduction. Hence from the results it can be concluded that treatment of erythromycin improved the silkworm growth and by suppressing the bacteria and also had no effect on haemolymph protein and silk cocoon characters hence may be considered as symbiotic bacteria. Therefore it is suggested that erythromycin could be used even in the normal rearing to avoid spread of pathogen and improve the silk quality and cocoon production.

REFERENCES

Anitha, J., Sathish, J. & Sujatha, K. (2015). Nutritional efficiency and economic traits in *Samia ricini* donovan reared on castor leaves fortified with probiotic agent. *Int. J. Inn. Res. Sci. Eng. Tech.,* 4: 2319-8753.

Anonymous (1996). By-products for better revenue. *Ind. Silk,* 35: 3.

Arrese, EL. & Soulages JL. (2010). Insect fat body energy, metabolism, and regulation, *Ann. Rev. Entomol.* 55: 207-225.

Cheng, D.J., Xia, Q.Y., Zhao, P., Wang, Z.L., Xu, H.F., Li, G.R., Lu, C. & Xiang, Z.H. (2006). EST-based profiling and comparison of gene expression in the silkworm fat body during metamorphosis. *Arch. Insect Biochem. Physiol*., 61: 10-23.

Chitra, C., Karanth, N.G.K., & Vasantharajan, V.N. (1975). Disease of the mulberry silkworm, *Bombyx mori* L. *J. Scient. Ind. Res*., 34: 386-401.

Dandin, S. B., Jayaswal, J. & Giridhar, K. (2003). Handbook of Sericulture Technologies. Central Silk Board Publication, 3rd edition Banglore, pp. 1-285.

Hamamoto, H., Kurokawa, K., Kaito, C., Kamura, K., Manitra, R.I., & Kusuhara, H. (2004). Quantitative evaluation of the therapeutic effects of antibiotics using silkworms infected with human pathogenic microorganisms. *Antimicro. Agents Chemoth.*., 48: 774-779.

Hammer, T.J. & Bowers, M.D. (2015). Gut microbes may facilitate insect herbivory of chemically defended plants. *Oecol.,* 179: 1-14.

Kawamoto, S., Shima, J., & Sato, R. (2002). Biochemical and genetic characterization of mundticin KS, an antilisterial peptide produced by *Enterococcus mundtii* NFRI 7393. *Appl. Environ. Microbiol*., 68: 3830-3840.

Liang, X., Sun, C., Chen, B., Du, K., Yu, T., Luang-In, V., Lu, X. & Shao, Y. (2018). Insect symbionts as valuable grist for the biotechnological Mill: An alkaliphilic silkworm gut bacterium for efficient lactic acid production. *Appl. Microbiol. Biotechnol.,* 102: 4951-4962

Lowry, O.H., Rosebrouh, N.J., Farr, A.L. & Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *The J. Bio. Che.,* 193(1): 265-275.

Mala, M. & Vijila, K. (2018). Beneficial effects of *Bacillus licheniformis* and *Bacillus niabensis* on growth and economic characteristics of silkworm, *Bombyx mori* L. *Int. J. Chem. Stud.*, 6: 1750-1754.

Mandal, M., Trivedy, K., & Kumar, S.N. (2007). The Silk proteins, sericin and fibroin in silkworm, *Bombyx mori* Linn. A review. *Caspian J. Environ. Sci*., 5(2): 63-76.

Matsumoto, E., Matsui, M. & Tamura, H.O. (2003). Identification and purification of sulfotransferases for 20-hydroxysteroid from the larval fat body of a fleshfly, *Sarcophaga peregrina*. *Biosci., Biotechnol. Biochem*., 67: 1780-1785.

Mitter, C., Davis, D.R., & Cummings, M.P. (2017). Phylogeny and evolution of Lepidoptera. *Annu. Rev. Entomol.,* 62: 265-283. Doi: 10.1146/annurev-ento-031616-035125.

Mohanta, M., Saha, A., Saleh, D. & Hasan, M. (2013). Bioefficacy of some plant extracts against pathogenic bacteria isolated from diseased silkworm larvae. *Univ. J. Zool.*, 32: 9-14.

Murthy, M.R.V., Shankarappa, B.S. & Sreenivasaya, M. (1951). Influence of antibiotics on the growth of silkworm, *B. mori*

L. *J. Curr. Sci.*, 20: 269-270.

Phillips, I., Case W.M., Cox, T., Groot, D., Brad F.C., & Ronald, J. (2004). Does the use of antibiotics in food animals pose a risk to human health? a critical review of published data. *J. Antimicro. Chemoth*., 54(1): 76-278.

Rajendra, B., Chandrashekhar, H.J. & Avhad, S.B. (2011). Prevalence of muscardine disease in different silkworm hybrid, *Bombyx mori* L. under agro-climatic conditions in aurangabad (ms), India. *J. Life Sci.*, 5: 136-138.

Robert, W.P. (1999). Insects and other arthropods used as drugs in Korean traditional medicine. *J. Ethno Pharmacol*., 65(3): 207-216.

Santos-Garcia, D., Mestre-Rincon, N., Zchori-Fein, E. & Morin, S. (2020). Inside out: Microbiota dynamics during host-plant adaptation of whiteflies. *IMSE J*. 14: 847-856.

Savanurmath, C.J., Basavarajappa, S., Hinchigeri, S.B., Ingalhalli, S.S., Singh, K.K. & Sanakal, R.D. (1992). Relative incidence of silkworm viral diseaes in agroclimatic zones of Northern Karnataka, India. In: Natl. Conf. Mulb. Seric. Res., CSR & RI, Mysore. December. 10 & 11. P. 123.

Shao, Y., Chen, B., Sun, C., Ishida, K., Hertweck, C., & Boland, W. (2017). Symbiont-derived antimicrobials contribute to the control of the lepidopteran gut microbiota. *Cell Chem. Biol*., 24: 66-75. Doi: 10.1016/*j.chembiol*.11.015.

Shyamala, M.B. & Bhat, J.V. (1961). On the relationship between panthothenate levels and the growth response of the silkworm to chloromycetin. *Intern. J. Curr. Res.*, 20: 333-335.

Sidhu, H. (2013). New hybrids for commercial crops for multivoltine silkworm. *J. Insect Physiol.*, Indonesia. 56(3): 897-911.

Sidhu, N.S. & Singh, N.K. (1968). Resistance of silkworm mutant strains and breeds and inductive factors leading to the development of grasserie and flacherie diseases in silkworm, *Bombyx mori.* L. *Ind. J. Seric*., 7: 27-31.

Sonnenburg, J.L., Chen, C.T.L. & Gordon, J.I. (2006). Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. *PLoS Biol*., 4: e413.

Tayade, D.S., Jawale, M.D. & Unchegaonkar, P.K. (1988). Effect of antibiotic on the growth of silkworm *Bombyx mori* L., *Ind. J. Sericul.*, 27(2): 69-72.

Trenczek, T. & Faye, I. (1988). Synthesis of immune proteins in primary cultures of fat body from *Hyalophora cecropia. Insect Biochem.,* 18: 299-312.

Vilanova, C., Baixeras, J., Latorre, A. & Porcar, M. (2016). The generalist inside the specialist: Gut bacterial communities of two insect species feeding on toxic plants are dominated by *Enterococcus sp. Front. Microbiol*., 7: 1005.

Xiang, H., Li, M., Zhao, Y., Zhao, L., Zhang, Y. & Huang, Y. (2007). Bacterial community in midguts of the silkworm larvae estimated by PCR/DGGE and 16S rDNA gene library analysis. *Jiangxi Sci.,* 50: 222-233.

Yadav, M., Shukla, A.K., Srivastva, N., Upadhyay, S.N. & Dubey S.K.. (2016). Utilization of microbial community potential for removal of chlorpyrifos: a review *Crit. Rev. Biotechnol.*, 36: 727-742.

Yeruva, T., Vankadara, S., Ramasamy, S. & Lingaiah, K. (2020). Identification of potential probiotics in the midgut of mulberry silkworm, B*ombyx mori* through metagenomic approach. *Probiotics Antimicro*., 12: 635-640.

Zhang, H. (2003). Alignment of BLAST high-scoring segment pairs based on the longest increasing subsequence algorithm. *Bioinformatics,* 19(11): 1391-1396.

Zhang, J., Shen, J., Tang, X., Xu, L. & Zhu, F. (2013). Isolation and idenfication of a pathogen, Providencia rattgeri, in *Bombyx mori. Global J. Bacteriol. Cytol. Nametol.,* 1(1): 49-55.

Zhang, X., Zhang, F. & Lu, X. (2022). Diversity and functional roles of the gut microbiota in Lepidopteran insects. *Microorg.,* 10(6): 1234.