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Degumming of muga cocoon with mycogenic extracellular protease and lipase enzyme - An alternative method for efficient reeling of silk

Indrani Sarma

The Energy and Resources Institute, North Eastern Regional Centre, Guwahati

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ABSTRACT

Sericulture is an agro-based industry with traditional living culture and plays an important role in the rural economy of the North-eastern region of India. Among various silk the Muga (Antheraea assamensis) silk has a tremendous demand as textile commodity. Reeling is the vital process of conversion of the cocoons into an industrial output yarn which involves various traditionally used chemicals including washing soda, sodium sulphite, sodium silicate etc. The uses of these chemicals are hazardous, cause environmental pollution and also reduce the quality of silk thread. As an alternative for degumming of muga cocoon the Fungal enzymes like protease and lipase are used which can hydrolytically cleave the peptide bonds of sericin without destroying the fibroin. In the present study 11 different fungal strains were isolated from soil and waste cocoon sample collected from different waste cocoon dumping site and waste cocoons of various muga reeling small scale industries of Kamrup district of Assam. Among them depending on the REA values only 6 strains were selected with different degrees of production of protease and lipase enzymes for enzymatic degumming. Among these the fungal isolates Aspergillus niger was found to be most effective for production of protease as well as lipase enzyme for degumming of Muga silk fibres. From the study of physical properties of the degummed muga fibre it was established that fungal secretes play an important role for degumming and recorded optimum properties with Aspergillus niger (Tenacity 3.837g/den, Toughness-0.986 g/den)which was followed by Aspergillus tamarii (Tenacity 3.787g/den, Toughness-0.985 g/den).

Keywords: Fungal enzymes, muga silk, *Antheraea assamensis*, tensile strength.

INTRODUCTION

Muga silk is obtained from the silkworm (*Antherea assamensis*) and popular for its natural golden colour, glossy fine texture and durability. Cocoon is a protective shell made up of a continuous protenious filament spun by the mature silk prior to pupation. The silkworm secretes continuous filament of core protein fibroin produced by its two salivary glands,

The continous filament of fibroin protein binds with another protein *i.e* sericin which is gummy in nature become herden together to form the muga silk.

Muga cocoon contains fibroin protein(70-80%), sericin(20-30%), carbohydrates(1.6%), waxy matter(0.4-0.8%) and inorganic matter(0.7%) [9, 16].

Reeling of silk is a process of unwinding the filaments from a number of cooked cocoon together to produce a single thread.

As to draw the silk filament by reeling process the cocoon is softened by boiling in hot water and decomposing or partially solubilising the sericin component, the proteinaceous silk gum, which binds the protein fibroin strands. Degumming is the removal of sericin by breaking the peptide linkage of amino acid in sericin structure into small water soluble molecule.

In this process other particles are also drawn from silk fibre which facilitate to obtain adequate quantity of reeling ends for the formation of a composite thread of high cohesion and size of yarn.

Although different methods are used for scouring the silk from cocoon but enzymatic treatments for silk refining process is limited as compared to other textile industry. So, there is an urgent need for scientific studies for potential application of mycogenic enzymes in cocoon cooking and degumming process.

During reeling process various chemicals like washing soda, sodium sulphite, sodium silicate, sodium lauryl sulphate, hydrogen peroxide etc. are traditionally used for drawing the silk thread from the cocoons. However, these chemical methods reduce the quality of silk thread, hazardous to the health of the workers and also cause environmental pollution. So, there is a scope for enzymatic degumming of silk as an eco-friendly alternative approach having advantages over other chemical methods. Eco friendly microbial enzymes, which can hydrolytically cleave the peptide bonds of sericin without destroying the fibroin may be utilized as a replacement of the harsh and energy demanding chemical treatments. The use of mycogenic protease and lipase enzyme will help in reeling of silk fibers which may enhance the productivity and improves the silk quality.

The combined effect of lipase and protease enzyme showed an effective de-waxing and degumming of silk fibres [4]. Degumming with fungal protease and lipase enzyme can be a viable alternative to the existing methods of degumming which is economically viable, chemically non- hazardous to the environment.

The current study shows the effectiveness of different fungal isolates for production of lipase and protease enzyme which is applicable for degumming of silk fibres.

MATERIALS AND METHODS

Isolation of Fungal isolates: Inoculum preparation from waste cocoon and Soil sample of waste cocoon dumping site: Waste cocoons were cleaned with tape water and then cut into small segments sizes 1 mm × 1 mm(approx.) dimensions for direct inoculation to the 0.1% ampiciline containing semi solid PDA (potato dextrose agar) medium. Soil sample (10.0 g) collected from the waste cocoon dumping site were put into a 100 ml sterile distilled water and shacked well (about 10 minutes) and made a serial dilution of 10-^{4}, 10^{-5}, 10^{-6}, $10^{-7}$and 10^{-8} which were inoculated to the semi solid PDA medium with 0.1% ampicillin to avoid bacterial growth. Fungal strains were also isolated by inoculating the degraded cocoon parts directly into the PDA medium containing ampicillin.

All the samples inoculated in the culture medium were placed in the BOD incubator and the temperature were maintained at 28°C for 3 -5 days and observed the mycelial growth.

Purification and identification of isolated fungi: The isolated fungi were purified by point inoculation on plates containing PDA medium and studied their purity under 40X-100X magnification using trinocular microscope. In the present study 11 different fungal strains were isolated from soil and waste cocoon sample collected from different waste cocoon dumping site and waste cocoons of various muga reeling small scale industry. The purified fungal isolates were identified based on morphological and microscopic observations by referring the standard. After ensuring purity, the cultures were sub-cultured on PDA slants and allowed to grow for a period of 5-7 d and subsequently stored at 4°C as stock cultures. The authenticity of these morphologically and microscopically identified Fungal isolates were validated finally at National Centre for Fungal Taxonomy (NCFT), New Delhi.

Primary Screening of Fungal isolates for alkaline protease production :
Fungal isolates were tested for their ability to produce protease enzyme using solid agar plate assay on Reese medium (containing KH2PO4-2g, (NH4)2SO4-1.5g, MgSO4. 7H2O-0.3g, Cacl2-0.3g, urea-0.3g, yeast extract-0.5g, glucose-2.5g, casein-5.0g, agar-20g, distilled water-1000ml, pH 9) [13]. The fungal strains were grown on Reese's medium containing 0.5% casein as protein substrate. The fungal isolates were inoculated under sterile laminar air flow condition and after that these plates were incubated in BOD incubator maintaining temperature at 28°C and allow the growth of tested fungi for another 4 days. Cultures were examined for the formation of zone of clearance around the colony. The zone were made clearer, by flooding the plates with a solution of 5% Trichloroacetic acid (TCA) and kept for 30 min to allow the precipitation of residual proteins in the medium. The diameter of the fungal colony and the total zone of enzyme activity including the growth diameter were measured for each fungal isolates and the 'Relative Enzyme Activity' (REA) was calculated.

The fungal isolates that gave biggest zone of inhibition was selected as the best producer of protease enzyme and grown in Reese broth maintained at 28°C for 96 h in the incubated shaker at 150 rpm. After incubation, the fungal biomass was removed by filtering through Whatman No. 1 filter paper. The culture filtrates were centrifuged at 5000

rpm for 10 min at 4°C temperature and the enzyme activity in the supernatant were re-checked by agar-well diffusion method in casein containing media.

Secondary screening: The fungal isolates that showed biggest zone of inhibition in the media were selected as the best producer of protease enzyme and were grown in alkaline Reese broth at 28°C for 96 h at 150 rpm. After the incubation period , the flasks were removed and filtered through Whatman No. 1 filter paper. The culture filtrates were centrifuged at 5000 rpm for 10 min and the enzyme activity in the supernatant was checked by agar-well diffusion method in the media enriched with casein [14].

Total protein concentration was determined by Lowry's method in the enzyme solution [6].

Assay of proteolytic activity of different fungal strains : Protease activity of each fungal strain secreted was measured using casein as a protein substrate[18]. 1 ml aliquot of culture supernatant was incubated at 40°C for 5 min. After that 1 ml of bicarbonate buffer (pH 10.0) containing 1% casein was added and mixed properly with the culture supernatant and incubated again at 40°C for another 10 min. The reaction was stopped by the addition of 3.0 ml 5% trichloroacetic acid (TCA). The mixture was allowed to stand at room temperature (25°C) for 30 min and then filtered through Whatman No.1 filter paper to remove the precipitates. 1 ml aliquot of the resulting filtrate was mixed with 5.0 ml 0.4 M sodium carbonate solution and added 1 N Folin-Ciocalteu reagent (0.5 ml) to the reaction mixture and then incubated at 37°C for 30 min. The absorbance of the fungal supernatant was measured at 660 nm against distilled water as blank by using Simadzu 1700 UV-Vis spectrophotometer and protease activity was estimated for every fungal strains by comparing with the standard curve of tyrosine (0 to 100 µg/L).

The enzyme activity was measured in U/ml. A blank was also used as a control of enzyme activity.

Optimization of different cultural parameters
Protease production was studied in Reese medium at different pH (5-9) level and at different temperature range (30-50°C). Effect of different carbon and nitrogen sources were also studied for protease production.

Influence of carbon sources on the production of extracellular protease enzyme:
Effect of different carbon sources on enzyme production in culture media were determined by using six different carbon sources viz. glucose, sucrose, starch, fructose, glycerol and tri-sodium citrate (2% w/v) in Yeast Extract broth media at 28°C. After 96 h of incubation, the crude extract was analyzed and studied for protease activity.

Influence of nitrogen sources on extracellular protease enzyme production: Effect of nitrogen source on enzyme production in culture media, seven different nitrogen sources viz. Yeast extract, peptone, glutamic acid, ammonium sulphate, ammonium nitrate and potassium nitrate (0.5%) were added to culture broth containing KCl (2%), Casein(1%), and Sucrose(2%). Crude extract was analyzed after 96 h incubation at 28°C.

Partial purification for enzyme characterization by Ammonium sulphate fractionation: Fungal supernatant was partially purified by ammonium sulphate precipitation and activity was again checked [1].
Solid ammonium sulphate was added to the crude extract to 40-80% saturation. The precipitate was collected by centrifugation, dissolved in minimal volume of 0.1% Tris-Hcl buffer (pH 9) and dialyzed against same buffer at 4°C.

Screening of fungal isolates for lipase production:
Fungal isolates were tested for their ability to produce Lipase enzyme using Malt Extract Agar medium (Malt extract-1%(w/v), NH4NO3-0.1%(w/v), KH2PO4-0.1%(w/v), MgSO4. H2O-0.05%(w/v), Yeast extract-0.1%(w/v), NaCl- 1.0%(w/v) Tween-20-2.0%(v/v), agar- 10 g, distilled water- 1000ml, pH 5.5).

Determination of lipase activity was done with the help of cup-plate method [16].

The medium contains peptone-10g, NaCl-5g, CaCl2.2H2O- 1.0g, Agar 20g and 10ml lipid substrate Tween-20, distilled water- 1000 ml were used. The pH of the medium was adjusted to 6. The medium were poured to pre-sterilized Petri plate and after solidifying the medium, well were made at the centre of each plate with the help of a cork borer (No.4) of 8mm diameter under sterile environment. These well were filled with 0.1 ml culture filtrate and plates were incubated at 28°C. After 24 hours, clear circular zone were observed which indicated lipase activity of the fungal strains. The fungal isolates with clear zone were maintained in incubator at 28°C for another 3 to 5 days that gave biggest zone of inhibition. From the treatment the strains with biggest zone were selected as the best producer of Lipase enzyme and grown in Malt extract broth medium(containing Malt extract-0.5-5.0%(w/v) and maintained at 28°C in a shaking incubator(at 150 rpm). The spore suspended 10 ml distilled water containing 10-8

per ml spore will be used as inoculum in 250 ml fresh broth medium.

Assay of lypolytic activity:
The lipase activities of the fungal strains were measured by titrimetric assay method [12]. The substrate (primary) emulsion was prepared with olive oil, gum arabic and water (4:1:2). Appropriate measure of olive oil and gum arabic was mixed thoroughly with pestle in a clean dry porcelain mortar. When well mixed, the water (vehicle) was added all at once and mixed vigorously to produce a thick homogenous white creamy emulsion; the primary emulsion. The supernatant of the culture (20µL) was added to 5mL of substrate emulsion and mixed with 2ml of 50mM phosphate buffer, pH 7.0 (Na2HPO4-KH2PO4) before incubating for 20 mins at 37°C with shaking (120rpm).

After completion of incubation, the reaction was stopped with the addition of 4mL of acetone-ethanol (1:1 v/v) containing 2-3drops of 0.09% phenophthalein as indicator. The same reaction was also carried out by adding distilled water in place of fungal filtrate in the control (excluding sample). The blank (B) and test samples (T) were titrated against 0.05M sodium hydroxide solution. Enzymatic activity were determined by titration of fatty acid released with 50mM NaOH [11]. All lipase activity assays were performed at least in triplicate. One unit of lipase were defined as the amount of enzyme that catalyses the release of 1µmol of fatty acids per minute at 37°C.

Lipase assay:
One unit of lipase activity was defined as the amount of enzyme releasing in one mole of free fatty acids in one minute under standard assay condition.

$$\text{Lipase activity (U/mL)} = \frac{\text{(Volume of alkali consumed)} \times \text{(Normality of NaOH)}}{\text{(Time of incubation)} \times \text{(Volume of enzyme solution)}}$$

$1\ U = 1\ \mu\ min^{-1}g^{-1}$

Optimization for degumming of cocoon:
Depending on the REA values among 11 fungal strains isolated only 6 strains were selected for evaluation of protease and lipase enzymes in muga silk degumming process. Different parameters were optimized for finding the optimum degumming procedure. Ten numbers of muga cocoon was degummed using 100 ml of the crude enzyme for each fungal isolates. For degumming treatment of the muga cocoon firstly the collected cocoons were boiled in water for 20 minute at 60°C. After cooling to room temperature the enzymatic treatments of the boiled cocoon were carried out. Three types of treatments like (i) fungal extracellular secretes (enzyme) containing individual fungal secrete alone (ii) fungal secrete with tender papaya latex and (iii) conventionally used sodium carbonate (Na_2CO_3) were used for degumming process.

Different fermented fungal enzymes were evaluated and reeling efficiency of the treated cocoon was evaluated by studying different physical properties of the degummed silk.

For dual treatment different concentrations of plant based papain were taken in 500 mL Borosil flask and fungal enzyme containing solutions were added in different quantity and 10 numbers of boiled cocoon were added to each treatment. All treatments were initially maintained at the pH 7.5. A set of boiled cocoon with distilled water was maintained as control. Cocoons were boiled in distilled water for 20 minutes and after cooling to room temperature the fungal secretes were added in different concentration and kept for 15 to 20 minutes. After 20 minute of the enzymatic treatment the cocoons were removed from the treating solution and proceed for reeling which was carried out at the Directorate of State Silk Board, Khanapara, Guwahati. The tenacity, percentage elongation at break, Young's modulus and toughness, weight loss, tensile strength were recorded by utilizing the Universal Testing Machine (UTM) (Model: 3343), from Instron Corporation, UK interfaced with a PC at Institute of Advance Study in Science and Technology(IASST), Boragaon, Guwahati, Assam. A gauge length of 5 cm and cross–head speed of 20 mm/min was chosen for conducting these tests. All the samples were mounted on the cross heads with a pre–tension of 0.02 g/den. 10 tests were performed on each fibre sample and the mean value was taken.

RESULTS

Isolation and Screening of Protease and Lipase enzyme producing fungi: Screening of protease and lipase producing fungi usually involves growth on the medium that contains protein and lipid respectively as the selective substrate. Alkaline protease activities of 11 test fungi were determined using 0.5% casein as protein substrate on solid Reese media. Six test fungi produced alkaline protease activity as clear zone on the casein enriched media indicating casein hydrolysis around the fungal colonies grown at pH 9 after 4 d of incubation at 28° C whereas

remaining 5 test fungi showed growth on casein containing medium (pH 9) but were unable to produce distinct zone of hydrolysis.

Extracellular lipase production of the selected fungal strains was observed by adding 10 ml/l of Tween -20 in the media which act as a surfactant. The Tween -20 enhances the extracellular lipase production due to changes in the permeability of the cell or surfactant effects on cell-bound lipase

Protease activity of Enzymes released from Fungal Isolates During Five Days Incubation Period:

Alkaline protease producing fungal isolates showed varied levels of Relative Enzyme activity (REA) ranging from 1.37 to 3.9. Protease production at different temperature was examined for 72 h keeping the other fermentation conditions constant. Determination of protease activity from the fungal strains showed that *Aspergillus niger* (REA3.9) had the highest activity at day 4 followed by *Aspergillus flavus* (REA-3.14) and *Aspergillus tamari* (REA-2.25), *Penicillium* sp. (REA-2.00), *Penicillium pinophylium*(REA-1.82) and *Aspergillus fumigatus* (REA-1.37)(Table-1).

The comparative quantitative parameters of the 6 fungal isolates *Aspergillus niger* showed maximum protease activity (REA 3.9) while *Aspergillus fumigatus* shows the minimum protease activity (REA 1.37) (Table -1).

Casein (0.5%) shows protease induction in six fungal isolates and the enzyme activity in the control samples was found to be negligible. Thus, it could be concluded that the presence of inducer like Casein is very much essential for production of protease. Even the maximum protease producing *Aspergillus niger* is unable to produce protease in the absence of inducer.

Among all the fungal strains studied for efficient degumming of muga cocoon the most potent fungal strain was identified as *Aspergillus niger*. The fungal strain showed whitish colonies and turned black with the maturity of the culture.

Effective production of Protease enzyme of *Aspergillus niger* was recorded in the range of pH 9 indicating the alkalinophilic nature of the fungus. The fungal enzyme production increases with the increasing incubation time and recorded to be optimum at 96 hours and after which the enzyme production decreases considerably. The present findings established that the optimum incubation time for enzyme production of fungal strains was recorded as 96 hours. After optimum enzyme production, declination starts which may be due to nutrient stress, aging of culture, accumulation of toxic end products, degradation of medium proteins by protease etc.

Table 1. Screening and evaluation of Alkaline protease activity of potential fungal strains with Relative Enzymatic Activity after 4 days of inoculation

Fungi	Fungal colony in diameter (mm)	Zone of clearance (mm)	Relative Protease Activity
Aspergillus flavus	7	29 ±1	3.14
Aspergillus tamarii	12	39 ±1	2.25
Aspergillus niger	10	49 ±1	3.9
Aspergillus fumigatus	16	38 ±1	1.37
Penicillium sp.	9	27 ±1	2
Penicillium pinophylim	28	79 ±1	1.82

**Relative enzyme activity (REA) = Clear zone diameter- Colony diameter(mm)/Colony diameter(mm)*

Lipase Activity of Enzymes Extracted From Fungal Isolates During Four Days Incubation Period:

Microbial lipases are mostly extracellular and their production is greatly influenced by medium composition besides physicochemical factors such as temperature, pH and dissolved oxygen. Tween-20 is used as a substrate in the media for lipase production. The substrate induces enzyme production in the media. Extracellular lipase production of the selected fungal strains was observed by adding 10 ml/l of Tween -20 in the media which act as a surfactant. The Tween -20 enhances the extracellular lipase production due to changes in the permeability of the cell or surfactant effects on cell-bound lipase. The Enzymatic hydrolysis for lipolytic activity was also recorded to be maximum in *Aspergillus niger* (REA 2.78) at 4th day of treatment followed by *Aspergillus tamari* (REA-2.75) and *Aspergillus flavus* (REA-2.3), *Penicillium pinophylium*(REA-2.1), *Penicillium* sp. (REA-1.21) and *Aspergillus fumigatus* (REA-1.11)(Table-2).

Table 2. Screening and evaluation of Lipase activity of potential fungal strains with Relative Enzymatic Activity after 4 days of inoculation

Fungi	Fungal colony in diameter (mm)	Zone of clearance (mm)	Relative Lipase Activity
Aspergillus flavus	10	43 ±1	2.3
Aspergillus tamarii	8	30±1	2.75
Aspergillus niger	9	34±1	2.78
Aspergillus fumigatus	15	36±1	1.11
Penicillium sp.	14	31±1	1.21
Penicillium pinophylim	20	62 ±1	2.1

Lipase activity of different fungal strains was significantly affected by Tween-20. The present studies showed that *Aspergillus niger* start to produce lipase from 24 hours up to 72 hours approximately 33.66 and 20.83 U /ml and turned to decrease when it was grown in 2.5% and 5% molasses mineral medium (Table-3).

The utilization of reducing sugar contents by *Aspergillus tamarii* decreases with increasing the time duration during the incubation process. The concentration of reducing sugars, protein, and mycelial mass decreased with the increased time duration in both the cultures. The final pH values of culture broth were observed increasing toward alkaline side in both cases.

It is concluded that highest production of lipase (approximately 33.66 Units / ml) was recorded by *Aspergillus niger*, when it was grown on MEA media enriched with Tween-20 as surfactant at 72 hours in comparison to other species of fungi. On the basis of higher production of lipase, it is suggested that the Tween-20 induces the production of lipase enzyme and *Aspergillus niger* is the best producer of lipase in comparison to other species of fungi studied.

Table 3. Alkaline Lipase and Protease activity of different screened fungi grown at 28°C for 4 days at 150 rpm

Fungal isolates	Lipase Activity(U/mL)	Amount of tyrosine equivalent released	Protease Enzyme Activity(U/mL)
Aspergillus niger	33.66	336.58	139.43
Aspergillus fumigatus	20.83	299.53	104.83
Aspergillus flavus	22.5	309.33	106.32
Aspergillus tamarii	27.17	294.6	102.62
Penacillium pinophylium	24.17	280.93	98.32
Penicillium sp.	30	330.36	112.48

Influence of carbon and nitrogen sources: The formation of extracellular enzymes is influenced by the availability of precursors for protein synthesis. Different carbon sources have different influences on extracellular enzyme production by different strains. Among the various substrates tested like casein, Yeast extract, mannitol, peptone were found to be the effective inducer for protease production. This study has shown that casein can influence markedly the production of extra cellular protease by fungal species. Few fungal strains including *Aspergillus niger*, *A. tamari*, *A. fumigatus*, *Penicillium* sp., *Penicillium pinophylium* were able to hydrolyse different protease inducing substrate. Among these Casein was found to be the most effective protease producing substrate.

It was also observed that although increase in colony size and growth a very low extracellular protease production was detected for few fungal species. Differences in the ability to utilize different protein substrates may be due to difference in substrate specificities of the enzyme produced or may be consequence of parameters known to affect extracellular protease production such as pH, medium composition and temperature.

The highest protease production was achieved by addition of glycerol among other carbon sources. It was observed that when the culture broth was supplemented with glucose, protease production declined (Table-4). The type of nitrogen sources also affect enzyme production as shown in Table-4 . The protease activity recorded to be highest for *A. niger* and recorded 256 U/ml/min in Casein containing medium. It was followed by the nitrogen source Yeast Extract (240U/ml) and Manitol(225U/ml).

Moderate to good levels of enzyme activities were obtained when peptone is used as nitrogen source. However, with the addition of inorganic nitrogen sources tested for protease production, it was observed that the protease formation by fungal strains declined.

Table 4 : Effect of carbon and nitrogen sources on the protease production of *Aspergillus niger*

Sl No	Carbon sources	Protease Activity U/ml/min
1	Glycerol	164
2	Glucose	127
3	Sucrose	162
4	Starch	155
	Nitrogen sources	
5	Yeast Extract	240
6	Peptone	212
7	Casein	256
8	Manitol	225
9	BSA	167
10	Amonium sulphate	136

Influence of temperature and pH on Protease production: Protease production at different temperature and pH was examined for 72 h keeping the other fermentation conditions constant. From the present findings it was also observed that the production of protease is influenced by the initial pH of the medium studied for the range of 7 to 9 indicating that this enzyme might be the alkaline protease. The optimum temperature for protease activity was determined by screening temperatures from 30° to 50°C using casein as a substrate dissolved in phosphate buffer at different pH ranged from 7-9. It was observed that the protease production increased with increase in temperature from 30 to 40°C and recorded best activity at 40°C in the pH 8.0. It was also observed that the fungal growth and protease production ceased at higher temperature (50°C) and increasing pH(9.0). The maximum production of protease for *Aspergillus niger* was obtained at 40°C at pH 9.0. It may be hypothesized that the environmental temperature and pH not only affects growth rates of fungal strains but also exhibit significant influence on the levels of protease enzyme production. Maximum protease production was found at pH 9.0 which indicated the alkalinophilic nature of the fungal strains.

Table 5 : Protease production at different temperatures and pH by *Aspergillus niger*

Sl No	Temperature °C	pH	Protease Activity U/ml/min	Fungal dry weight(g/100ml)
1	30	7	138	16
2	30	9	264	28
3	30	8	212	25
4	40	7	236	27
5	40	8	277	46
6	40	9	252	34
7	50	7	116	22
8	50	8	153	26
9	50	9	122	22

Degumming of Muga Cocoon

Degumming with fungal enzyme can be a viable alternative to the existing methods of degumming. The degumming treatments like individual fungal secretes, fungal secrete with tender papaya latex and conventionally used Na2CO3 were carried out in laboratory condition. Different fungal enzymes were evaluated for degumming of muga cocoon by studying different physical properties of the degummed silk. It was observed that the processing of silk under mycogenic enzyme treatment retain lustre and softness of silk. By studying different physical properties of the degummed silk it was found that fungal extracellular secretes of *Aspergillus niger* was recorded to be an effective degumming agent.

Characterization of Muga Fibers after enzymatic treatments

The quality parameters of the degummed silk fibers and yarns were measured by studying different physical properties like variation of the tensile properties including strain, stress, tenacity, Young's modulus. In the present study, extracellular fungal protease and lipase enzyme are used as degumming agent. The efficiency of these enzymes has been studied in terms of strength of the fibres and other physical properties. It was observed that the treatment of different fungal enzymes have diverse effects on the tensile properties of degummed muga silk fibres obtained from cocoons. It was observed that the Alkaline fungal secretes performed better than acidic and neutral ones in terms of complete and uniform sericin removal, retention of tensile properties and improvement of surface smoothness, handle and lustre of silk.

The effect of fungal secretes with proteolytic and lypolytic activity of *Aspergillus niger* was found to be best for degumming of Muga silk fibres with higher tensile properties (Table-6). The efficient degumming is probably due to effective release of both protease and lipase enzymes by *Aspergillus niger* in natural environment.

From the study of physical properties of the degummed muga fiber it was established that fungal secretes play an important role for degumming and recorded optimum properties with the fungal strain *Aspergillus niger* (Tenacity 3.837g/den, Toughness-0.986 g/den)which was followed by *Aspergillus tamarii* (Tenacity 3.787g/den, Toughness-0.985 g/den). Enzymatic treatment with *A. niger* extracellular secretes significantly increases the tensile properties of the fibres especially the tenacity and toughness. The tenacity values indicate that treatment of fungal secretes of *Aspergillous niger* able to increase the tenacity of silk .

From the present findings it was also established that the tenacity of the traditional Na2Co3 treated muga silk is not significantly higher than the untreated silk. The insignificant loss of tenacity of the silk fibres upon treatment was recorded with the rest of the extracts treated for degumming experiment.

The strain percent of the silk samples is found to increase after treatments with *Aspergillus niger*(28.220g) which is followed by *Aspergillus flavus* (26.981g), *Aspergillus fumigatus* (26.981g), Papaya (24.007g), Na2Co3(17.426g) , Papaya latex (24.007g) and Papaya latex with *A. niger* enzyme (17.673g) treatments (Table-6). It was also observed that there is no significant variation of Young's modulus of the silk fibres after treatment with these extracts. Toughness of the muga silk sample has also been found to increase after treatment with *Aspergillus niger*. On the other hand, after Na2CO3 and papaya latex treatments, the silk loses its toughness significantly.

The breaking loads of the fungal enzyme treated muga silk fibres are shown in Table -6. It is seen that the *Aspergillus niger* and papaya latex with enzymes treated silks gained significant breaking load after treatment. All these treatments influence the fibres' chemical, physical and mechanical properties. The changes of tenacity may be due to the variation of moisture content and the change of degree of crystallinity after treating with the extracts.

Remarkable changes observed in breaking load after treatments which indicates that the treatment give rise to close packing of fibre molecule with an increase in the intermolecular forces. The fungal enzyme treated fine silk fibres have higher tensile strength and Young's modulus in comparison to other treatments. Higher tensile property of treated fibre indicate the prospect of uses of mycogenic enzymes in the textile industries.

Table 6. Tensile properties of *Antherea assamensis* silk samples treated with different mycogenic enzyme treatment

Treatments	Load at maximum (g)	% Strain at maximum (g)	Modulous (Auto young) (g/den)	Tenacity at Break(g/den)	Tenacity at Maximum (g/den)	Toughness (g/den)
Aspergillus niger	22.312	28.22	65.772	3.736	3.837	0.986
Aspergillus fumigatus	22.147	26.884	56.105	3.699	3.699	0.958
Aspergillus flavus	20.002	26.981	54.985	3.207	3.223	0.984
Aspergillus tamarii	22.543	20.947	56.571	3.605	3.787	0.985
Penacillium pinophylium	22.141	21.06	39.906	3.689	3.689	0.973
Na2Co3	20.778	17.426	46.804	3.395	3.395	0.944
Papaya latex	20.266	24.007	52.689	2.812	3.736	0.885
Papaya+ A. niger enzyme	22.587	17.673	55.636	2.621	2.797	0.829

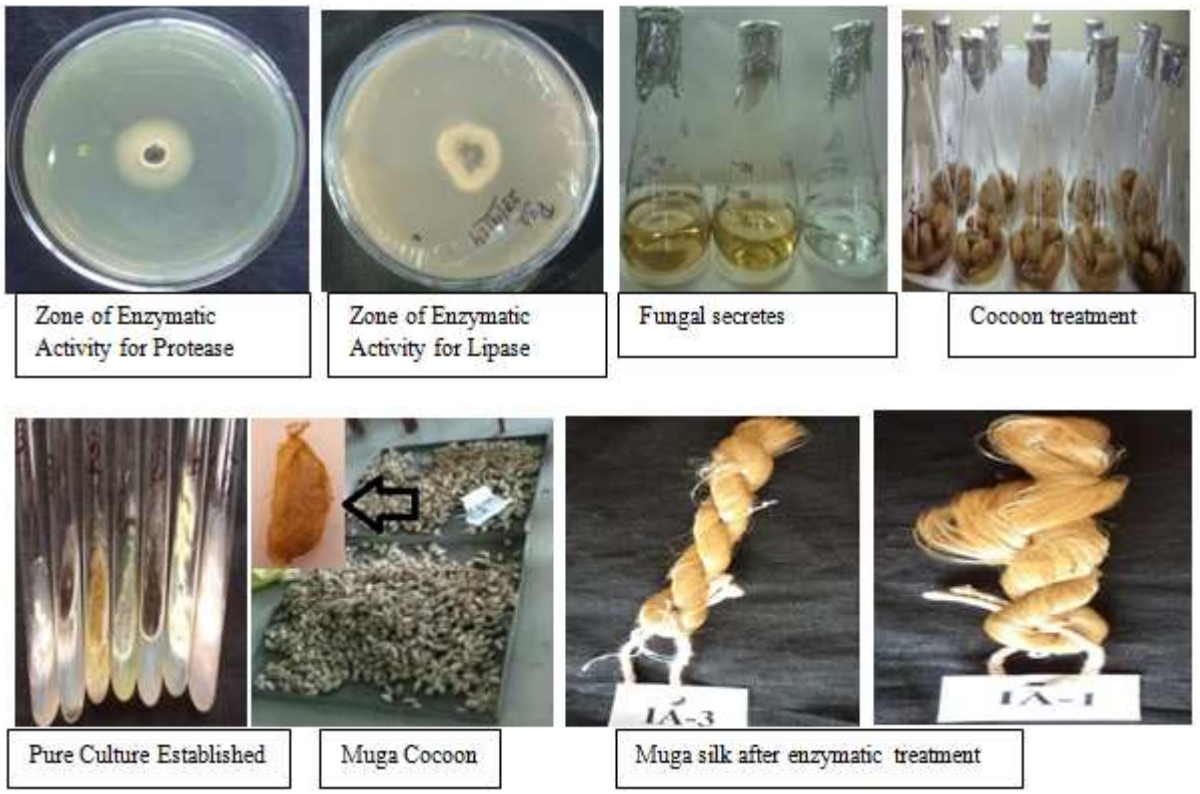

Fig: Isolation, Screening, and enzymatic degumming of Muga cocoon

DISCUSSION

Depending on the Relative Enzyme activity (REA) out of 11 only 6 fungal isolates were selected for further degumming studies of muga silk. Among the studied fungal isolates, the *Aspergillus niger* showed greater enzymatic activity in screening test. The alkaline protease activity in culture filtrate was studied and among the six potent fungal isolates *Aspergillus niger* showed higher enzyme activity. In the present study, maximum activity of alkaline protease was recorded in culture fluids of *A. niger*. Other workers have also reported alkaline protease production by their tested strains of this species [5, 7, 8]. Isolates of *A. tamarii* produced more than 102.62 U/mL activity of alkaline protease which is inconformity with the earlier studies [15]. Other workers also reported maximum alkaline protease activity from *Aspergillus* sp. using modified Reese medium [10]. In the present study, isolates of *Penicillium* sp. were found to produce alkaline protease in its cultures grown on casein containing medium with maximum activity (112.48U/mL) was recorded in 4 days culture and followed by the enzyme activity of *Aspergillus flavus* (106.32U/ml) enzyme production. In contrast to that, acid protease production by some strains of *Aspergillus* and *Penicillium* reported by other worker [2]. The results obtained from the present investigation and those reported earlier by other workers indicates that *Aspergillus* is one of the important genera for the production of mycogenic alkaline enzymes of commercial importance.

The addition of Tween- 20 and Tween- 80 in the media could stimulate lipase production since some authors place these compounds in the category of artificial lipids [18]. In this study the enzymatic hydrolysis for lipolytic activity was recorded to be maximum in *Aspergillus niger* (REA2.78) which was followed by *Aspergillus tamari* (REA-2.75) and *Aspergillus flavus* (REA-2.3). Similar results for improvement of lipase secretion with addition of surfactants have been reported in several microorganisms with different results. It was reported that the addition of surfactant like Tween-20 favoured best extracellular lipase production in *Penicillium citrinum* (Jayaprakash and Ebenezer., 2012). Addition of Tween-20 in the media increased lipase production (33.66 Units / ml) in *Aspergillus niger* in our study. Other worker reported a low biomass of *Candida rugosa* when Tween- 80 was the sole carbon source [3]. The silk fibres were evaluated both visually for appearance, colour, luster and studying other physical parameters like Tenacity, Strain, Toughness etc. after enzymatic degumming. Mycogenic Enzyme treated muga fibres were found better morphologically as well as other physical properties than to the conventionally degummed fibers. The silk degummed using extracted protease and lipase enzyme produced by *Aspergillus niger* was found to be good in general appearance, colour, texture and lustre. The fungal enzyme treated fine silk fibres have higher tensile strength and Young's modulus in comparison to other treatments. Earlier reports also showed similar findings of both protease and lipase enzyme [4]. Higher tensile property of treated fibre indicated the prospect of

uses of mycogenic enzymes for degumming and quality of silk fibre.

CONCLUSION

Sericulture being a major agro based industry playing an important role in the rural economy in India, the health hazards of the workers as well as the environmental pollution associated with the use of chemicals in silk reeling industries needs special attention. In recent day's enzymatic degumming have become an integral part of silk finishing process. With the increasingly important requirement for proper degumming to reduce environmental health hazards, the use of microbial enzymes in the chemical processing of fibres is rapidly gaining wider recognition because of their non-toxic and eco-friendly characteristics. The Muga cocoon degummed using extracted protease and lipase enzyme produced by *Aspergillus niger* was found to be best among other fungal isolates studied in respect to their degumming efficacy and other physical properties. Moreover, the commercial enzymatic preparations are expensive and not readily accessible to the reelers and weavers, therefore, mycogenic enzyme may solve this problem and also saving resources like energy and chemicals and improving the quality of silk. Hence this study, which was based upon the use of mycogenic enzymes for degumming has thrown light on silk quality, reduction of cost and energy. Moreover, the strength loss was minimum in enzymatic method and maximum in conventional method and hence it could be concluded that the enzymatic treatment reduces the loss of tensile strength unlike the chemical treated fibres.

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