

Editorial

Cocoonase Enzyme: Current and Future Perspectives

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Content

The tropical tasar silkworm, *Antheraea mylitta* Drury produces tasar silk that have very much commercial importance giving livelihood to more than 1,00,000 poor people. This insect is widely distributed in diverse geographical regions of India. Cocoons obtained from tasar silkworm have the highest capacity of silk production and the largest among all the other known non-mulberry silk producing insects [1]. *Terminalia tomentosa* and *T. arjuna* are primary food plants for the *A. mylitta* larvae. Recently it has been reported that jamun (*Syzygium cumini* L.) is another potential host of tropical tasar silkworm, *A. mylitta* drury [2]. Silk fiber produced by silkworm is a composite material formed by fibroin protein which is again surrounded by sericin protein [3]. It is reported that tasar cocoons are comparatively harder than cocoons of other sericigenous insects and it's softening for reeling is relatively more complicated. To obtain the silk from cocoons sericin removal is necessary. Predominantly, tasar cocoon sericin removal (cooking/softening/degumming) is being carried out in highly alkaline (pH 8.8 to 11.5) condition which influences the natural unique color and softness of tasar silk.

Silk fiber contains 67-75% fibroin protein and 22-25% sericin protein. There is qualitative and quantitative variation in sericin of silk fiber obtained from different species of silk moth. Gelatin like characteristic of sericin helps in adhering on outer surface of fibroin that resulted irregular fashion of cocoon [4,5]. Several methodologies have been used by investigators [6-8,3] for softening of cocoons and silk degumming. As a ruling practice, softening of tasar cocoon is generally used to perform in alkaline solution by using soap, soda, H₂O₂, alkali etc. that adversely affects the natural color and softness of tasar silk [9]. However, several enzymes like lipase, protease etc. have also been tried for cocoon cooking and degumming of silk [10,6,8]. Recently a study on purification and detailed characterization of cocoonase from the silkworm *Bombyx mori* has been reported [11]. However, few study on enzyme based technology for tasar cocoon cooking in comparatively eco-friendly manner has been carried out. Therefore, development of enzyme based method for cocoon softening is required.

A review on biotechnological application of proteolytic enzymes in post cocoon technology [12] and dissolution properties of silk

cocoon shells and degummed fibers from African wild silk moths have been published [13]. Several sericigenous insects including *A. mylitta* exude a proteolytic enzyme cocoonase when they reached the final stages of their metamorphosis. This proteolytic enzyme is able to soften the anterior portion of cocoon that enables the moth to exit from cocoon [9]. This creates an idea that cocoonase enzyme is capable to soften anterior portion of cocoon during adult emergence. Hence, this enzyme can be utilized for softening/cooking of tasar cocoons. Reports on cocoonase from different sericigenous insects are available [14-21]. However, their extensive possible-efficacy in cocoon cooking/softening has not yet analyzed.

Previously enzymatic mechanism for moth escapes and collection of the cocoonase by means of a capillary tube from the face of a newly emerged *A. pernyi* moth was reported [22]. Till now several methods for the collection of cocoonase were tried (like direct collection during secretion, collection from newly pierced cocoons, collection by dissecting the galea and collecting enzyme in crystal form) to examine the possible ability of cocoonase in cocoon softening [14,9]. Although these investigators were able to collect cocoonase but methods adopted by these researchers were very hard and labor intensive. Therefore, till today studies on cocoonase enzyme could not be made effectively along with its utilization in cocoon softening.

Studies on secretory organs of cocoonase and silk moth-vomiting fluid of silkworm, *Bombyx mori* have been also made [16]. Rapid determination of cocoonase contents in maxilla of four silkworm breeds by dot blotting has also been studied [20]. Study on cloning and expression of the cocoonase gene from *B. mori* have been tried and its eukaryotic expression and biological activities of the expressed product also evaluated [18]. Expression of cocoonase in *B. mori* cells by using a recombinant *Baculovirus* and its bioactivity assay have been made [21]. Study on cloning and expression of a *B. mori* cocoonase and its possible role on cocoon softening and removal of sericin has been discussed [23]. Recently an active recombinant cocoonase from the silkworm *B. mori* has been produced and bleaching, degumming as well as sericin degrading activities of this recombinant cocoonase also reported [24]. In contrary studies of *A. mylitta* cocoonase in cocoon softening has not been made extensively. Although possible efficacy of cocoonase in tasar cocoon have been initiated where SDS-PAGE analysis of freshly collected cocoonase showed molecular weight around 26 kDa [9]. Experimental analysis of *A. mylitta* cocoonase indicated that Tasar silk softened by this cocoonase enzyme retain natural and unique tasar silk color, soft texture and luster of tasar silk yarn. Also cocoonase directly acts on the sericin protein without affecting the fibroin protein indicating that sericin is excellent natural substrate of cocoonase [9]. Detailed computational study about the presence of cocoonase enzymes, cocoonase nucleotide sequences and their evolutionary relationships among other insects, presence of any conserved domains and its 3D structure has been reported [25].

It has been concluded that recombinant *B. mori* cocoonase is very much useful in silk degumming [23]. Hence, efforts should be made

to explore an enzymes based technique for tasar cocoon cooking. As enzymes are known for specific and mild action hence it is expected that enzyme based cocoon softening will be useful in preserving natural color and softness of tasar silk in comparatively eco-friendly. Therefore, there is need to develop enzyme based technology for cocoon softening in order to get tasar silk with natural color and soft texture. Extensive study also need to carryout to check the quality, tensile strength, durability of cocoonase treated tasar silk as well as its international demand. Detailed molecular and biochemical characterization of cocoonase enzyme is also need to be carried out. Such a technology will be having immense use in post cocoon sector of tasar silk industry and it will lead to give value addition to tasar silk where poor farmers will be benefited. Further, collection of cocoonase from insect is having its own limitation because it cannot be collected throughout the year due to unavailability of moth having eclosion stage. Therefore, efforts towards cocoon availability should also be made.

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