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Studies on *Antheraea mylilla* Cocoonase and its Use in Cocoons Cooking

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ABSTRACT

Cocoonase which is secreted as a natural phenomenon has its direct utility in softening of cocoons for reeling without altering the very organic nature of tasar silk. In the present study, efforts have been made to comprehend and utilize cocoonase for its future use in cocoon cooking. The emerging moth gradually release cocoonase from galea of their mouth parts in anterior inner portion of the cocoon (peduncle region). After releasing adequate volume of enzyme (around 400-600 μL) for softening the peduncle region of cocoon, with the help of appendages emerging moth create outlet near the peduncle and escape from cocoon shell. Around 1000 mL cocoonase from 2000 eclosion stage *A. mylitta* have been collected and centrifuged in cold condition (4°C) at 10000 rpm to minimise the impurity. To maintain buffer conditions, cocoonase was collected in pre chilled Tris buffer pH 9.2 and stored at -4°C temperature for further use in cocoon cooking. When cocoons of *A. mylitta* (Daba ecorace) were subjected to initial water boiling at 100°C on leisurely flame for 30-40 min followed by cocoon soaking in cocoonase for 20-24 h at 37°C, the 80-90% softening of cocoon shell was found. Silk filament obtained from the cocoons cooked in cocoonase maintains natural tasar silk colour, softness and structure. The 50-52% silk recovery was observer in cocoonase cocoon cooking. By centrifuging used reaction mixture at 9000 rmp in cold condition to remove impurities and adding 10-15% volume of fresh cocoonase in reaction mixture left over enzyme solution can be reused once. More study is required to get better cocoonase cooking efficiency and consistent cooking with higher or comparable silk recovery than the ruling practices.

Key words: *Antheraea mylitta*, enzyme, cocoonase, cocoon, sericin

INTRODUCTION

The *A. mylitta* is an economically important sericigenous insect producing tasar silk having elite color, matchless quality and has massive demands at national and International level. It is interesting to note that tasar silkworm cocoons have the utmost capacity of silk production being the largest among all the other known non-mulberry silk producing insects (Akai, 2000). Insects principally belong to two families, viz., Saturnidae and Bombycidae, which spins silk fibre. It is reported that *Bombyx mori* belongs to Bombycidae produces a delicate twin thread of silk fibroin, which is coated by a protective cover of sericin. Silk protein is a kind of protein like collagen, elastin, keratin, fibroin, sporgin etc., is an essential constituent of cocoon filament (Komatsu, 1975). It is reported that silkworm commence spinning to form cocoon which is a complex material shaped mainly by fibroin and sericin protein chain. For cooking/softening of sericigenous insects cocoons,

several methods have been tried by using hydrogen peroxide as a softening agent (Moon *et al*., 1996) development of a simple cooking method (Tikoo and Goel, 1987) and to study the technical conditions of silkworm cocoon fibroin hydrolyzed with enzymes (Liu and Li, 2002). Subsequently several attempts have been made to study the fibroin and sericin protein (Mondal *et al*., 2007; Srihanam *et al*., 2009; Srisuwan *et al*., 2009; Prasong *et al*., 2009; Teshome *et al*., 2011). Insect proteolytic enzyme basic study was also conducted by researchers by using immunochemistry of an insect protease, cocoonase and its zymogen (Berger and Kafatos, 1971) and preliminary characterization of cocoonase from silkmoths (Kafatos *et al*., 1967). The proteolytic enzyme cocoonase is produced by silkworms during pupal-adult transformation, in close proximity to the final stages of their metamorphosis. It makes fibre of cocoon softer; by this means of *A. mylitta* to facilitate the emergence of the adult insect (Kafatos *et al*., 1967; Berger and Kafatos, 1971; Felsted *et al*., 1973). Although, this phenomenon was reported around six decade back and basic nature of research work was conducted by several investigators (Felsted *et al*., 1973) but elaborate studies on *A. mylitta* cocoonase in cocoon cooking is not carried out much. Therefore, in the present study, efforts have been made to study the activity of *A. mylitta* cocoonase in order to use for cocoon cooking.

MATERIALS AND METHODS

The larvae of *A. mylitta* Drury (Daba ecorace) were reared in outdoor conditions on leaves of *Terminalia tomentosa* and *Terminalia arjuna*. Subsequent to spinning, cocoons were collected and stored at room temperature in grainage laboratory and allowed to complete pupal period and utilized for present study at Silkworm Physiology Laboratory, Central Tasar Research and Training Institute Ranchi Jharkhand India J. Specific stage of pupae was identified on the basis of change in pupae integument color and softness. These pupae were allowed to emerge in grainage. Cocoons were collected from the laboratory grainage and anterior wet portion of the cocoon (peduncle region) was washed and squeezed in buffer. To maintain buffer conditions, generally cocoonase was collected in pre chilled Tris buffer pH 9.2 and stored at -4°C temperature for further use in cocoon cooking. Around 1000 mL cocoonase from 2000 eclosion stage *A. mylitta* and freshly pierced cocoons have been collected and centrifuged in cold condition (4°C) at 10000 rpm to minimize the impurity. Cocoons of *A. mylitta* (Daba ecorace) were used for cocoon cooking experiment and it was boiled initially at 100°C on leisurely flame for 30-40 min followed by cocoon soaking in cocoonase for 20-24 h at 37°C after completion of cocoon cooking, the cocoon samples deflossed and reeling was performed on an epprouvette machine/CTR and TI Developed Charakha in the post cocoon Technology Section of our Institute. The silk recovery percentage was calculated by using standard formula (weight of reeled silk/weight of reeled silk+weight of waste silk×100) (Ramesha *et al*., 2009). Alternatively, by centrifuging used above reaction mixture at 9000 rmp in cold condition to remove impurities and adding 10-15% volume of fresh cocoonase in reaction mixture left over enzyme solution were reused once and reeling was conducted.

Data analysis: The data was subjected to the statistical analysis by using Student's "t" test.

RESULTS AND DISCUSSION

In the present study, efforts have been made to comprehend the cocoonase secretion and utilize this proteolytic enzyme for its future use in cocoon cooking. It is found that emerging moth gradually release cocoonase from galea of their mouth parts (Fig. 1a and d) in anterior inner

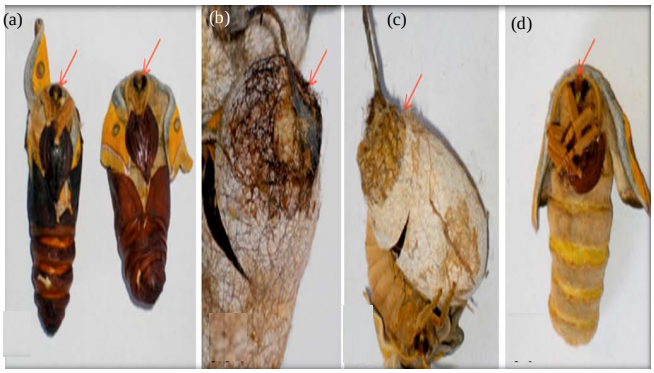

Fig. 1(a-d): Cocoonase discharge by (a and d) emerging *A. mylitta* moth and (b and c) softening of anterior portion of cocoon shell

Fig. 2: Showing collection of *A. mylitta* cocoonase for its utilization in cocoon cooking

portion of the cocoon (peduncle region) (Fig. 1b, c). Cocoonase was collected from emerging *A. mylitta* (Fig. 2) for its use in cocoon cooking.

After releasing adequate volume of enzyme (around 400-600 µL) for softening the peduncle region of cocoon, with the help of appendages emerging moth create outlet near the peduncle and escape from cocoons. Around 1000 mL cocoonase from 2000 eclosion stage *A. mylitta* have been collected and centrifuged in cold condition (4°C) at 10000 rpm to minimise the impurity. To maintain buffer conditions, cocoonase was collected in pre chilled Tris buffer pH 9.2 and stored at -4°C temperature for further use in cocoon cooking. When cocoons of *A. mylitta* (Daba ecorace) were subjected to initial water boiling at 100°C on leisurely flame for 30-40 min followed by

Fig. 3(a-e): Softening of cocoon in *A. mylitta* cocoonase and their reeled filament, (a) Pierced cocoons and anterior portion opening due to action of cocoonase, (b) Cocoon softening in cocoonase for activity test, (c and d) Cocoonase soften cocoons and (e) tasar silk filaments obtained after cocoon cooking in cocoonase

Fig. 4(a-d): Showing comparative appearance of silk obtained from cocoonase cooking and cooking in existing method, (a) Silk filament obtained after cocoon cooking with help of synthetic inorganic chemical with hot water treatment, (b) Silk filament obtained from the cocoons cooked in cocoonase which maintains natural tasar silk color, softness and shine, (c) Cocoon cooked in Cocoonase reeled by using CTR and TI developed charkha and (d) Twisting of silk yarn by using spinning machine which was obtained after cocoon cooking in chemical (thin arrow) and in cocoonase (thick arrow)

cocoon soaking in cocoonase for 20-24 h at 37°C, the 80-90% softening of cocoon shell was found (Fig. 3a-e). Silk filament obtained from the cocoons cooked in cocoonase maintains natural tasar silk colour, softness and shine (Fig. 4a-d).

The 52-53% silk recovery was observed in cocoonase cocoon cooking. By centrifuging used reaction mixture at 9000 rmp in cold condition to remove impurities and adding 10-15% volume of fresh cocoonase in reaction mixture left over enzyme solution can be reused once. For cooking/softening of sericigenous insects cocoons, several methods have been tried by using hydrogen peroxide as a softening agent (Moon *et al*., 1996) development of a simple cooking method (Tikoo and Goel, 1987) and to study on technical conditions of silkworm cocoon fibroin hydrolyzed with enzymes (Liu and Li, 2002). Subsequently several attempts have been made to study the fibroin and sericin protein (Mondal *et al*., 2007; Srihanam *et al*., 2009; Srisuwan *et al*., 2009; Prasong *et al*., 2009; Teshome *et al*., 2011). Insect proteolytic enzyme basic study also conducted by researchers by using immunochemistry of an insect protease, cocoonase and its zymogen (Berger and Kafatos, 1971) and preliminary characterization of cocoonase from silkmoths (Kafatos *et al*., 1967).

The *A. mylitta* is an economically very important sericigenous insect manufacture tasar silk having elite colour and it is interesting to note that tasar silkworm cocoons have the utmost capacity of silk production being the largest among all the other known non-mulberry silk producing insects (Akai, 2000). Insects principally belong to two families, viz., Saturnidae and Bombycidae, which spins silk fibre. It is reported that silkworm commence spinning to form cocoon which is a complex material shaped mainly by fibroin and sericin protein chain. Sericigenous insect produces a delicate twin thread of silk fibroin, which is coated by a protective cover of sericin. Silk protein is a kind of protein like collagen, elastin, keratin, fibroin, sporgin etc., is an essential constituent of cocoon filament (Komatsu, 1975). The proteolytic enzyme cocoonase is produced by silkworms during pupal-adult transformation, in close proximity to the final stages of their metamorphosis have reported in several sericigenous insects. It is reported that spit liquid is produced by *Bombyx mori* during their eclosion stage is also cocoonase .It softens and dissolves cocoon layer so that the adult can escape from the cocoon. It makes fibre of cocoon softer; by this means *A. mylitta* facilitate the emergence of the adult insect (Kafatos *et al*., 1967; Felsted *et al*., 1973). In the present study *A. mylitta* cocoonase was used for cooking of cocoons of its Daba ecorace which semi-domesticated ecorace. *A. mylitta* cocoonase could able to soften these cocoons and showed good proteolytic activity. In order to use cocoonase for cocoon cooking, more study is required to get better cocoonase cooking efficiency and consistent cooking with higher or comparable silk recovery than the ruling practices. It is expected that the yarn obtained from the soften tasar cocoons with cocoonase will be a natural organic tasar silk retaining its natural sheen with higher economic value.

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