Multidisciplinary Sciences Reports

Review

New Molecular Biomarkers used for biological pest control

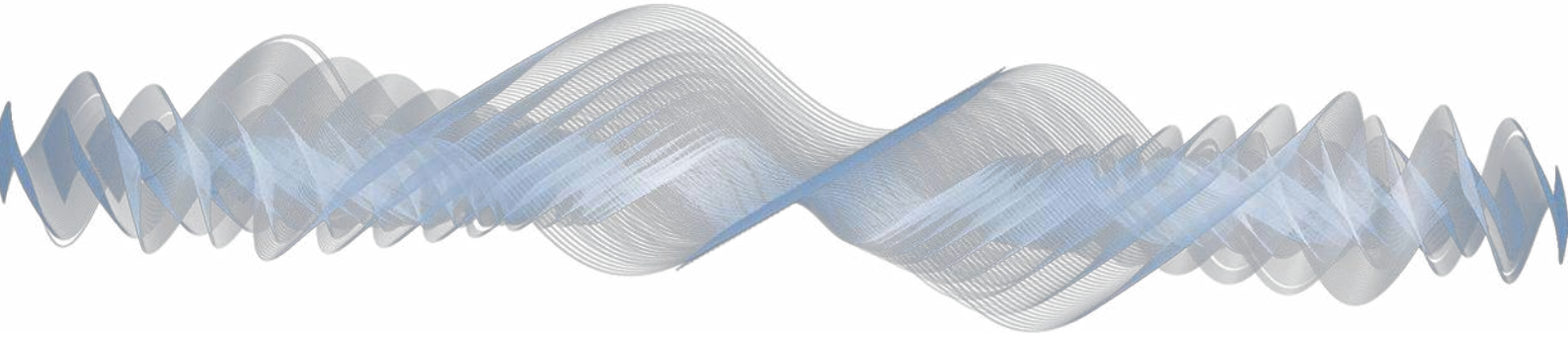
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ABSTRACT

Palm trees are part of the Arecaceae botanical family. These have great economic importance, mainly because they have products for food, as well as shelter, food, and reproduction of various animals, including arthropods. *Attalea phalerata* is distributed in several Brazilian states and its length varies between 5-10m. The main pest for this type of palm tree are the insects of the sub-family Bruchinae. Insects have digestive enzymes that help them obtain nutrients, including alpha amylase. In obtaining food, they end up destroying the seeds, nuts, or regions of the plant, which served as an economic source to produce oils and carbohydrates. Etc. *Pachymerus nucleorum*, an example of this family of insects, has a larva in one of its stages, which grows and develops through the assimilation of palm nuts. As a result, the economic loss is very high. In this sense, the study and discovery of the peculiarities of the digestive enzymes of this insect can bring benefits to biological control, being more effective, simpler, and causing less damage to other organisms. Among these main tools of biological control, we have enzymatic biomarkers (amylase and ATPases) that may have subtle differences between organisms.

KEYWORDS: Control. Amylase. Palm trees. Bruchinae. *Pachymerus nucleorum*.

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GENERAL ASPECTS – INTRODUCTION

The term biomarker comes from the definition of an analyte or substance with objective characteristics and easy measurement. It can be evaluated as an indicator of a normal biological, pathogenic, or pharmacological response to a therapeutic intervention (1,2). In addition to being easy to measure, it must be safe and generate accurate and consistent results regardless of groups or conditions (1–3). In recent years, the applicability of molecular biomarkers has grown, mainly involving metabolic issues, by measuring enzymes, generated, or produced analytes, etc. (2,4–7).

The use of this tool for prediction, response to environmental monitoring and prevention of damage or exposure to environmental agents can bring benefits for pest control of certain species of palm trees.

Babassu Palm

Babassu palm trees (*Orbinya spp*) are plants found in Brazil, Bolivia, Colombia, Mexico, and other parts of Latin America. In Brazil, it is typical of the transition region between the Cerrado, the Amazon Forest, and the semi-arid northeast, concentrating in Maranhão in floodplain areas and river valleys (8–12).

From this plant, up to 64 products can be obtained industrially, ranging from oil, coal, and food to the production of hammocks, baskets, and ropes (8–12). However, the majority used consists of almonds, which correspond to only 6% of the weight of the fruit, the remainder made up of epicarp (15%), mesocarp (20%), and endocarp (59%) end up being discarded or used to a lesser extent. Studies have shown that there is an enormous potential for using these compounds (13). In the wood-based panel industry, for example, reforestable species such as *Pinus sp.*, *Eucalyptus sp.*, and *Acácia mearnsii*, however, it has been shown that the mixture of particles from the babassu coconut shell (epicarp) together with Portland cement produces quality panels (14).

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The endocarp produces charcoal with characteristics like eucalyptus with a carbonization temperature of 600 ºC, low volatile and carbon monoxide content, and other carbonization byproducts, in addition to producing twice as much energy with the same volume as other coals. Other advantages of babassu charcoal are its low sulfur composition (reduces SO_2 emissions) and low phosphorus composition. In this way, the charcoal produced from babassu endocarp when used in industry produces high-quality steel (15).

Starch, the most important plant energy reserve polysaccharide, represents 50 to 68 % of the mesocarp. One of the problems faced in its use is the high fiber content, between 10 and 30 %, which can hinder the access of enzymes to starch molecules during the industrialization process. On the other hand, industrial gelatinization processes cause a reduction of 10 to 2 % of fibers. Therefore, the production of ethanol from babassu coconuts of the *Orbignya phalerata* species is technically and economically viable (13,16).

The mesocarp of *Orbignya phalerata* contains 99 % carbohydrates and 1 % protein. From carbohydrates, a fraction containing glucose and other monosaccharides is obtained, which influences the reticuloendothelial system, increasing phagocytic activity, suggesting an immunomodulatory property. This fraction also inhibits the increase in vascular permeability, which is the first stage of the anti-inflammatory reaction (17). Other plants in this genus also apparently have medical properties. Alcoholic extracts of *O. phalerata*, for example, may inhibit Ehrlich carcinoma (18).

Oils extracted from almonds are used as a cheap source of carbon for high production of extracellular biosurfactants with emulsification activity, components that exhibit high active surface and emulsifying activities (hydrophilic and hydrophobic domains), from *Candida lipolytica* (19). Furthermore, they are also used as a cheap source for “solid state” fermentation systems (SSF). This type of fermentation using *Penicillium restrictum* and palm of the species *Orbynya oleifera* enabled the production of different enzymes through appropriate supplementation, such as lipases that can be used in the biodegradation of plastics (20).

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As a taxonomic marker, there is an ether (Triterpene methyl) that is commonly extracted from grasses and has been observed in species of *Orbinya phalerata*, O. speciosa, *O. cohune*, in percentages exceeding 50%, except for the species *O. phalerata*, which contains only 34% of Triterpene methyl (21).

This work aimed to carry out an integrative review of the problem faced in pest control, addressing new molecular tools used as a solution, to obtain an overview of current knowledge on the topic.

METHODS

The search was carried out in the main search bases for scientific articles, Scielo, Medline, Google, Science direct, using the keywords “Palm trees”, “babaçu coconut”, “Acre”, “Bruchinae”, “*Pachymerus nucleorum*”, “Enzyme”, “Control” “Amylase”. Terms in Portuguese were used for the search, as well as words translated into English. Articles were found covering the period from 1967 to 2023, with a total of 82 articles used. We organized the data presenting the main species found in Acre, Brazil, and the problem palm tree species have concerning the natural predator (the beetle). Their way of preying on palm trees and harming the culture and trade of nuts and coconuts. Finally, we present the main possible tools as biomarkers for controlling and protecting the palm tree.

DEVELOPMENT, RESULTS AND DISCUSSION

In Acre, we found 26 genera and 76 native species of palm trees (22,23).

The Arecaceae botanical family is represented by around 200 genera and 1,500 species distributed throughout the world. (22).

Representatives of the Arecaceae family are characteristic of tropical flora and can transmit luxury and fascination to these regions, which is why these characteristics are part of the national landscaping (24). Other examples include “puninha” (*Bactris gasipaes*) and “guariroba” (*Euterpe oleracea*), suppliers of “heart of palm” that can be

consumed both sweet and bitter. In the extractive form, we have the “babaçu do Maranhão” (*Attalea speciosa*) providing edible and industrial oil from almonds (24).

Attalea phalerata is distributed in the southwestern part of the Peruvian and Bolivian Amazon rainforest. In Brazil it is found in Acre, Goiás, Maranhão, Mato Grosso, Pará, Rondônia and Tocantins (22,25).

Known by the common names of Aricuri, Uricuri, Red Uricuri in Acre (26) and in other regions of Brazil known as Bacuri, Acuri, Urucuri (22,27). In the state of Acre, it is distributed in the municipalities of Marechal Taumaturgo, Plácido de Castro, Rodrigues Alves, Rio Branco, Sena Madureira, Tarauacá and Xapuri (26).

Attalea phalerata has a solitary stipe 5-10 m long, 60-75 cm in diameter, and sometimes the stipe is covered with leaf sheaths. Its leaves measure more than 6 m in length. Its fruits are arranged in inflorescences, measuring 5-6.5 cm in length and 3-5 cm in diameter with an ellipsoid-oblong shape; the epicarp is fibrous with a light brown color; fleshy yellowish mesocarp; endocarp with distinctly grouped fibers; each fruit can bear 2-4 seeds (24,25). Its usefulness is linked to the leaves them used to cover huts. In some Amazonian states, its endocarp is a source of charcoal and the mesocarp is edible. It is used for landscaping purposes. Wood is used for rural constructions and the “heart of palm” is edible. (Sin. *Scheelea phalerata* (Mart.) Burret) (Lorenzi et al., 2010) (28).

Works like Santos et al. (2003) (29) shows the importance of palm trees for shelter, feeding, and reproduction of various animals, including arthropods. Among these, the greatest abundance is in the Orders Coleoptera, Formicidae, Collembola, Psocoptera, Diptera, and Araneae. Within the Order Coleoptera, the family that most stood out the were Curculionidae, Staphylinidae, and Chrysomelidae, as also shown in the work of Meik J, Dobie P. (1986) (30) e Basset (2001) (31).

In addition to the diversity of arthropods associated with the *Attalea phalerata* canopy, a striking factor is the variety of individuals found in periods of flood and drought, (29,32). Other studies were carried out only with the orders Formicide and Araneae, (33,34), respectively.

The larvae of the subfamily Bruchinae are predators of 33 plant families. The majority are from the legume family, followed by Arecaceae, Convolvulaceae, Malvaceae and other plant families (35).

The species of the *Pachymerini tribe* become economically important, as they feed almost exclusively on palm seeds and are commonly called palm beetles. Among these palm trees, there are carnaúba (*Copernicia cerifera* Mart.), Bahia coconut (*Cocos nucifera* L.), licuri (*Syagrus coronata* (Mart.) Becc., piassava (*Attalea funifera* Martius) and babassu (*Orbignya phalerata* Mart.)(21).

Insects, like most organisms, produce digestive enzymes to obtain their essential nutrients. Among the most studied digestive enzymes are alpha-amylase (36). Alpha amylases are enzymes classified as endo amylases that catalyze α-1,4 glycosidic bonds of starch (37,38). Starch is one of the main components of seeds and grains (39).

THE PROBLEM – INSECTS OF THE BRUCHINAE SUBFAMILY

Bruchinae is a subfamily of Coleoptera included in the family Chrysomelidae. Until recently this subfamily was known as the Bruchidae family. Insects from the subfamily Bruchinae are predators of legume seeds (30,40), and other species of the Chrysomelidae family attack tropical plant crowns such as *Acácia farnesiana* (41,42), *Erythrina abyssinica* (43) and *Attalea phalerata* (29).

Members of this family are characterized by having a shortened elytrum, leaving the end of the abdomen (pygidium) unprotected. Their bodies are oval, with a free head, a short and flat rostrum, and antennae with 11 segments (44). The coleoptera *Pachymerus nucleorum* (Fabricius, 1792), a member of this family, is a very common insect throughout northern Brazil. It measures 12 to 15 mm in length and 5 to 7 mm in width. They are gray in color, striated elytra, ovoid and toothed posterior thighs, being one of the largest bruchids known (Figure 1). Of every 5 to 10 eggs laid per fruit, only 1 or 2 of the larvae that hatch manage to enter through the sap channels or hilum.

The larva *P. nucleorum* (Figure 2) parasitizes babassu palm trees (*Orbinya spp*) growing and developing through the assimilation of nut oil and proteins (45). After complete development, measuring 20 mm in length, they weave a cocoon (Figure 3). After emerging, the adults remain inside the coconuts for two weeks, exiting through the 5 mm diameter hole opening in the posterior part of the fruit insertion. As they attack the pulp of the fruits, they end up making them unusable for commerce. (44).

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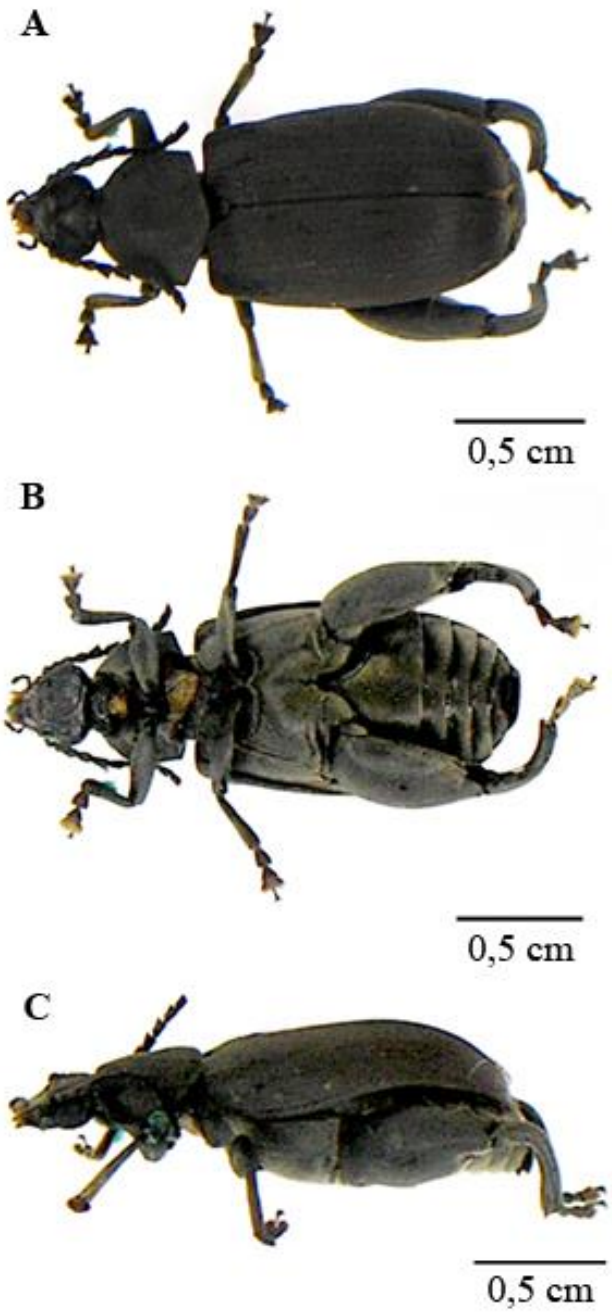


Figure 1: Adult specimen of the species *Pachymerus nucleorum*. Bruchins of the largest known size, 15 mm long and 7 mm wide, have a gray color, striated elytra, ovoid, and toothed posterior thighs. (A) Dorsal view. (B) Ventral view. (C) Side view.

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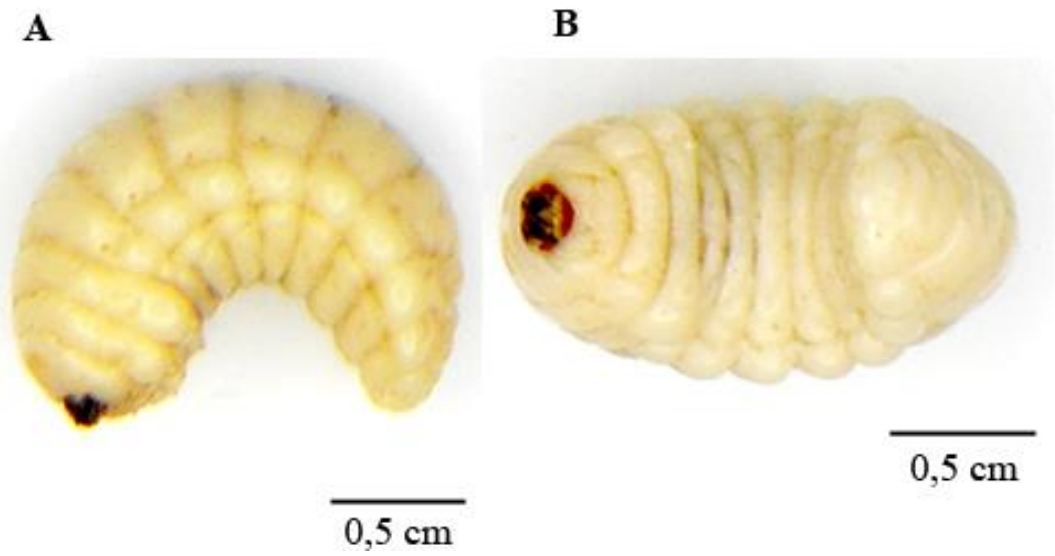


Figure 2: Specimen of larvae of the species Pachymerus nucleorum. Larva is used in this work. (A) Side view. (B) Ventral view.

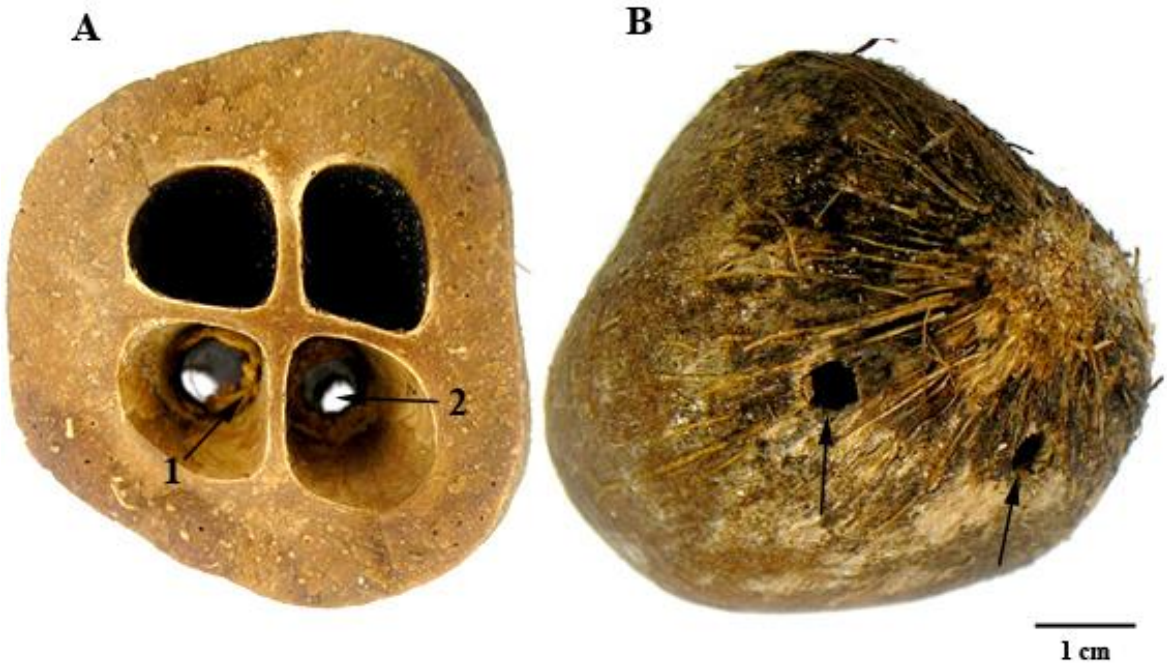


Figure 3: Specimen of babassu fruit (Orbignya sp.). The fruit has been sectioned and the nuts are not present. (A) Cross section. 1. Part of the cocoon that the larva weaves after feeding on the nut. 2. Hole made by the adult beetle to exit the fruit. (B) View of the anterior part of the fruit, which connects to the cluster. Arrows indicate holes made by beetles.

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Due to the nature of the species attacked by this type of Coleoptera, which have high levels of serine proteinase inhibitors, bruchids have developed special attack abilities, such as resistance to toxic alkaloids and free amino acids, as well as the nature of their digestive enzymes (41,42,46).

Zabrotes subfasciatus, another species of bruchid, has little dispersal capacity and attacks stored grains. The success of the species *Z. subfasciatus* in attacking common beans also depends on factors such as temperature, humidity, storage location, and competition with other bruchids (30). Proteinases found in *Z. subfasciatus* are resistant to trypsin and chymotrypsin inhibitors, being sensitive to cysteine proteinase inhibitors such as E-64 (47).

Control of these bruchids is normally carried out using insecticides that are toxic to animals and humans. However, it has been observed that certain plants have natural resistance to this type of pest, mechanisms acquired during evolution. Arcabose, isoarcabose, cyclodextrin are examples of non-protein α-amylase inhibitors found in plants. Due to their similarity with the substrate of this enzyme, they bind to the catalytic site, inhibiting amylase activity (37). Protein inhibitors are present in cereals such as wheat. Wheat extract (*Triticum aestivum*) has polypeptides around 14 kDa that inhibit amylases in some insects, such as *Acanthoscelides obtectus, Callosobruchus maculatus and Zabrotes subfasciatus* (37,38).

Phaseolamine, a 49 kDa glycoprotein found in species of *Phaseolus vugaris*, is commonly used to inhibit amylases in both insects and humans. This protein acts reversibly, without chelating action on calcium (48). Other genera of the Phaseolus species also have components with an inhibitory nature on the amylase activity of insects. Fractions extracted from species of *Phaseolus acutifolius* present polypeptides of approximately 14-18, 28, 35, and 40 kDa that inhibited amylases of *Callosobruchus chinensis, Callosobruchus maculatus e Zabrotes subfasciatus* (49). Some lectins significantly affect the amylase activity of *Z. subfasciatus* but do not affect the amylase activity of *Acanthoscelides obtectus* gus (50). In addition to lectins found in *P. vulgaris*, a lectin isolated from *Talisia esculenta*, a species of Sapindaceae, causes weight

reduction and increases mortality in larvae of *Z. subfasciatus* e *C. maculatus* (51) (Macedo et al.,2002)

Another means of plant resistance is through disordered growth outside of meristematic regions, a neoplastic phenomenon, preventing the entry of the larvae. In pea pods of the species *Pisum sativum* L., for example, there is an Np gene (Neoplastic gene) that is induced by the presence of esters released by the oviposition of females of *Bruchus pisorum* L.(52).

THE TOOL FOR CONTROL – BIOMARKERS

Amylases

Starch is the most important plant energy reserve polysaccharide and is present in cereal seeds such as corn, barley, wheat, and rice and in tubers or roots such as potatoes and cassava, etc. Its degradation has been widely studied and characterized and is mainly used by the food, paper, textile, chemical, and biological control industries (53).

Amylases are enzymes that degrade starch, hydrolyzing its glycosidic bonds. These are classified into endo amylases, exo amylases, debranching, and transfer enzymes depending on the type of bond cleaved (53).

α-Amylases (EC 3.2.1.1), classified as endoamylase, are enzymes that hydrolyze the α-1,4-glycosidic bonds of starch. Its catalytic site is composed of 3 domains (A, B, and C). The A domain has the Asp, Glu, and Asp residues that are arranged in a (b/α) 8 barrel or TIM (triose isomerase) barrel structure (Figure 4). In α-amylases from *Aspergillus oryzae* (Taka-amylases) these correspond to Asp-206, Glu-230, and Asp-270. The other domains (B and C) also have these residues in conserved regions, and unlike domain A, they are not directly involved in substrate catalysis (54). At the interface of domains A and B, a conserved region in the structure of α-amylases, Taka-amylases, *Bacillus amyloliquefaciens,* and CGTases, there is the presence of calcium ion, promoting greater stability and integrity of the active site (54–56).

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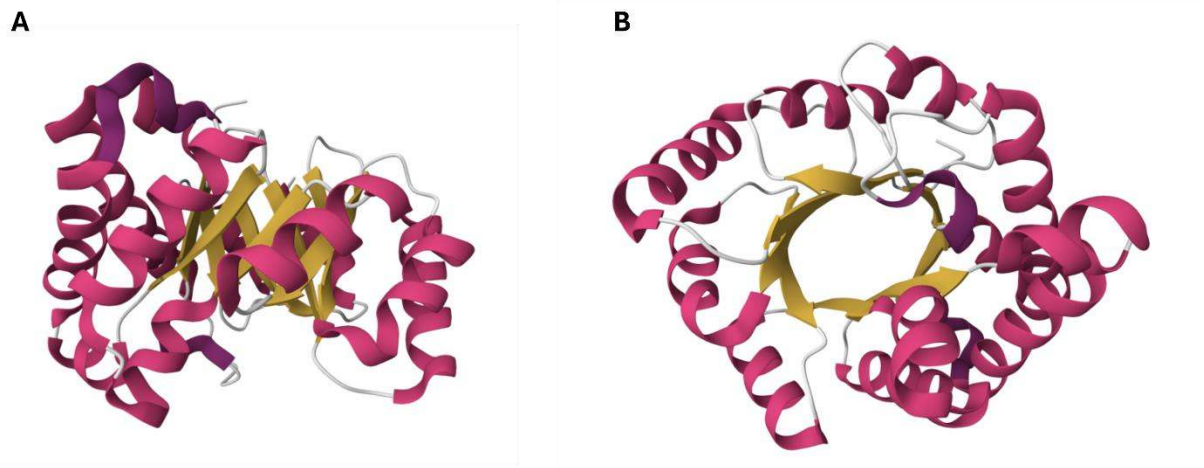


Figure 4: Structure of (α/b)8-Barrel. Catalytic domain present in the structure of enzymes belonging to the α-amylases family. (A) Side view. C-terminal end facing the entire page. (B) View from below. C-terminal end leaving the plane of the paper (Extracted RCSB.org, PDB ID: 4PC8 - **https://doi.org/10.2210/pdb4PC8/pdb)** (57)

The role of calcium in Taka-amylases is related to the reactivation of reduced enzymes. In native α-amylases from *Aspergillus oryzae* there are 4 disulfide bridges and a free sulfhydryl group. In some situations, disulfide bonds are reduced, and re-pairing may occur incorrectly. At concentrations of 5 mM calcium, an excess of 5 times the EDTA concentration, excellent renaturation occurs, recovering practically all activity. However, at high concentrations (100 mM), calcium can act in an inhibitory manner, binding to the second calcium-binding site present in Taka-amylases (54,58).

α-Amylases from *Bacillus amyloliquefaciens*, in the presence of calcium, present a higher denaturing temperature. At concentrations of 6 mM of the cation, there is an increase of 4 ºC in the denaturation temperature, varying from 72 to 76 ºC (59). On the other hand, calcium does not affect the activity and stability of *Bacillus sp* α-amylases at high temperatures (60). In a 75 kDa amylase from yeast (*Cryptococcus flavus*), the presence of calcium does not affect either the activity or stability of the enzyme (61).

Hyperthermophilic α-amylases from *Pyrococcus furiousus* do not require metal ions and show activity and stability around 100 ºC. This, unlike some bacterial

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amylases, from plants and many other archaebacteria, presents calcium and zinc binding sites close to the active site, at the interface of domain B and A. Zinc, in concentrations greater than 3 mM, inhibits more than 90% the activity of this enzyme, not being affected by calcium (62).

Differently, α-amylases from *Bacillus sp* (AmyK38), adapted to alkaline conditions and resistant to oxidizing reagents, do not bind calcium and are therefore not inhibited by chelating reagents. On the contrary, in site 1, a highly conserved and calcium-binding region, there is the presence of sodium (Na^+), acting in the same way in maintaining the functional structure of the AmyK38 molecule (63).

In *Callosobruchus maculatus*, α-amylase isoforms with 56, 45, and 35 kDa showed different inactivation profiles in relation to temperature. The 35 kDa amylase showed greater sensitivity to heat, while the 56 kDa one showed greater thermostability, but all were completely inactivated at 65 ºC for less than 10 minutes (64). Of these, none were inhibited by the α-amylase inhibitor (αAI-1), extracted from common bean (*Phaseolus vulgaris*) seeds, but were inhibited by inhibitors extracted from wheat (65).

Amylases isolated from *Zabrotes subfasciatus* were sensitive to heat, being slightly inhibited after 20 minutes at 60 ºC, but activity was reestablished after the addition of 20 mM NaCl or 1.0 mM $CaCl_2$ (47). In 1999, Silva and collaborators identified 3 α-amylase isoforms (95, 85 and 65 kDa) in *Z. subfasciatus*. The 85 kDa isoform is sensitive to heat, being completely inactivated after 30 minutes of incubation at 65 ºC, while the 65 kDa isoform was more thermostable, remaining active after 2 hours. The 95 kDa isoform showed intermediate thermostability, inactivating between 90 and 120 minutes.

ATPases

ATPases hydrolyze ATP into ADP and inorganic phosphate using the energy released in different cellular processes such as muscle contraction, the transport of

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ions across membranes, the beating of cilia and flagella and the movement of cellular vesicles (66–68). Some ATPases are integral membrane proteins such as Ca-ATPases and Na/K-ATPase, while others are found in the cytoplasm, such as myosins, dyneins and kinesins participating in movements throughout the cytoskeleton (69,70).

The latter, called molecular motors (Figure 5), carry out transport along F-actin filaments in the case of myosins and microtubules, formed by heterodimers of α, β tubulin, in the case of dyneins and kinesins. The "cargo transported" by molecular motors includes membranous organelles, protein complexes, nucleic acids and a variety of other structures present within the cell. (71).

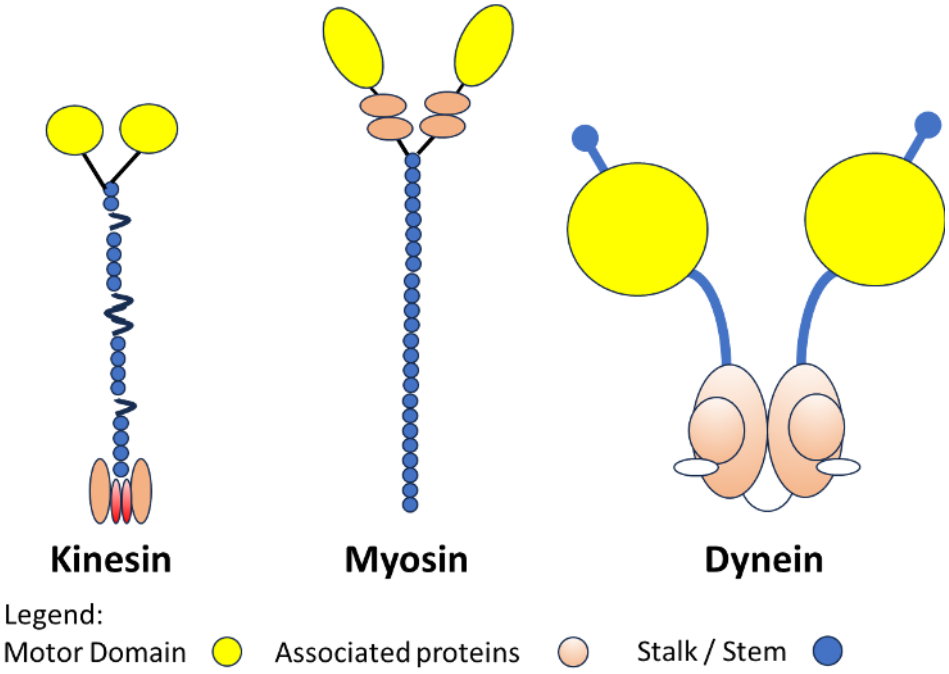


Figure 5: Three types of molecular motors, highlighting the similarities in the domain structures.

There are 10 classes of kinesins, 2 of dyneins, and 20 of myosins that are different in some respects. They all have a structural division into 2 domains, the motor domain, which hydrolyzes ATP and connects to the cytoskeleton, and the tail domain, which is a molecular structure that binds to the "transported cargo", and which can play some regulatory role in the motor (68,71).

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Kinesins are the most abundant motors in different types of cells and are composed of 144 motor domains in 31 species. Cargos transported by kinesins include vesicles, organelles, mitotic spindle, and chromosomes (72). The direction of movement carried out by kinesins can be either towards the end (-) or towards the end (+) of the microtubules (73).

Dyneins are divided into 2 groups, axonemal and cytoplasmic, with movement towards the (-) end of microtubules (70). These molecular motors are involved in the transport of vesicles from the Golgi complex to the endoplasmic reticulum, aiding the mitotic spindle and thus potentially cytokinesis, in the case of cytoplasmic dyneins; and beating of cilia and flagella in the case of axonemal dyneins (74,75).

Myosins are enzymes that are characterized by having three functional subdomains: a globular N-terminal domain (called head, or motor domain), which can bind to actin, hydrolyze ATP and translocate along actin filaments; a neck (or regulatory) domain, which consists of a calmodulin binding sequence and/or light chains, forming the IQ motifs, which can vary from zero to six in the different classes of myosins. Finally, a C-terminal region (tail domain) arranged in an α-helix with the ability to interact with the tails of other myosin molecules to form bipolar filaments (76,77).

The C-terminal region, which is specific to each myosin class (69), it can act as an “anchor” for the positioning of the head domain in the interaction with actin. This region also appears to have a regulatory and/or targeting function for myosin with its cargo (69,77).

Myosins are related to several cellular functions such as the transport of melanosome vesicles or RNA in the case of myosin V, endo- or exocytosis or phagocytosis in the case of myosin I (69,77).

In insects, there are numerous cellular functions, responsible for the maintenance and development of life, which are performed by both integral membrane and soluble ATPases (78,79). In locust intestines, Na/K-ATPasic activities were detected, which presented an optimum pH of 7.5 and whose peak activity occurs at

equimolar concentrations of Mg and ATP. In immunoblot, this ATPase presented subunits similar to the ATPase of the V-ATPases family (79).

Manduca sexta, tobacco hornworm, has a 40 kDa polypeptide that, through immunoblot and cDNA expressions, showed high similarity with ATPase subunits belonging to the V-ATPase family (80). In *Manduca sexta* you can also find members of ATPases associated with various cellular activities (AAA Families) that have one or two ATP-binding domains. MsNSF (Manduca sexta fusion protein sensitive to N-ethylmaleimide) is a homo-oligomeric ATPase, classified as type II, as it has two ATP binding and hydrolysis domains, the first domain highly related to the transport function, while domain 2 is required for hexamerization of NSF, a functional holoenzyme in transport (81).

ATPases can still act as biological control tools, as there are natural insecticides that cause inhibition of specific ATPases and, as a result, cause the death of the insect. Compounds such as 1,5-diphenyl-2-penten-1-one (dp-B), first isolated from *Stellera chamaejasme* (a plant typical of China), act on the nervous system of locusts, inhibiting their Ca-Mg-ATPasic activity to the detriment of other ATPases (82).

FINAL CONSIDERATIONS

Given the economic impact arising from the pest/problem of predation of insects from the bruchinae subfamily on palm trees, the search for new biomarkers used as biological control criteria, taking advantage of the biodiversity of plants and resources found in the Amazon is very important. The detection of key enzymes in insect metabolism, its functioning, and especially its metabolic diversity concerning other animals, insects, plants, and especially humans can be much more economical, simple, effective, and a new pest control tool/product. All this and not least, the action without adverse reactions to the other organisms contained in that habitat.

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