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Hydrolases in the hypopharyngeal glands of workers of *Scaptotrigona postica* and *Apis mellifera* (Hymenoptera, Apinae)

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ABSTRACT. Hydrolytic enzymes from hypopharyngeal gland extracts of newly emerged, nurse and foraging workers of two eusocial bees, *Scaptotrigona postica*, a native Brazilian stingless bee, and the Africanized honey bee (*Apis mellifera*) in Brazil, were compared. The hypopharyngeal gland is rich in enzymes in both species. Fifteen different enzymes were found in the extracts, with only a few quantitative differences between the species. Some of the enzymes present in the extracts may have intracellular functions, while others seem to be digestive enzymes. *Scaptotrigona postica*, had lower β-glucosidase and higher lipase esterase activities than *A. mellifera*. The differences may be due to different feeding habits and behavioral peculiarities of the two species.

Key words: Apidae, Apini, Enzymes, Esterase lipase, Glucosidases, Africanized honeybee, Meliponini, Stingless bee

INTRODUCTION

In *Scaptotrigona postica*, differently from *Apis mellifera*, the hypopharyngeal glands are present in males, queens and workers; the morphological organization is similar in these different sexes and castes (Costa and Cruz-Landim, 1977, 1999a), although it is more developed in workers. In queens and males, the hypopharyngeal glands are more highly developed in the newly emerged individuals (Costa and Cruz-Landim, 1999a); while in workers, they are most developed during the nursering phase.

In nurse workers of *A. mellifera*, the hypopharyngeal glands produce most of the proteins of the royal jelly (Patel et al., 1960; Klaudiny et al., 1994; Kubo et al., 1996), which is the food given mainly to young larvae and queens, but also to males and workers (Crailsheim, 1992; DeGrandi-Hoffman and Hagler, 2000). In *S. postica*, it is not yet known whether there is hypopharyngeal gland secretion in the larval food (Costa and Cruz-Landim, 2002a,b). Although tropholaxis occurs among the colony members, the workers do not feed queens and males with secretions. Nevertheless, the glands of *S. postica* workers go through developmental cycles, and they are similar to those of *A. mellifera* in other ways (Costa and Cruz-Landim, 2000, 2001) but their function is still not completely understood.

The social organization of the highly eusocial bees is characterized by an age-based division of labor among the workers (polyethism), in which different tasks are assumed as the individual matures. Therefore, except for the fact that a certain behavioral plasticity is maintained on behalf of the colony needs, the different tasks accomplished by the workers are preceded by changes in their physiological conditions, which change with age.

The secretory cycle of the hypopharyngeal glands of *A. mellifera* is closely related to the brood nourishment function accomplished by nurse workers between 3 and 18 days old (Rösch, 1925; Lindauer, 1952; Sakagami, 1953; Halberstadt, 1966). In *S. postica,* the division of labor among workers follows a similar pattern (Hebling et al., 1964), but there are differences in the types and sequences of behaviors. While in *A. mellifera*, the nurse workers visit the larvae several times a day to feed them (Ribbands, 1953), in *S. postica* they build brood cells, supply them with food and close them soon after egg oviposition (Sakagami and Zucchi, 1963, 1966).

There is little information available about the composition of the food supplied to the brood of the meliponines; however, the food contains honey, pollen and a fluid portion similar to honey bee royal jelly (Michener, 1974). It is presumed that the hypopharyngeal glands of meliponines produce all or part of the fluid fraction of the larval food, similarly to *A. mellifera*, based on the resemblances in the glands' secretory cycles (Simões and Bego, 1979; Bego, 1983; Costa and Cruz-Landim, 1999a, 2000), in the ultrastructural features (Costa and Cruz-Landim, 2000), in the pattern of division of labor of workers, and in the electrophoretic protein pattern of the glandular extracts (Silva-de-Moraes et al., 1996). In *A. mellifera*, the hypopharyngeal glands also produce invertase and glucose oxidase (White Jr. et al., 1963; Simpson et al., 1968; Takenaka et al., 1990a,b; Ohashi et al., 1996, 1999).

Though the hypopharyngeal glands of *S. postica* and *A. mellifera* regress as they pass from brood care tasks to other functions, they still may remain active, as evidenced by the glandular cell ultrastructure of foragers (Costa and Cruz-Landim, 2000; Cruz-Landim et al., 2000). We examined the enzymes in the hypopharyngeal glands of meliponine workers to look for possible functions, and we compared enzymatic activities of hypopharyngeal gland extracts from workers of *S. postica* and *A. mellifera*.

MATERIAL AND METHODS

Specimens of *S. postica* were collected from medium-size colonies, maintained in wood hives designed for meliponines (Nogueira-Neto, 1997) in Rio Claro, SP, Brazil. *Apis mellifera* workers were captured from a single colony maintained in the same apiary.

The following groups of bees were analyzed: newly emerged, nurse and foraging workers of *S. postica* and *A. mellifera* (50 individuals of each group). The hypopharyngeal glands from these individuals were dissected and macerated in saline solution, and 1,350 µL of bidistilled water was added to each sample for the Api Zym test.

The enzymatic tests were done with substrates of the Api Zym kit (Bio Mèrieux), which semiquantitatively identified 19 different enzymes. The Api Zym kit was developed to assay enzymes present in raw extracts of organs, without purification, and initially was used to determine enzymatic production in the alimentary canal (Plantevin and Nardon, 1972) and in glands of the salivary system of bees (Arnold and Delage-Darchen, 1978; Delage-Darchen et al., 1979, 1982; Delage-Darchen and Darchen, 1982). It consists of a gallery of 20 pits, each one containing a specific substrate for the enzyme to be tested. The result of the enzyme-substrate reaction is a colored product. The color intensity is directly correlated to the degree of substrate hydrolysis. The approximate amount (in nmol) of the enzyme is given by comparison with a colored scale that comes with the kit. The assays were repeated twice for each sample; in all cases the values were the same.

RESULTS

Among the 19 enzymes tested with the Api Zym kit, 15 were detected in both species; the enzymes that gave values between 0 and 5 nmol were considered absent and those with 5 nmol activity were considered to be present in trace amounts (Table 1). Among the enzymes considered as having a possible function, numbers 10-17 (Table 1) had different concentrations in the two bee species; only enzymes 18 and 19 had similar patterns in the three phases of the workers of the two species. The main differences found between the species were in the enzymes that digest carbohydrates, including β-galactosidase, which is present at higher activity levels in newly emerged and nurse workers of *S. postica*, while it was present in significant amounts only in newly emerged workers of *A. mellifera*. β-glucosidase was absent in *S. postica* and was present only in nurse bees and foragers of *A. mellifera*. The enzymes α-glucosidase and N-acetyl-β-glucosaminidase were also present at different levels in the two species. The esterase lipase (C8) was found only in *S. postica*.

DISCUSSION

The hypopharyngeal glands are only found in the Hymenoptera, where they are present in both sexes, except in a few species such as *A. mellifera*, in which males and queens have no functional hypopharyngeal glands. The function of this gland in most of the Hymenoptera is unknown, but it apparently produces enzymes in most species, and in social species it produces food for immature and mature nest mates (Cruz-Landim and Costa, 1998).

Although digestive enzyme production for food digestion in insects mainly occurs in the midgut, the glands in the salivary system, linked to the bucal cavity or bucal appendages, also

Table 1. Enzymatic activity tested by the Apizym (Bio Mèrieux) system of hypopharyngeal gland extracts from workers of *Scaptotrigona postica* and *Apis mellifera*, in nmol of digested substrat.

Enzymes	*Scaptotrigona postica*			*Apis mellifera*		
	Newly emerged	Nurse	Forager	Newly emerged	Nurse	Forager
1. Trypsin	0	0	0	0	0	0
2. α-Chymotrypsin	0	0	0	0	0	0
3. α-Galactosidase	0	0	0	0	0	0
4. β-Glucuronidase	0	0	0	0-5	0	0
5. Lipase (C14)	0-5	0-5	0-5	0-5	0-5	0-5
6. Alkaline phosphatase	5	5	5	0-5	0-5	0-5
7. α-Fucosidase	5	0-5	5	5	0-5	0
8. α-Mannosidase	0-5	5	5	5	5	5
9. Cystine arylamidase	5	5	5	5	5	0-5
10. Esterase (C4)	10	10	10	5	5	10
11. β-Glucosidase	0-5	0-5	0-5	5	30	30
12. Valine arylamidase	5	≥40	5	5	5	0-5
13. β-Galactosidase	≥40	30	5	20	5	0-5
14. Esterase lipase (C8)	20	30	≥40	5	5	5
15. α-Glucosidase	5	≥40	5	10	5	≥40
16. N-acetyl-β-glucosaminidase	≥40	≥40	5	30	5	10
17. Acid phosphatase	≥40	≥40	5	20	20	≥40
18. Naphthol AS-BI-phosphohydrolase	≥40	≥40	10	≥40	≥40	10
19. Leucine arylamidase	≥40	≥40	≥40	≥40	≥40	30

produce some of these enzymes in several insect species (Chapman, 1998). Simpson et al. (1968), Takenaka et al. (1990a) and Ohashi et al. (1996) found invertase and glucose oxidase in the secretion of the hypopharyngeal glands of *A. mellifera*, as well as other inhibines, which has germicide activity (Takenaka et al., 1990a,b; Ohashi et al., 1999).

The hypopharyngeal gland extract of *Scaptotrigona mexicana* hydrolyzed 10 of the 19 substrates when tested with a kit similar to the Api Zym kit that we used (Delage-Darchen et al., 1982). Larger differences in the activity levels of the esterase lipase were found between *S. mexicana* and *A. mellifera* than we found between *S. postica* and *A. mellifera*.

We found greater quantities of β-galactosidase and N-acetyl-β-glucosaminidase in the hypopharyngeal glands of *S. postica* than was reported for *S. mexicana* and *A. mellifera* (Delage-Darchen et al., 1982). The β-galactosidase activity was high in newly emerged workers and nurse bees of *S. postica*, while in *A. mellifera* it was present in functional quantites only in newly emerged workers (Table 1). This corresponds with a greater consumption of pollen by the younger workers. The β-galactosidases in the midgut of lepidopterans catalyze the hydrolysis of glycolipids originating from plant tissues, being used in the synthesis of hemolymph and tissue lipid trehalose (Turunen, 1992). The same can occur in bees that are, also, phytophagous. As well, variations in the amount of enzymes along the worker life phases may occur because these are produced only when their substrates are present and in quantities corresponding to it.

The enzyme α-glucosidase was found in hypopharyngeal gland extracts of *A. mellifera* by Plantevin and Nardon (1972). In fact, these glands are the only ones in the salivary system of

A. mellifera that have α-glucosidase activity (Arnold and Delage-Darchen, 1978). The α-glucosidase catalyzes polysaccharide digestion and performs the final steps of starch digestion (Bergmeyer, 1984). This enzyme is highly active in the hypopharyngeal glands of foragers of *A. mellifera*, a phase in which the workers are collecting nectar, which is processed into honey by invertase, which is a type of α-glucosidase. Nevertheless, in *S. postica* the α-glucosidases were only found in large amounts in the nurse workers (≥40 nmol), a phase in which the workers are not collecting nectar, though they may be processing it in the hive.

The α-glucosidases can be constituent elements of the hypopharyngeal gland secretion or they may act in cellular processes. Inside of the cells, they are found in large amounts in lysosomes (Pitt, 1975), where the final steps of the digestion of disaccharides and oligosaccharides found in the food occur (Terra et al., 1996).

β-glucosidases hydrolyze sugars, such as cellobiose, hemicellulose, gentianose, and carbohydrate moieties of glucoproteins (Terra et al., 1996). These enzymes were found in large amounts only in nurse and forager workers of *A. mellifera*, being absent from the gland extracts of *S. postica*. This result is in accordance with the data of Arnold and Delage-Darchen (1978), Delage-Darchen et al. (1979, 1982) and Delage-Darchen and Darchen (1982) although these enzymes are frequently found in phytophagous insects. The results indicate that the process of digestion of the pollen may be different in *A. mellifera* compared to *S. postica*.

Similar to what is known for *S. mexicana* and *Melipona beecheii* (Delage-Darchen and Darchen, 1982; Delage-Darchen et al., 1982), the hypopharyngeal gland of *S. postica* was found to have considerable esterase lipase. In foraging workers of *S. postica*, this enzyme was present in larger quantities (≥40 nmol) than in the other age groups of the two species (20-30 nmol). In *S. postica*, the activity of the esterase lipase increased progressively, beginning with 20 nmol in newly emerged workers, increasing to 30 nmol in nurse workers and to ≥40 nmol in the foraging workers. Bees may use floral lipids as food or in nest building (Roubik, 1992). The esterase lipases in the hypopharyngeal gland extracts of *S. postica* may be for one of these uses, which are not common in *A. mellifera*; this may explain the differences found between the species (Table 1). Also, the large amounts of lipase in the midgut of some predacious ants seem be related to their habit of feeding on the fat of dead animals (Ayre, 1967). The predominant digestive enzymes of most of the carnivorous and necrophagous beetles are proteases and lipases (Müller, 1938). Several Trigonini related to *S. postica* are necrophagous and *S. postica* has the habit of carrying vertebrate fecal material to the hive (Nogueira-Neto, 1997), which according to Roubik (1992) may be used for feeding, nest building or germicide production; this may explain the presence of this enzyme.

Among the three arylamidases tested, only leucine arylamidase was found in significant amounts in the two species (Table 1); while high activity of valine arylamidase was only found in nurse workers of *S. postica*.

Changes in food consumption and in duties performed by the bees are correlated with substances circulating in the hemolymph (Crailsheim, 1986). Leucine is one of the 10 most important essential amino acids for maximal growth of the young honey bee (Groot, 1953). Protein metabolism is fast in young bees (newly emerged and nurse workers), therefore demanding large quantities of leucine arylamidase. Foraging workers also have considerable protein metabolism, and the turnover rate of body protein is highest in this group (Crailsheim, 1986). In *S. postica*, the quantity of leucine arylamidase was the same (≥40 nmol) in all worker classes; however, in *A. mellifera* the quantities varied (30 in forager workers and ≥40 in newly emerged

and nurse workers). Perhaps the foraging workers of *S. postica* are more active than those of *A. mellifera*, accomplishing similar duties more intensely or executing some additional activity, demanding larger amounts of leucine arylamidase.

In most animal tissues, acid phosphatase occurs inside lysosomes (Chayen et al., 1973) and is involved with intracellular digestion, protein synthesis or cell reabsorption. The acid phosphatase levels showed opposite profiles in *S. postica* and *A. mellifera*, occurring in larger amounts in the younger workers of *S. postica* and in the foraging workers of *A. mellifera*. The acid phosphatase detected in the hypopharyngeal gland extracts of *S. postica* seems to act in the cellular metabolic processes, rather than being a component of the glandular secretion; while, the enzyme activities in the *A. mellifera* foragers were expected to be based on lysosomal activity and due to the involutive process in the glands of foraging workers (Cruz-Landim and Hadek, 1969). The occurrence of larger amounts of acid phosphatase in the hypopharyngeal glands of newly emerged and nurse workers of *S. postica* could be related to its utilization in gland differentiation and secretion elaboration, respectively. A similar situation could occur with naphthol AS-BI phosphohydrolase, since it is also a phosphatase and their profiles are almost identical.

The hypopharyngeal glands of workers of *A. mellifera* produce several of the proteins of royal jelly (Patel et al., 1960; Yatsumami et al., 1987; Hanes and Simúth, 1992; Klaudiny et al., 1994; Kubo et al., 1996), besides producing digestive enzymes, such as glucose oxidase (Takenaka et al., 1990a,b), amylase and invertase (Simpson et al., 1968; Takenaka et al., 1990a; Ohashi et al., 1996, 1999; Costa and Cruz-Landim, 2003). In *A. mellifera*, glandular activation seems to be limited to the nurse and foraging workers, while in *S. postica* it involves all classes of workers and young queens and males (Costa and Cruz-Landim, 1999b). However, in both species the hypopharyngeal glands are hypertrophic in nurse workers, which apparently is related to the function of producing brood food. In the other classes of individuals, the main function seems to be enzyme production. We still do not know how these enzymes are being used. They may be used for digesting the food that they consume, being given to other individuals during trophollaxis, or they may be deposited for storage in pots, for nectar transformation into honey or for predigestion of pollen.

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