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The gut of Guatemalan passalid beetles: a habitat colonized by cellobiose- and xylose-fermenting yeasts☆

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ABSTRACT

The gut of insects is a productive environment for discovering undescribed species of yeasts, and the gut of wood-feeding insects of several families is especially rich in yeasts that carry out the fermentation of cellobiose and xylose. Passalid beetles (Passalidae, Coleoptera) live in dead wood that they ingest as their primary food source. We report the isolation, molecular identification and physiological characterization of 771 yeast cultures isolated from the gut of 16 species of passalids collected in nine localities in Guatemala. Ascomycete yeasts were present in the gut of every passalid studied, and the xylose-fermenting (X-F) yeasts *Scheffersomyces shehatae* and *Scheffersomyces stipitis* were the most abundant taxa isolated. The gut of the beetles also contained undescribed cellobiose-fermenting and X-F species in the *Lodderomyces*, *Scheffersomyces* and *Spathaspora*, and undescribed species in *Sugiyamaella* clades as well as rare yeast species in the *Phaffomyces* and *Spencermartinsiella* clades. Basidiomycete yeasts in the genera *Cryptococcus* and *Trichosporon* were also common. The yeast species richness was influenced by the host species and the substrate, and gut-inhabiting yeasts have the ability to survive the differing physiological conditions of several gut compartments.

Introduction

Yeast isolations from the gut of insects have led to discovery of a large number of new species of yeasts (Suh et al., 2003, 2004a, 2005a, 2006; Suh and Blackwell, 2005, 2006; Berkov et al., 2007; Rivera et al., 2009; Grunwald et al., 2010; Houseknecht et al., 2011; Calderon and Berkov, 2012; Urbina et al., 2013). Despite these efforts the diversity of gut-inhabiting yeasts remains understudied. Suh et al. (2005a) suggested that only 50 % of the gut-inhabiting yeasts from certain Panamanian mushroom-feeding beetles have been discovered after extensive sampling from more than 20 insect families. The new yeasts are important because they fill taxon-sampling gaps, help to understand the phylogenic relationships among members of Saccharomycotina, and synthesize enzymes, vitamins and other products that could be useful in biotechnological processes.

Recent rising fuel costs have stimulated the search for new yeasts capable of fermenting cellobiose and D-xylose, which can be used in the production of bioethanol. Many of the yeasts discovered were isolated from soil, plant tissues and the gut

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of wood-feeding beetles (e.g. Cerambycidae, Curculionidae, Lucanidae, Passalidae) (Suh et al., 2003, 2005a; Zhang et al., 2003; Suh and Blackwell, 2004; Berkov et al., 2007; Cadete et al., 2009; Tanahashi et al., 2010; Santos et al., 2011; Calderon and Berkov, 2012). In general the characterization of yeasts associated with the gut of lignicolous insects confirmed the consistent association between xylose-fermenting (X-F) yeasts and insects.

Passalid beetles (Passalidae, Coleoptera) mostly feed on rotting wood and spend most of their lives inside rotting logs (Reyes-Castillo, 1970; Boucher, 2005; Schuster, 2006). The beetle family comprises approximately 960 species, and in the most recent taxonomic reclassification of the family, besides their distribution, external morphological characters and biology, the macro-morphology of the anterior hindgut is another character proposed to distinguish the five subfamilies in Passalidae (Fonseca et al., 2011). The subfamilies Passalinae and Proculinae are distributed exclusively in the New World, while Aulacocyclinae, Macrolininae and Solenocyclinae occur in Asia, Africa and Australia (Boucher, 2005; Fonseca et al., 2011).

The majority of passalids exhibit subsocial behavior that includes parental care by the feeding of a mixture of digested wood and feces to larvae and juveniles. Adults also envelop the larvae at the time of metamorphosis to pupae with a covering of frass and pre-digested wood, the pupal chamber, that becomes the first meal for juveniles as they emerge (Reyes-Castillo, 1970; Tallamy and Wood, 1986). Such behavior suggests that horizontal transfer of microbes is required for the complete metamorphosis from larvae to adults, and apparently larvae cannot survive when fed only sterilized pulverized rotten wood (Pearse et al., 1936; Reyes-Castillo, 1970; Nardi et al., 2006; Berkov et al., 2007; Rao et al., 2007). These findings emphasize the important role of the passalid gut microbiota in the digestion of the substrate in wood-feeding insects.

The biology, ecology and physiology of passalids are best studied for *Odontotaenius disjunctus*, a common species in the southeastern United States (Pearse et al., 1936; Roberts, 1952; Bryan, 1954; Hiznay and Krause, 1955; Ferguson and Land, 1961; Robertson, 1962; Collings, 1966; Burnett et al., 1969; Ward, 1971; Delfinado and Baker, 1975; Dismukes and Mason, 1975; Schuster, 1975; Gibson, 1977; Rains and Dimock, 1978; Buchler et al., 1981; Mason et al., 1983; Tafuri and Tafuri, 1983; Wit et al., 1984; Sawvel et al., 1992; MacGown and MacGown, 1996; King and Fashing, 2007; Punzo, 2007; Jackson et al., 2009; Wicknick and Miskelly, 2009). Nardi et al. (2006) characterized the morphological and cellular transformations of the hindgut region of the digestive system from larva to adult of *O. disjunctus*, and described the physical distribution and arrangement of the microbiota in the gut compartments.

The digestive system of an adult *O. disjunctus* is often over 10 cm long and a complex organ, at least twice as long as the length of an individual. The three regions of the gut (foregut, midgut and hindgut) are conspicuously differentiated and, in addition, the hindgut has readily distinguishable anterior and posterior compartments (Nardi et al., 2006). The gut regions also differ in their physiological conditions of O_2, CO_2 and pH (Ceja-Navarro et al., 2013). Another structure, a conspicuous diverticulum, is present at the anterior end of the hindgut. In addition to bacteria and yeasts, trichomycetes, amoebae, nematodes, flagellated protists and filamentous fungi may be present in the gut of *O. disjunctus*. Most notable, a variety of bacteria attach to form conspicuous surface films in the anterior hindgut, while filamentous yeasts almost exclusively colonize the posterior hindgut attached by a holdfast (Suh et al., 2003; Nardi et al., 2006; Ceja-Navarro et al., 2013).

The ascomycete yeasts inhabiting the gut of *O. disjunctus* have been characterized by molecular and physiological means. Previous studies have confirmed the predominant and consistent presence of the X-F yeast *Scheffersomyces stipitis* and other less abundant X-F yeasts including *Scheffersomyces shehatae*, *Candida maltosa* (*Lodderomyces* clade) (Suh et al., 2008) and the cellobiose-fermenting (C-F) yeast *Scheffersomyces ergatensis* (Suh et al., 2003; Zhang et al., 2003; Nardi et al., 2006). The X-F yeasts *Spathaspora passalidarum* and *Candida jeffriesii* (*Spathaspora* clade) (Nguyen et al., 2006), the trehalose-fermenting yeast *Kazachstania intestinalis* (Suh and Zhou, 2011), the D-xylose-assimilating yeast *Sugiyamaella bullrunensis* (Houseknecht et al., 2011) and the basidiomycete yeast *Trichosporon xylopini* (Gujjari et al., 2011) were all first described in association with *O. disjunctus*. The gut of this single passalid species has been a source for isolation of at least nine previously unknown ascomycete and basidiomycete yeasts that exhibit the ability to utilize several wood components.

Only a few studies have characterized yeasts from the gut of other passalids such as *Paxillus leachi*, *Passalus interstitialis*, *Ptichopus angulatus, Verres hageni*, *Verres sternbergianus* and *Veturius platyrhinus*, which occur in Panama and Peru. Species closely related to *Candida mycetangii* (*Wickerhamomyces* clade) and *S. stipitis*, as well as other yeasts including *Candida parapsilosis* (*Lodderomyces* clade), *Candida temnochilae* (*Yamadazyma* clade) and the basidiomycete yeast *Trichosporon insectorum* were isolated from these beetles (Suh et al., 2003, 2005a,b; 2008; Fuentefria et al., 2008). The diversity of yeasts associated with the gut of most passalid beetles is, however, poorly known considering the worldwide distribution of the group.

The principal aim of this study was to expand the knowledge of diversity of ascomycete and basidiomycete yeasts in the gut of Guatemalan passalids. This aim was achieved through the isolation, identification and characterization of 771 yeast isolates from the gut of 16 species of passalids collected at nine localities in Guatemala. Our results confirm the association of passalids and X-F yeasts, including undescribed species in the *Phaffomyces*, *Scheffersomyces*, *Spathaspora*, *Spencermartinsiella* and *Sugiyamaella* clades.

Materials and methods

Passalid collection

A total of 47 adult specimens identified as 16 species of passalids in two subfamilies, Passalinae and Proculinae were collected at nine localities in Guatemala (Fig 1, Tables 1 and 2). The localities were selected based on the endemic passalid zones described previously (Schuster, 1991, 1992a,b, 1993, 2002, 2006; Schuster et al., 2000, 2003). Targeted searches in passalid galleries in rotted logs were effective for collecting passalid individuals, and only one passalid specimen was collected at a light trap.