Insect science

Insect Science (2010) 17, 175–183, DOI 10.1111/j.1744-7917.2010.01320.x

REVIEW

The scarab gut: A potential bioreactor for bio-fuel production

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Abstract Cellulose and hemicelluloses are the most prevalent sources of carbon in nature. Currently many approaches employ micro-organisms and their enzyme products to degrade plant feedstocks for production of bioenergy. Scarab larvae are one such model. They consume celluloses from a variety of sources including plant roots, soil organic matter and decaying wood, and are able to extract nutrients and energy from these sources. In this paper, we review the physicochemical properties of the scarab larval gut, the diversity and digestive role that microflora play in the scarab gut and discuss the potential for applying these digestive processes in bioreactors for improving bio-fuel production. Scarab larvae are characterised by their highly alkaline midgut which is dominated by serine proteinase enzymes, and a modified hindgut which harbors the majority of the intestinal microbiota under anaerobic conditions. Evidence suggests that digestion of recalcitrant organic matter in scarab larvae likely results from a combination of endogenous gut proteinases and cellulolytic enzymes produced by symbiotic micro-organisms. Most of the easily digestible proteins are mobilized and absorbed in the midgut by endogenous proteinases. The hindgut contents of scarab larvae are characterized by high concentrations of volatile fatty acids, the presence of fermenting bacteria, and typical anaerobic activities, such as methanogenesis. The hindgut typically contains a wide diversity of micro-organisms, some of which appear to be obligate symbionts with cellulolytic potential. As a result, the scarab larval gut can be regarded as a small bioreactor resembling the rumen of sheep or cattle, where solid food particles composed of cellulose, hemicellulose, pectin and polysaccharides are degraded through enzymatic and fermentation processes. Together these observations suggest scarab larvae have potential to assist the bio-fuel industry by providing new sources of (hemi)cellulolytic bacteria and bacterial (hemi)cellulolytic enzymes.

Key words bio-fuel, bioreactor, cellulolytic enzymes, microflora, Scarabaeidae

Introduction

The order Coleoptera is the largest within the insect world and contains the family Scarabaeidae, whose members predominate in grassland soils (Lavelle *et al*., 1997) and other environments where decaying plant materials form a high proportion of biomass.

The larvae of scarabaeids are almost exclusively herbivorous or saprophagous (Crowson, 1981), and many species live in soils and feed on plant roots or organic matter of low nutritive value (McQuillan & Webb, 1994; Zhang & Jackson, 2008). The typical intestinal tract of humivorous scarabaeid beetle larvae has several characteristic physicochemical properties, including: a long midgut occupying most of the length of the body cavity (Terra, 1990); a modified and expanded hindgut (often referred

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to as a fermentation chamber) (Terra, 1990); a highly alkaline midgut with multiple alkali-stable hydrolases (Li & Brune, 2005); and specific gut microbiota (Egert *et al.*, 2003; Zhang & Jackson, 2008). While several scarab larvae are important agriculture and forestry insect pests, many scarab larvae play an important role in decomposing cattle dung, or decaying wood, and therefore may provide an effective resource for allowing the utilization of other organic biowastes in the future (Koyama *et al.*, 2003).

In this review, we have assembled available information on digestive enzymes and gut bacteria in scarab larvae in order to understand the digestion process of (hemi)celluloses (refers to both hemicelluloses and celluloses) in the scarab larval gut. Through this work we hope to offer new ideas and insights toward creating future bioreactors for bio-fuel production.

Morphology and biochemistry of the scarab gut

Gut morphology

A typical larval alimentary tract of a Scarabaeidae is divided into three major sections: a foregut containing a small crop, a long midgut that occupies a high proportion of the body cavity, and a modified expanded hindgut (Terra, 1990; Zhang & Jackson, 2008) (Fig. 1).

The foregut is lined with cuticle and extends from the oral cavity to the cardiac valve (valvula cardiaca), which marks the transition to the midgut (mesenteron). The crop is expandable and can be used for food storage with circular muscles at the cardiac valve that are able to restrict entry of food particles into the midgut. The midgut consists of a tube of epithelial cells and extends for most of the body length, occupying a large proportion of the body cavity, and it is distinguished by the presence of three rows of caecae circling the tract. Two rows are found at about one-third of the distance from the anterior end and one row is located at the terminal portion of the midgut. The point of division between midgut and hindgut (proctodeum, ileum) is marked by the entry of the malphigian tubules. The anterior portion of the hindgut is the highly muscular pyloric sphincter, which controls movement of food materials between the mid- and hindguts. Beyond the sphincter, the tract expands into a distinctive chamber, described as a fermentation chamber, which is lined with cuticle and covered with distinctive lobe-like structures extending into the lumen from the cuticular surface. The alimentary tract reverses direction before entering the fermentation chamber, which consists of two large

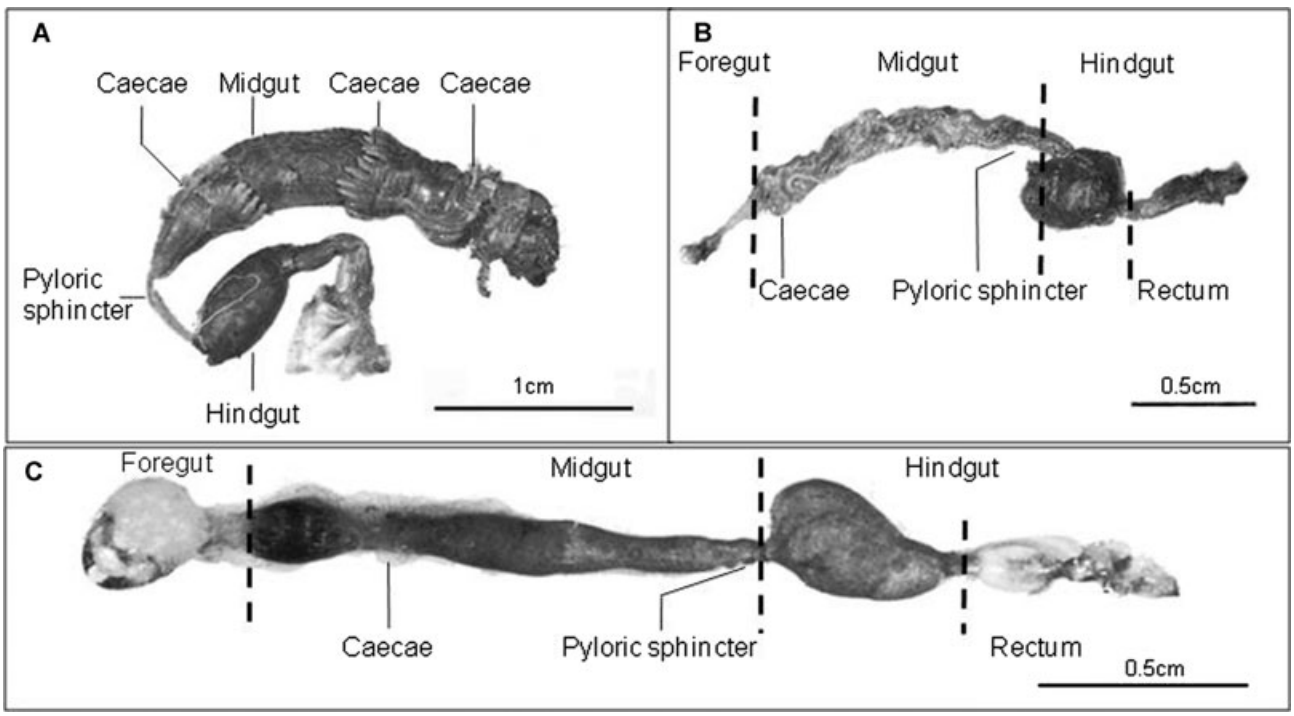

Fig. 1 View of the alimentary tract of scarab larvae showing relative locations of the foregut, midgut and hindgut for (A) *Papuana huebneri*, (B) *Holotrichia parallela*, (C) *Costelytra zealandica*.

sections lying on each side of the midgut. Interestingly, the hindgut fermentation chamber (Fig. 1) is relatively larger in the decaying wood and humus feeding larvae of the scarabaeid subfamily Dynastinae than their root-feeding counterparts in the subfamily Melolonthinae. On leaving the fermentation tract the alimentary tract returns to the anterior–posterior direction and extends into the cuticle-lined rectum and finally the anus (Fig. 1).

Physicochemical properties of the scarab larval gut

Gut pH

The alkaline midgut of scarabaeid larva differs from most other coleopteran species. Biggs and McGregor (1996) found the pH of the larval *Costelytra zealandica* midgut and hindgut to be > 8, and that this varied depending on the location within these gut regions. In the precaecal region the pH was 8.4 ± 0.69; it rose to 10.8 ± 1.28 in the anterior part of the remaining midgut, 10.9 ± 0.95 in the middle of the midgut, and then dropped to 9.3 ± 0.68 at the posterior end of the midgut. The pH of the hindgut was 8.2 ± 0.35 (Biggs & McGregor, 1996). Studies of *Pachnoda ephippiata* larvae also revealed a gut pH > 8. The pH of the anterior midgut of *P. ephippiata* reached a maximum between 10.1 and 10.7, before declining to 8.4 ± 0.1 beyond the third row of caecae. The hindgut of first instar *P. ephippiata* larvae maintained a constant, slightly alkaline pH value, although values could drop to neutral toward the rectum in the second and third instars (Lemke *et al.*, 2003).The midgut of *Pachnoda marginata* similarly is strongly alkaline with a pH of 9.5–11.7 (Cazemier *et al.*, 1997b). For *Melolontha melolontha* larvae the midgut pH was fairly constant (pH 7.9–8.2), but increased to slightly alkaline (pH 8.6) in the hindgut paunch before falling again to neutral pH conditions in the colon and rectum (Egert *et al.*, 2005). Similar findings were recorded for *Sericesthis geminate* larvae, where the midgut pH was 9.0 and hindgut pH was 7.5 (Soo Hoo & Dudzinski, 1967).

These observations show a consistent pattern among the different scarab larvae with a high pH recorded in the midgut, falling to a lower pH in the hindgut. This suggests that the pH is maintained to assist the digestive process through the scarab larval gut in a manner that facilitates efficient absorption of nutrients (Terra & Ferreira, 1994; Elpidina *et al.*, 2001; Oppert *et al.*, 2006). The alkaline environment in the gut of scarab larvae has been proposed as a way of helping to increase the solubility of organic polymers in humus, assist with extraction of hemicelluloses from plant cell walls, and possibly aid the breaking up of stable organo-mineral complexes (Biggs & McGregor, 1996; Kappler & Brune, 1999). Zhang and Brune (2004) found that the proteolytic activities of the midgut proteinases in *P. ephippiata* were higher at pH 12 than at pH 7, and the midgut extract had higher activity in digesting the proteinaceous component of the model humic acid at pH 12 than at pH 7, indicating an adaptation of proteinases to the physiological pH of the *P. ephippiata* midgut. Thus, gut alkalinity may represent an important nutritional adaptation for the uptake of essential or limiting nutrients from plant roots and other organic matter consumed by scarab larvae (Felton & Duffey, 1991).

Gut redox potential

Microbial activity partly determines physiological gut conditions such as pH level, redox potential, and oxygen concentration (Zimmer & Topp, 1998; Kappler & Brune, 2002). The oxygen status determines whether anaerobic fermentation or aerobic oxidation of nutrients prevails (Zimmer & Brune, 2005). For example, reducing conditions are essential for the development of some strictly anaerobic methanogenic bacteria found in the proctodeum of *Oryctes* spp. (Bayon & Etiévant, 1980).

In *Oryctes nasicornis* larvae, the electric potential of the midgut contents varied between $+50$ mV and -10 mV, while the proctodeum (fermentation chamber) contents ranged between -40 mV and -100 mV. Redox potential values within these ranges are conducive to establishment of a reducing environment within the intestine (Bayon & Etiévant, 1980). In *P. ephippiata*, the midgut of first-instar larvae favored oxidizing conditions; whereas the redox potential shifted toward reducing conditions in larvae of the second and third instars, demonstrating that the redox potential can change considerably during larval midgut development. However, reducing conditions prevailed in the fermentation chamber across all larval instars ($\sim -100 \pm 11$ mV) (Lemke *et al.*, 2003). Investigation of the *P. marginata* larval digestive tract showed that redox potentials in freshly prepared midguts and hindguts were in the range of -100 to -200 mV, which is indicative of an anaerobic environment (Cazemier *et al.*, 2003). In contrast to *P. marginata*, the redox potential in the midgut of *M. melolontha* larvae ranged from $+220$ to $+340$ mV, and dropped sharply at the midgut–hindgut junction, to attain a minimum (0 mV) in the anterior hindgut, and increased again toward the rectum (Egert *et al*., 2005). Negative redox potentials have also been detected in wood-feeding cockroaches and termites, and in herbivorous lepidopteran larvae (Veivers *et al*., 1982; Appel & Martin, 1990).

The chemical and enzymatic reactions associated with digestion, absorption and detoxification of food in the

insect gut are dynamic and sensitive to changes in pH, redox potential and other physicochemical parameters (Johnson & Felton, 1996a). Redox potential and pH affected the oxidative state of tannins, phenolics and other ingested compounds in the gut, as well as the activity of digestive enzymes, by imposing physiological constraints on the effectiveness of some classes of digestive enzymes (Christeller *et al*., 1989; Johnson & Felton, 1996a, 1996b). It is also found that redox conditions can alter the effects of plant allelochemicals on insect herbivores (Appel & Martin, 1990).

The significance of redox potential in the gut can be illustrated through comparison with biodegradation processes in the soil. Paar (1969) distinguished characteristic stages of organic matter degradation in soil that are associated with redox potential values. Values of less than +300 mV promoted the reducing environment required for degradation of organic matter and accelerated the rate that soluble products could be accumulated. As negative redox values were reached, reducing conditions were strengthened and a strict anaerobic environment would prevail; at redox values below −100 mV, carbohydrates were completely reduced and methane could be generated. Thus, the redox values typically found in the hindgut of scarab larvae ($\sim -100 \pm 11$ mV) provide an environment that is compatible with the formation of methane. Similar physiochemical conditions exist in ruminants, except that the redox potential in the stomach of ruminants reaches values in the order of −300 mV and the methane yield is generally 20 times higher than for scarab insects (Bayon & Etiévant, 1980; Lemke *et al.*, 2003; Egert *et al.*, 2005).

Gut microflora

Scarab larvae have a microbe-rich alimentary tract with most organisms concentrated in the enlarged hindgut (fermentation sac) (Fig. 2). The scarab hindgut has been considered analogous to the micro-organism-rich rumen of higher mammals, which is the primary site of microbial fermentation for digestion of plant organic material.

Previous studies of microbes in the hindgut of *C. zealandica* revealed a dense population of flagellate protozoa and bacteria, ranging from $2–5 \times 10^{10}$ bacteria per g wet weight of gut, with some of these microbes able to digest pectin and xylan substrates (Bauchop & Clarke, 1975). Using plate counts of microbes and polymerase chain reaction (PCR) – denaturing gradient gel electrophoresis (DGGE) fingerprinting, Zhang and Jackson (2008) further investigated the bacterial diversity within the larval gut of *C. zealandica*. Results showed that numbers of cultured bacteria were significantly ($P < 0.05$) higher in the hindgut than the midgut. The bacterial

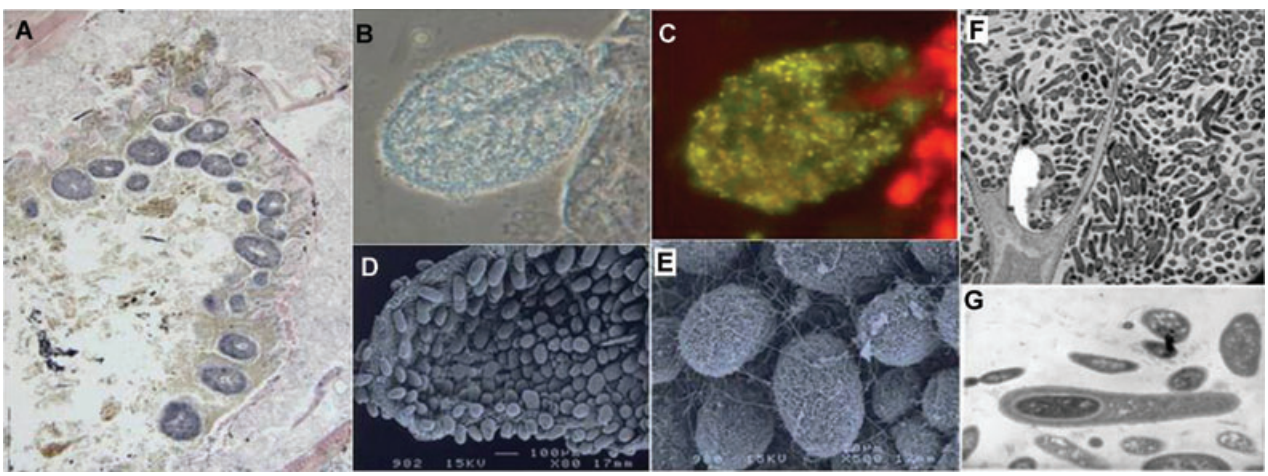

Fig. 2 Features of the scarab hindgut and associated bacteria. (A) Thin section of a *C. zealandica* hindgut (also referred to as the fermentation sac), showing the characteristic lobe-like structures. (B) Wet mount of a lobe from first instar *C. zealandica* larva 5 days post-eclosion observed under phase contrast microscopy. (C) Fluorescence image of the lobe from photo B after staining with LIVE/DEAD Baclight (molecular probes); bacteria in the lobe appear green (SYTO9) and the hindgut cells appear red (propidium iodide). (D) and (E) Scanning electron micrographs of the *C. zealandica* hindgut lobes displaying tree-like cuticular growths surrounded by a cuticular meshwork. (F) Transmission electron micrograph of a cross section through the *C. zealandica* hindgut showing a portion of the tree-like lobe structure (bottom left) and the diverse bacterial morphotypes. (G) Transmission electron micrograph showing Clostridia-form bacteria from the hindgut lobes of *C. zealandica*.

community composition within the midgut was highly variable and changed with site of collection or diet, while the hindgut bacterial species community was less affected by external factors and had more species diversity. The dominant species identified by PCR-DGGE were affiliated with the Clostridiales with the predominant bacteria affiliated to the genus *Clostridium*. The remaining bacteria were aligned to the β-proteobacteria, δ-proteobacteria, and Bacteroidetes. The research on bacterial community profiles associated with the hindgut walls of individual *Dermolepida albohirtum* third-instar larvae indicated that a number of taxa were found consistently in all *D. albohirtum* larvae. These taxa included representatives from the "Endomicrobia", Firmicutes, Proteobacteria and Actinobacteria, and closely related hindgut bacteria found in *C. zealandica* (Pittman *et al.*, 2008).

By using terminal restriction fragment length polymorphism (T-RFLP) to analyze bacterial 16S rRNA genes from gut samples, Egert *et al.* (2005) found that *M. melolontha* midgut samples from individual larvae possessed a simple but variable and probably nonspecific community structure. The hindgut samples were more diverse but highly similar, especially in the wall fraction, which is suggestive of a gut-specific community involved in digestion. Analysis of the microbiota from the hindgut of *M. melolontha* showed it was dominated by clones related to the Clostridiales. Clones related to the Actinobacteria, Bacillales, Lactobacillales and γ-proteobacteria were confined to the lumen, whereas clones related to the β- and δ-proteobacteria were found only on the hindgut wall, suggesting that specific bacterial species were restricted to different compartments. Both the midgut and hindgut had high acetate concentrations, indicating microbial fermentation was likely to occur in both compartments.

Egert *et al.* (2003) studied the digestive tract of *P. ephippiata*, and found these larvae also contain a complex gut microbiota. The species composition differed markedly between midgut and hindgut, and was clearly distinct from the microbiota identified in the surrounding soil. Four fermentative metabolism bacteria groups (Lactobacillales, Clostridiales, Bacillales and Cytophaga-Flavobacterium-Bacteroides [CFB] phylum) were the dominant phylogenetic groups identified from the gut of *P. ephippiata*. 4′,6-diamidino-2-phenylindole (DAPI) counts showed cell densities of $8.9 \pm 3.5 \times 10^9$ cells/g and $4.0 \pm 1.4 \times 10^{10}$ cells/g in the midgut and hindgut, respectively. Based on 16S rRNA gene frequencies, Actinobacteria dominated the alkaline midgut, while the hindgut was dominated by members of the CFB phylum. Investigation of the related scarab, *P. marginata*, showed that the numbers of cultivable bacteria in the midgut and hindgut were $0.95 \pm 0.3 \times 10^9$/mL gut and $2.0 \pm 0.47 \times 10^{10}$/mL gut respectively, which was similar to *P. ephippiata* (Cazemier *et al.*, 1997a).

In vertebrates with rumen or hindgut fermentation that relies almost completely on microbial activity for the digestion of food, bacterial numbers in the intestinal tract range up to 10^{11}/mL (Hungate, 1966). Comparable numbers of bacteria are also present in the hindgut of scarab larvae (Cazemier *et al.*, 1997a; Egert *et al*., 2003), suggesting that these bacteria are important for food digestion in scarabs (Cazemier *et al.*, 1997a). In the gut of *Pachnoda* spp. larvae, the apparent dominance of fermenting bacteria (i.e., Lactobacillales, Clostridiales, members of the CFB phylum, and clones related to *Turicibacter sanguinis*) correlates with the high lactate and acetate concentrations in hindgut (Lemke *et al*., 2003). Likewise, the high acetate concentration in *M. melolontha* larval hindguts indicates the presence of microbial fermentation in this compartment, and methanogenesis was found to be confined to the hindgut (Egert *et al*., 2005).

In *C. zealandica*, the most common bacterial species were affiliated with the order Clostridiales (Zhang & Jackson, 2008). Egert *et al*. (2005) found that the hindgut of *M. melolontha* was also dominated by specific groupings of the Clostridiales. Even though the hindgut of *P. ephippiata* was dominated by members of the CFB phylum, a high proportion of Clostridiales was also identified in the hindgut by 16S rRNA gene sequence and T-RFLP profile analysis (Egert *et al*., 2003). These observations seem to imply that members of the Clostridiales have developed a symbiotic relationship with scarab larvae (Zhang & Jackson, 2008).

(Hemi)cellulose digestion in the scarab gut

Lignocellulose is the predominant component of woody plant material, as well as being the most abundant form of biomass in terrestrial ecosystems. Fungi, bacteria and soil invertebrates are the major lignocellulose decomposers (Ohkuma, 2003). Earlier studies have demonstrated fibre digestion in some scarab beetle larvae. Bayon and Mathelin (1980) injected ^{14}C-labelled cellulose into isolated midgut and proctodeal segments of *O. nasicornis* for *in vitro* incubation, and estimated that 25%–39% of the ingested pure cellulose was degraded. By comparing the fibre content in the diet and the feces, Cazemier *et al*. (1997b) demonstrated that *P. marginata* digested 65% of neutral detergent fibres present in their diets. Digestion of (hemi)cellulose is thought to occur in the enlarged hindgut paunch with the assistance of micro-organisms (Bayon, 1980;

Martin, 1983; Cazemier *et al.,* 2003; Zhang & Jackson, 2008; Douglas, 2009).

Because bacteria with (hemi)cellulolytic activity appear to be absent in the midgut, it seems unlikely that microbial degradation of cellulose and hemicellulose occurs in this part of the intestinal tract (Cazemier *et al*., 2003). While carboxymethyl cellulase (CMC-ase), β-glucosidase and xylanase activities have been detected in the midgut of *O. nasicornis* larvae and *P. marginata*, their activities were low. In *P. marginata*, activity of CMC-ase in midgut extracts was 0.69 $\pm$ 0.23 U/mL gut, while β-glucosidase activity was 0.28 $\pm$ 0.19 U/mL gut, and xylanase activity was 0.18 $\pm$ 0.11 U/mL gut. All three activities were one to two orders of magnitude lower than the other wood-feeding arthropods examined (*Pycnoscelus surinamensis*, *Mastotermes darwiniensis*, *Hylotrupes bajules*, etc.) (Cazemier *et al*., 1997b). Furthermore, it is not clear whether these enzymes are functional under the alkaline conditions of the midgut (Bayon & Mathelin, 1980; Cazemier *et al*., 1997b, 2003). Therefore, it has been proposed that the midgut likely serves a predigestive function for lignocellulose digestion, with the anaerobic conditions of the hindgut facilitating processes required for further biodegradation of the lignocellulosic material (Cazemier *et al*., 2003; Zverlov *et al*., 2003).

Based on the high number of fermentative bacteria present in the hindgut of scarab larvae, the CMC-ase activity 0.29 $\pm$ 0.35 U/mL gut, β-glucosidase activity 0.13 $\pm$ 0.09 U/mL gut and xylanase activity 0.6 $\pm$ 0.4 U/mL gut in hindgut extracts of *P. marginata* (Cazemier *et al*., 1997b), and the existence of cellulolysis in intestinal contents of proctodeal segments of *O. nasicornis* larvae (demonstrated by injection of ^{14}C-cellulose into the gut; Bayon & Mathelin, 1980), it has been suggested that these intestinal bacteria were responsible for cellulose degradation (Bayon & Mathelin, 1980; Martin *et al*., 1991; Cazemier *et al*., 1997b; Zhang & Jackson, 2008). As noted above, Clostridiales is the dominant bacteria within the hindgut of *C. zealandica* and *M. melolontha* larvae (Egert *et al*., 2003, 2005; Zhang & Jackson, 2008). Since the genus Clostridium contains a wide range of species able to ferment sugars and more complex molecules including cellulose, hemicellulose, pectin and polysaccharides (Hippe *et al*., 1992; Varel *et al*., 1995; Weber *et al*., 2001; Zhang & Jackson, 2008), it seems likely that the *Clostridium* spp. identified from scarab hindguts have an important role in the degradation of the roots and organic matter consumed by scarab larvae (Egert *et al*., 2005; Zhang & Jackson, 2008).

The anaerobic conditions and production of methane in the fermentation chamber of scarab larvae parallels digestion in ruminants. The breakdown of cellulose by extracellular symbiotic organisms leads to the formation of volatile fatty acids (VFA), such as acetic acid (Bayon & Mathelin, 1980) and by-products that can act as substrates for methanogenic bacteria (Bayon & Etiévant, 1980). The diffusion of small molecules, such as acetic acid, is uninhibited in structures involved in absorption and the cuticlar membrane. The production of methane took place exclusively in the hindgut of *O. nasicornis* larvae (Bayon, 1980). Methanogens were closely associated with the chitinous lobe-like structures formed within the host's fermentation sac in the hindgut of *P. ephippiata* (Egert *et al.*, 2003). In addition, accumulation of microbial fermentation products had been detected in the hindgut of *P. ephippiata* larvae (Lemke *et al*., 2003). These experiments demonstrated that the high level of cellulolytic activity was associated with bacteria in the hindgut of scarab larvae.

In fact, as early as 1926, a cellulolytic bacterial species, *Bacillus cellulosam fermentans*, was isolated from enrichment cultures inoculated with hindgut contents of a rose chafer, *Potosia cuprea*; unfortunately specific experiments demonstrating *in vivo* cellulolytic activity were not conducted for this bacterium (Werner, 1926). However, a number of facultative anaerobic and strictly anaerobic bacteria with (hemi)cellulolytic activity have been isolated from the hindgut of *P. marginata* (Cazemier *et al*., 2003). The dominant (hemi)cellulolytic species was *Promicromonospora pachnodae*, which can produce xylanase and endoglucanase activities against several plant-derived polymers under both aerobic and anaerobic conditions. Moreover, the (hemi)cellulolytic bacterium, *Cellulomonas pachnodae*, was isolated from the hindgut of *P. marginata* larvae, and two new xylanase-encoding genes, named *xyn11A* and *xyn10B*, were isolated from a genomic library and expressed in *Escherichia coli* (Cazemier *et al*., 1997b, 1999, 2003). Geissinger *et al.* (2009) isolated *Elusimicrobium minutum*, the first cultivated representative of the termite group 1 phylum from a humivorous scarab beetle larva. It can grow heterotrophically on sugars, and is able to ferment D-galactose, D-glucose, D-fructose, D-glucosamine, and N-acetyl-D-glucosamine to acetate, ethanol, hydrogen, and alanine as major products (Geissinger *et al.*, 2009).

The successful isolation of bacteria with (hemi)cellulolytic activity from scarab hindguts shows that micro-organisms participate in the digestion of cellulose. The absence of cellulases from hindgut fluid suggests that cellulose digestion is due to the activity of cell-bound enzymes produced by the bacteria, rather than from secreted extracellular enzymes (Martin, 1983). Indeed, bacteria possessing (hemi)cellulolytic activity have been found attached to the fragments of plant tissue

present within scarab hindguts (Martin, 1983). The final product of cellulose degradation is mainly acetic acid, which appears to pass through the hindgut wall for subsequent utilization by the insect (Bayon & Mathelin, 1980).

With the identification and isolation of bacteria possessing cellulolytic and hemicellulolytic activities from scarab digestive tracts, a new source of (hemi)cellulolytic enzymes has been established. Future work will no doubt characterize specific cellulase and hemicellulase genes and enzymes, such as xylanases and endoglucanases, from bacteria identified from scarab hindguts (Cazemier *et al*., 1999), and this research will help develop the potential of scarab-associated (hemi)cellulolytic activities for use within the bio-fuel industry.

Conclusion

Larvae from the Scarabaeidae family consume soil, wood and organic matter, the nutrients of which are extracted with the assistance of digestive enzymes present within the mid- and hindgut. The hindgut is the main region for digestion of (hemi)celluloses in scarab larvae as evidenced by high concentrations of volatile fatty acids, the presence of fermenting bacteria and the occurrence of typical anaerobic activity, such as methanogenesis (Bayon, 1980; Brune, 1998; Egert *et al.*, 2003). (Hemi)cellulose digestion is probably mediated by hindgut bacteria (Werner, 1926; Cazemier *et al*., 1997b), and the expanded hindgut structure with its modified lobes appears to have evolved to function as a refuge for these symbiotic microbes. Novel (hemi)cellulolytic bacteria, such as *P. pachnodae* and *E. minutum* have now been isolated from the larvae hindgut of scarab larvae (Cazemier *et al*., 2003; Geissinger *et al.*, 2009). These findings indicate that the larval scarab gut is not only a fruitful source of microbial biodiversity and functional novelty, but also a new source of micro-organisms and enzyme activities that will aid the function and design of future bioreactors for the bio-fuel industry.

Acknowledgments

This research was supported by National Natural Science Foundation of China (30671404), the earmarked fund for Modern Agro-industry Technology Research System of China, the Research Fund for the Doctoral Program of Higher Education of China (200805040010) and the New Zealand Foundation for Research Science and Technology. The authors would also like to thank Sue Zydenbos for editorial assistance.

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Accepted December 11, 2009