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Diversity and Function of Insect Gut Microbiome

昆虫肠道微生物的多样性与功能

The role of gut symbionts from termites: a unique hidden player from yeasts

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Abstract: The capability to degrade lignocellulose in a variety of termite species is important for global carbon recycling on lignocellulosic biomass. This unique capability from termites relies on their special "gut digestome" such as bacterial, archaeal, yeast, or other eukaryotic symbionts. However, to date, their highly specialized gut systems of such insects are not yet fully understood regarding their unique symbiont functions and their potential values applied in biotechnology or other relevant fields, particularly for the group of various yeast symbionts. In recent years, there is a growing evidence to demonstrate the symbiotic yeast importance and their widespread distributions in various termite digestive systems, as well as their unique functions played in termite-symbiont interactions. Along with more yeast species to be identified from insect gut systems in the past two decades, their potential functions and relevant mechanisms interacted with host insects and other gut symbionts have been reported, which may be related to host nutrition, essential enzymes for biomass processing, intermediate chemical compounds, suppressing external pathogens, even colony outbreeding, etc. This review paper would mainly focus on the recent research work on yeast symbionts identified from termite gut and their potential values and functions applied in different fields.

Keywords: termites, fungi, yeasts, symbiosis, lignocellulose, biotechnology

Recently, there has been an increase in the number of studies on the gut symbionts of xylophagous insects like termites, with the objective of determining their role in the metabolic and digestive processes of these insects. Termites thrive in great abundance in the terrestrial ecosystems. Their ability to degrade lignocellulose gives termites an important role in global carbon recycling^[1]; and thus, lignocellulose-degrading enzymes from the "termite digestome", both termites and their gut symbionts have many potential bioenergy applications that warrant careful consideration^[2]. With the removal of cellulose (74%-99%), hemicellulose (65%-87%) as well as lignin (5%-83%), the digestion of lignocellulose by termites is far more efficient which typically achieving over 95% bioconversion within a day^[3]. Termites have developed a unique microflora of

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archaea, archaezoa, bacteria, yeasts and probably fungi^[4]. This specialized microflora enables the termites to efficiently digest lignocellulose and led to a tremendous evolutionary success (Figure 1). Of these symbionts, the anaerobic flagellates produce acetate, CO₂ and H₂ from cellulose. Acetate is also formed from CO₂ and H₂ by acetogenic bacteria^[5]. Acetate is absorbed by the gut epithelium and functions as a major carbon source of termites. The heterotrophic bacteria fulfill several functions in the hindgut. They fix dinitrogen and recycle the nitrogen of uric acid, maintain a low redox potential, synthesize amino acids, produce acetate and other lower fatty acids, and protect the hindgut from foreign bacteria^[6]. Yeasts are single-celled fungi and currently they are becoming the organisms of choice in studies aimed at their potential applications^[7]. They are frequent in guts of insects that feed on detritus or wood, and they likely play a part in digestion^[8].

In contrast to previous studies on lignin breakdown, which gave no convincing evidence of microbial degradation of lignin in the termite intestinal tract^[9], the recent investigations have shown that the paunch of termites harbors a significant number of yeasts and bacteria with hemicellulose-degrading activities^[10–11]. Clearly, between 10^7 and 5×10^8 yeast cells were found per mL gut content in Zootermopsis angusticollis and Neotermes castaneus^[10]. These yeasts were related the genera Candida, Pichia, Sporothrix, and Debaromyces. Even though none of these yeasts produced all the required hydrolytic enzymes to completely digest hemicelluloses, they play a significant role in the breakdown of hemicellulose in the termite gut, probably through synergistic activities of their glycolytic enzymes with other gut symbionts (Figure 1). In another study^[12], Debaryomyces hansenii and Sporothrix albicans as well as species of Trichosporon and Rhodosporidium could also be found in the hindgut of the termites from families: Mastotermitidae, Hodotermitidae, Kalotermitidae and Rhinotermitidae. Therefore, one may regard the termite gut as a complex ecosystem

with free-living and attached microorganisms.

Cellulose and hemicellulose are digested to a large extent during passage of food through the digestive tract by both lower and higher termites, but with different digestive strategies. In the phylogenetically "lower" termites, the gut symbionts include bacteria and protozoa. Many of protozoa are capable of ingesting wood particles and are cellulolytic^[13]. However, in the phylogenetically "higher" termites which constitute about 75% of all termite species, the gut microbiota essentially consist of bacteria alone. The confirmed role of bacteria in cellulose digestion is still unclear in lower as well as in higher termites, because animals themselves also produce cellulases^[14], and the interesting question: what role do symbionts play? On the other hand, fungi are found in the gut content of termites but do not appear to be involved in digestion^[14] cellulose SO another interesting questions are: (i) does the termites produce a cellulase themselves? (ii) does it ingest fungal enzymes? and (iii) what role do yeasts play?

Termite host tissues, via recent transcriptomic efforts, have been shown to produce a litany of cellulases and hemicellulases, as well as lignases, and contribute to lignocellulose degradation^[3]. The association with the lignin-degrading fungi enables fungus-cultivating termites to degrade the lignocellulose nearly completely, as reflected in the small volume of their final feces^[10]. The key activities attributed to the fungal partner in the mutualistic symbiosis of fungus-cultivating termites are extensive delignification of the substrate and the conversion of plant fiber to fungal biomass^[15]. The "acquired enzyme hypothesis" of Martin and Martin^[16] was confirmed by evidence for an activity within the gut of fungal cellulases ingested by the termites together with the fungus comb material^[17–18]. However, claims that the fungal cellulases are essential for cellulose digestion in the termite guts remain controversial, especially in view of the recently discovered ability of termites to produce their own cellulases^[3].

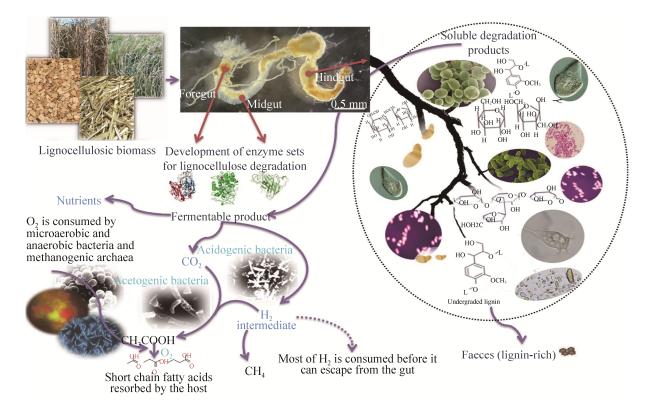


Figure 1. Consequences of lignocellulose breakdown by synergistic activities of yeasts and other termites' hindgut symbionts.

1 Yeast biodiversity

The "yeast" word is broadly used to represent a fungal growth form described by single-cell microorganisms^[19]. Still despite their widespread presence, it is thought that only 1% of the diversity of yeast species has been described so far, demonstrating that there is a huge number of undescribed species^[20]. At least 1500 yeast species, belonging to the Ascomycota and Basidiomycota phyla, are known^[21]. Of Ascomycota, almost 700 yeast species described in 93 genera are called the "true veasts" belong the and to class Saccharomycetes^[19]. These genera include Candida, Metschnikowia, Saccharomyces, Pichia. and *Kluveromyces*^[22]. *Cystofilobasidium*, Tremella, Cryptococcus, Fellomyces, and Ustilago are yeast genera belonging to Basidiomycota^[21,23].

Most of the known yeast symbionts harbored in

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the gut of insects belong to Candida, Pichia, *Metschnikowia*, *Pseudozyma*, and *Cryptococcus*^[21]. In most cases, these symbionts are grown intercellularly in their host guts^[19]. Clearly, true yeasts represent most of the gut symbionts although there are a group of fungal endosymbionts, "yeast-like symbionts" (YLS), that differs from true yeasts. YLS are characterized by evolutionary reduced yeast forms from the Pezizomycotina sub-phylum^[19]. However, the diversity and the association between YLS and insects seem to be more limited comparing with true yeasts and insects. To date, the association between YLS and insects have been observed mainly in some species of planthopper, aphid, and anobiid beetles^[24]. Overall, it appears mutualistic association between true yeast, YLS and insects which can vary with different classification^[25]. Generally, taxonomic the specificity in this association could be correlated to

the services provided by the insect to the yeast or vice versa^[24].

2 Evolutionary considerations of the intestinal yeasts

Termites are well known for their specific prokaryotic and eukaryotic gut symbionts^[26]. Despite their wide distribution of the investigated termites in different continents, the majority of yeasts isolated from lower termites comprise a homogeneous assemblage of representatives from the Endomycetales^[27]. Based on a genotypic characterization, Trichosporon mycotoxinivorans sp. nov., was the only Basidiomycetous yeast isolated from lower termites. This yeast species is useful in biological detoxification of various mycotoxins. Additionally, a highly symbiotic yeast consortium in the hindgut of the *Pachyiulus flavipes* was detected^[28]. The yeast consortia consist almost exclusively of DTZ-group of Ascomycetes identified to be Debaryomyces hansenii, Torulaspora delbrueckii, and Zvgowilliopsis californica^[29]. Beside the DTZ-group, other yeast species were minor components in the excrement, and include Pichia membranaefaciens, Trichosporon cutaneum, and Geotrichum candidum. The cell walls of such yeasts probably resist the enzymatic attack in the digestive tract of the insects^[30]. In contrast to lower termites, the symbiotic Basidiomycetous yeast species were commonly detected in the hindgut of the higher termite Odontodermes obesus. These species contained xylose in their cell walls and identified to the genera *Tremella* and *Filobasidium*^[27].

Based on the 18S rDNA sequences and fossil record, Berbee and Taylor^[32] reported that the Endomycetales are a sister group from the filamentous Ascomycetes. Considering a new concept of the carbohydrate composition of yeast cells, sexuality and mating type evolution, Prillinger et al.^[31] interpreted the Endomycetales as primitive fungi which have lost their filamentous stage based

on the extinction of the hosts^[33]. However, arguments to the later interpretation were added by Kurtzman^[34]. The presence of Endomycetes yeast symbionts representing the mannose-glucose carbohydrate pattern in lower termite documents that the "*Saccharomyces* type" is most probably ancestral to the *Ustilago*, *Microbotryum*, *Protomyces*, and *Tremella* type^[31].

Although intimate associations are known between filamentous Ascomycetes or Agaricales and higher termites, there is no information with respect to the yeast mycoflora in termites and its usefulness to trace the phylogeny of Ascomycetous and Basidiomycetous yeasts. If the speculation of Martynov^[35] about the evolution of termites is correct, coevolution between termites and yeasts may be extremely useful to trace the phylogeny of filamentous Ascomycetous and Basidiomycetous yeasts^[30]. Based on the study of Prillinger et al.^[27] about yeast symbionts in the hindgut of termites, it may be concluded that an amalgamation of information obtained from symbiotic associations, ribosomal DNA sequencing, yeast cell wall sugars, fermentation and the fossil record might be a more reliable indicator for fungal phylogeny than evolutionary trees based on ribosomal DNA sequencing alone to trace fungal phylogeny back to early Paleozoic periods^[30].

3 The possible benefits from the yeast-termite association

3.1 Benefits for the yeasts

Termites can provide yeasts with many benefits (Figure 2). The survival of yeast symbionts is widely demonstrated and is expected to be similar for bacterial symbionts^[8]. Generally, it is considered that symbionts are, first, acquired by ingestion. Then, they colonized the insect guts, and finally being released from feces for dispersion of microorganisms. Depending on the insect behavior,

yeast cells can be inoculated in eggs by females or they can be re-ingested by colonization of food sources or through trophallaxis and coprophagy^[8].

Contrary to filamentous fungi, yeasts elaborate spore cell types are able to survive numerous extreme environmental conditions in a laboratory experiment, such as high and low extremes of pH, high salt concentrations, heat shocks, growth at 42 °C, and ether vapor^[36]. These characters demonstrated that spores are more adapted than vegetative cells to endure environmental harsh conditions^[37]. The cell wall structure of the spore confers their resistance to stresses associated with digestion and allowing the yeasts to be dispersed by oviposition or insect feeding^[38]. Another benefit to yeasts after their consumption by termites is the putative role of digestion in the promotion of outbreeding^[37]. This role is fundamental to increase the probability of adaptation and to maintain genetic variation among the microbial progeny. Frequently, inbreeding between the four ascospores housed in the same ascus is observed during sexual reproduction of yeasts. To release these ascospores and avoid inbreeding, enzymes are required to break up the ascus. It has been proposed that the host enzymes of termite guts accomplish this function by breaking down the haploid ascospore tetrads and promoting outbreeding^[39].

3.2 Benefits for the termites

Yeast symbionts can also provide insects with many benefits (Figure 2). Vage et al.^[19] reported that the role of yeasts in insect nutrition is very important, where insect performance would decrease due to the absence of yeast symbionts. Yeasts are sources of B3 and B5 vitamins, amino acids, proteins, and trace metals that could be easily assimilated through simple digestion. In addition, yeast cells contain about 8.5% nitrogen by dry weight, thus insect feeding on yeasts represents a better nitrogen source than the plant tissue itself^[19,40]. Becher et al.^[41] reported the nutritional importance of yeasts for

Drosophila flies during egg maturation and larval development due to sufficient amounts of nitrogen, vitamins, lipids that yeasts provide. Additionally, previous studies reported the nutritional role of nitrogen, vitamins, essential amino acids, and sterols provided by the YLS on Symbiotaphrina and anobiid beetles^[42-43]. Furthermore, the role of yeast symbionts as facilitators of insect host nutrition has been demonstrated^[19,40]. These yeasts play a major role in the production of digestive enzymes that degrade plant lignocellulose into molecules with nutritional value for herbivores. Different types of enzymes are produced by termite-associated yeasts including peptidases, lipases and hydrolases. On the other hand, yeast symbionts in the Lasioderma serricorne. Ernobius mollis and Xestobium piumbeum beetles produce glucosidases lipases, phosphatases and trypsin in order to degrade cellobiose^[44]. Recently, research on the role of yeast enzymes has focused on the degradation of complex polymers into simple sugars that the termite can absorb directly. True yeast species of the genus Candida have the ability to degrade wood components such as cellulose, glucosides, and pectin. Therefore, wood-feeding termites might rely on their associated yeast species to degrade cellulose, and symbionts their produce glucosidases and pectinases^[45].

3.3 Some roles of the symbiotic yeasts isolated from the termite guts and their potential applications

Termite gut symbionts might be interesting due to their pronounced solvent tolerance or may be a future source of industrially useful solvent-tolerant enzymes. In addition these microbiota may detoxify allelochemicals or may be responsible for the biosynthesis of essential compounds such as vitamins, sterols, or nitrogen-containing constituents. Generally these microorganisms may be the source of novel metabolic capabilities such as defense substances and antibiotics or may fixate atmospheric

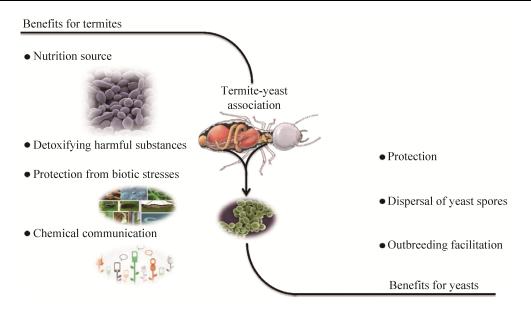


Figure 2. Symbiotic associations between termites and their yeast symbionts.

nitrogen. It has been known for many years that enzymes in the gut which originate from ingested fungal tissue or gut microbes may mediate cellulolysis. The data on yeasts in the termite guts are scarce. Nevertheless, the first investigations showed that the yeast isolates occur at high numbers in the guts of lower and higher termites of different systematic positions. Since they have been constantly isolated, they belong most probably to the autochthonous symbiotic microbiota of the paunch. They produce cellulolytic and hemicellulolytic enzymes and may play a significant role in the degradation of lignocellulose besides the protists, which thrive only in lower termites and bacteria^[46].

Termites play a major role in the recycling of photosynthetically fixed carbon. With the aid of their gut symbionts they degrade to a high extent the wood constituents cellulose and hemicellulose. Nevertheless, the microbial species involved in the degradation of cellulose and hemicelluloses have been yet poorly defined before the investigations of Schäfer et al.^[10] and Wenzel et al.^[47]. They identified different yeast strains with cellulose- or xylan-degrading capability. A series of different hemicellulose-degrading yeasts were isolated for the first time from the termite gut^[10]. Some of yeast species; namely Candida blankii, Candida edax, Pichia pini, Sporothrix schenkii and Debaryomyces hansenii var fabryii isolated from the lower termites Mastotermis darwiniensis. Neotermes jouteli, Reticulitermes santonensis, **Zootermopsis** angusticollis and Zootermopsis nevadensis produced clear zones on agar plates containing insoluble xylan (oat spelt) or dye-linked xylan or solubilized xylan (oat spelt) in liquid media^[27]. Reports on cellulase production by yeast wild-type strains are rare^[48]. Some of the yeast isolates from termites produced hemicellulases^[10] and some were also able to degrade cellulose^[47]. Some cellulolytic yeast strains, Sporothrix schenkii and Trichosporon sp. were isolated from Zootermopsis nevadensis and Mastotermes darwiniensis, respectively^[27]. From a biotechnological perspective, termite gut yeasts constitute promising and mostly untapped sources for potential applications in industrial processes, agriculture, medicine, and bioremediation (Figure 3). A number of approaches based on yeasts from termite guts have been successfully implemented (Table 1).

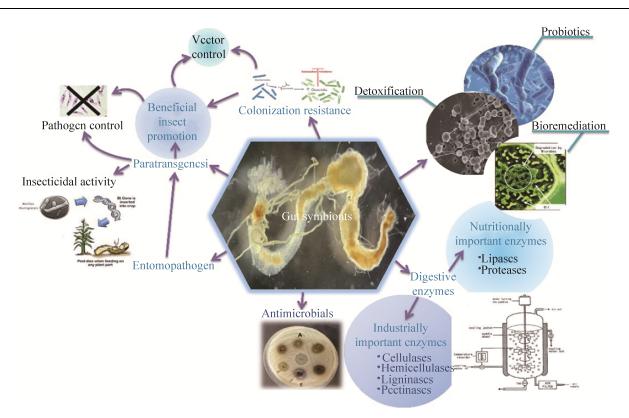


Figure 3. A schematic biotechnological applications potentially for yeasts-associated termites.

Table 1.	Some roles of the symbiol	ic yeasts isolated from termite gut syste	ms and their potential applications.
Amplication	Immortance	Example	Pafaranaa

Application	Importance	Example	Reference
Digestive enzymes	Biotechnologically relevant digestive enzymes produced by symbiotic yeasts in termites to overcome the host's nutritional limitations.	*Novel yeasts with potential xylanase activity have been isolated from termites, including <i>Candida pseudorhagii</i> sp. nov. strain SSA-1542 ^T , <i>Hamamotoa lignophila</i> sp. nov. strain SSA-1576 ^T , <i>Meyerozyma guilliermondii</i> sp. nov. strain SSA-1522 ^T , and <i>Sugiyamaella</i> sp.1 nov. strain SSA-1592 ^T from <i>Reticulitermes chinenesis</i> .	[11]
		*Termite guts of <i>Mastotermes darwiniensis</i> . *Termite guts of <i>Mastotermes darwiniensis</i> and <i>Odontotermes obesus</i> revealed novel yeast species such as <i>Sugiyamaella mastotermitis</i> sp. nov. strain MD39V ^T and <i>Papiliotrema odontotermitis</i> f.a. sp. nov. strain OO5 ^T for xylanase production, hydrolysis of methylumbelliferyl β-xylobiose, hydrolysis of methylumbelliferyl β-xylotriose, and production of vitamins. *Cellulases and ligninases production by novel yeast species; namely <i>Meyerozyma guilliermondii</i> sp. nov. strain SSA-1543 ^T and <i>Starmera dryadoides</i> Sp. nov. strain SSA-1549 ^T were detected in <i>Coptotermes</i> <i>formosanus</i> .	[49]
		*Several wood-feeding termites host symbiotic soft-rot fungi in their guts, either to degrade lignin itself or to enable efficient utilization of cellulose as a source of carbon and energy.	[50]

		Table 1.	Continued
Paratransgenesis	Using genetically engineered microorganisms to express and deliver gene products in a host organism.	*Genetically engineered yeast that expresses a synthetic protozoacidal lytic peptide (Hecate) coupled to a ligand that specifically binds the lytic peptide to protozoa. When lytic peptide expressing yeast was fed to <i>Formosan</i> <i>subterranean</i> termites in a bait, workers lost all their gut protozoa within three weeks and the termite lab colonies died within two weeks thereafter.	[51]
Bioremediation	Transformation, elimination, or attenuation of polluting substances by the use of biological processes.	*Melittin, a natural lytic peptide, expressing yeast did kill protozoa in the termite gut within 56 days of exposure. *The two ligninolytic yeast strains, <i>Debaryomyces hansenii</i> and <i>Sporothrix albicans</i> , were found in the hindgut of the lower termites <i>Mastotermes darwiniensis</i> , <i>Zootermopsis</i> <i>angusticollis</i> , <i>Zootermopsis nevadensis</i> , <i>Neotermes jouteli</i> , <i>Reticulitermes santonensis</i> , and <i>Heterotermes indicola</i> had a high ability for phenol removal.	[52] [53]
		*Lignin-modifying enzymes have long been used in palm oil mill effluent (POME) treatment to remove the dark brown color resulting from phenolic contamination. This study investigated a cost-effective industrial application method for optimizing phenol removal from POME using the termite-associated yeast <i>Galactomyces reessii</i> obtained from the subterranean termite under laboratory conditions.	[54]
Antimicrobial activity	Termite-associated yeasts present promising sources of novel antimicrobial secondary metabolites that find important applications in agriculture and human medicines.	<i>Candida lusitaniae</i> strain TERM73 obtained from the termite gut, <i>Cornitermes cumulans</i> was capable of efficient silver/silver chloride nanoparticles (Ag/AgCl-NPs) production. Exposure of Gram-positive <i>Staphylococcus aureus</i> and Gram-negative <i>Klebsiella pneumoniae</i> cultures to Ag/AgCl-NPs led to a strong growth inhibition with a strong potential for use in the biotechnology industry, particularly for biomedical applications.	[49]
Detoxification	Detoxifying enzymes providing resistance against toxic natural products as well as noxious compounds from human activities.	The newly yeast strain, <i>Trichosporon mycotoxinivorans</i> sp. nov., isolated from the hindgut of the lower termite <i>Mastotermes darwiniensis</i> had potential application in biological detoxification of various mycotoxins such as ochratoxin A and zearalenone. Therefore this strain can be used for the deactivation of the respective mycotoxins in animal feeds.	[28]
Beneficial insect promotion	Development of new biological control strategies to manage pests.	A survey of the mycoflora associated with <i>Reticulitermes flavipes</i> subterranean termite suggests that interactions among fungi may suppress pathogenic effects and promote termite survival.	[55]

4 Conclusion

Termite gut symbionts, including other insect gut symbionts, constitute a rich and mostly untapped source of bioactive small molecules as well as a of digestive variety enzymes for potential biotechnological values. In addition to exploiting their metabolic capabilities, it has been suggested that the main roles offered by insect gut symbionts are recognized for a nutritional contribution to their diets, promote insect health as well as to suppress pathogens that might hamper the insect development. Consequently, the study of how insects detect microbes has been focused on insects that depend on microorganisms for their dietary requirements. For this reason in the case of yeast-insect symbiosis, most of the available knowledge was derived from the beetles and Drosophilids, while other insects, such as termites, have been greatly ignored. Hence, the study of termites and the association with their yeast symbionts not only promise to uncover interesting new yeast symbionts but may also prove valuable in the continued efforts to find new sources of biotechnologically important molecules and enzymes. Specifically, targeted searches for compounds with particular application values, such as antimicrobial agents, detoxifying enzymes, industrial enzymes (e.g., xylanases, ligninases, cellulases, etc.) may benefit from being guided by the knowledge on the ecology of insect-symbiont interactions, which would have a potential to predict a promising system for future exploration.

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白蚁肠道共生微生物的作用:酵母,一个"隐身"的特别菌群

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摘要:长期以来,白蚁对木质纤维素的降解能力令人惊叹,毫无疑问,其在全球碳循环中扮演着一个十 分重要的角色。这一强大功能的实现极大地依赖于一种特别的肠道"消化液(digestome)",它的构成不仅 包括了来自白蚁自身产生的木质纤维素降解酶系统,还来源于独特与多样的肠道共生微生物的贡献(包 括了古细菌、细菌、酵母以及其他真核生物),它们的协同作用能有效地将木质纤维素生物质高效转化 为乙酸、甲烷、二氧化碳、氢气等物质。然而,到目前为止,我们对这类昆虫的独特肠道生物转化系统 的认识还很不深入,特别是针对肠道内的那些各类共生微生物菌群的功能、白蚁与共生微生物间的相互 关系、以及潜在的科学与应用价值还无法给予明确的科学解释,更不用说针对其肠道中的共生酵母菌群, 一类通常被忽略的独特微生物。近 20 多年来,越来越多的研究证据表明,白蚁肠道共生酵母在与寄主的 关系中表现了不可或缺的重要性与独特功能,已被证明广泛分布于不同白蚁及许多其他昆虫的肠道中。 随着近 20 年来越来越多昆虫肠道共生微生物酵母群被发现和鉴定,他们潜在的功能以及与寄主的共生机 制被逐步解析,这些研究结果进一步揭示了"隐身"的昆虫肠道酵母类微生物菌群与寄主的营养、关键生 物质转化过程中的重要酶系统、转化过程中的关键中间产物的转化与利用、抵御外源性的重要病原物, 甚至对白蚁种群繁衍的远缘交配等方面均可能发挥了重要和不可缺少的作用。本文将试图归纳相关研究 的最新进展,系统总结与解析白蚁肠道来源共生酵母的重要科学价值及其在不同领域的潜在应用前景。

关键词: 白蚁, 真菌, 酵母, 共生, 木质纤维素, 生物技术

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孙建中,美国路易斯安那州立大学昆虫学博士,曾在美国学习工作 15 年,其中,2004 - 2009 年任美国密西西比州立大学昆虫系/海岸研究与推广中心助理教授和博士生导师; 美国华盛顿州立大学系统生物工程系兼职教授;2009 年 7 月任江苏大学特聘教授、博导、 江苏大学生物质能源研究所所长。目前主要从事消化木质纤维素类自然生物系统(如白蚁) 的生物过程仿生、白蚁肠道共生微生物资源利用等方面的研究。现已发表各类学术论文 近 100 多篇,主编或参与 9 部著作的撰写,作为特邀主编,2010 年出版了"Insects and Biofuels"的研究特别专刊(Insects Science, 2010,17:163-333); 受英国皇家化学协会(RSC) 的邀请担任主编,2013 年出版了白蚁生物过程仿生方面的学术专著"Biological conversion of biomass for fuels and chemicals—Explorations from natural utilization systems"。



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