2017-08-25 DOI: 10.3724/SP.J.1145.2016.09016 http://www.cibj.com/
应用与环境生物学报 Chin J Appl Environ Biol 2017, 23 (4): 0764-0770

白蚁肠道微生物多样性和作用研究进展*

孙新新 宁 娜 谭慧军 倪金凤**
山东大学微生物技术国家重点实验室 济南 250000

摘 要 白蚁在热带森林系统碳循环和氮循环中起重要作用. 白蚁肠道中的微生物包括原生生物、细菌、古菌和真菌, 帮助白蚁消化食物. 本文主要概括近年来白蚁肠道微生物多样性研究方面的新进展, 介绍白蚁肠道微生物的组成和作用. 白蚁种类繁多, 根据其后肠内是否含有原生生物, 分为低等白蚁和高等白蚁两大类群. 不同白蚁食性不同, 其肠道微生物的组成也不同. 白蚁肠道微生物对于宿主具有重要的作用, 帮助宿主分解木质纤维素, 发酵产生乙酸、甲烷和氢气(中间产物), 除此之外, 在热带以土壤为食白蚁的腐殖质矿化作用有助于氮循环. 白蚁与微生物的共生关系是目前较受关注的研究热点, 不仅有助于人们了解白蚁共生菌群落间的互作及其与宿主间的关系, 而且可能为工业生产纤维素乙醇提供潜在的方法. 分离鉴定方法和高通量测序技术的发展将有助于进一步深入了解白蚁肠道微生物的结构组成和功能. (表2 参81)

关键词 白蚁; 肠道微生物; 多样性; 食性; 碳氮循环
CLC Q938 : Q958.122.3

Research progress of diversity and function of symbiotic microbes in the gut of termites*

SUN Xinxin, NING Na, TAN Huijun & NI Jinfeng**
State Key Laboratory of Microbial Technology, Shandong University, Jinan 250000, China

Abstract Termites are important contributors to carbon and nitrogen cycling in tropical ecosystems. Termites depend on gut microbes for digesting food, and these gut microbes include protists, bacteria, archaea and fungi. Here, the recent advances in microbial diversity of termite gut is summarized and the roles of gut microorganisms are described. There are many different kinds of termites. According to the presence or absence of protists in the hindgut, termites are conventionally classified into two groups: lower termites and higher termites. Different termites have different feeding diets and the composition of the gut microbes also varies. Gut microbes play important roles in nutrition and energy metabolism of termites. They help the host in decomposing lignocellulose, producing acetic acid, methane and hydrogen (intermediate). In addition, humus mineralization of the soil-feeding termites in the tropics is also helpful to the nitrogen cycle. Currently, the relationship between termites and symbiotic microorganisms has been a research focus. The studies on gut microbes not only facilitate our understanding of the symbiotic relationship between termites and microbes, the interaction among microbial communities, but also offer potential means for industrial production of cellulosic ethanol. With developments in the isolation methods and high-throughput sequencing technology, the structure and function of symbiotic microbes in the gut of termites will be further researched.

Keywords termite; symbiotic microorganism; diversity; feeding diet; carbon and nitrogen cycling

白蚁是社会性昆虫, 具有复杂的社会系统, 属于等翅目昆虫, 与蟑螂具有非常近的进化关系[1]. 白蚁主要分布于温带到热带地区, 是木质纤维素的主要降解者, 有助于生态系统的碳循环[2]. 根据白蚁后肠是否含有共生原生动物, 可以分为低等白蚁(有共生原生动物)和高等白蚁(无共生原生动物)两大类群[3]. 分类学上将其分为7个科, 低等白蚁包括6个科, 即草白蚁科(Hodotermitidae)、原白蚁科(Termopsidae)、澳白蚁科(Mastotermitidae)、木白蚁科(Kalotermitidae)、齿白蚁科(Serritermitidae)、鼻白蚁科(Rhinotermitidae); 高等白蚁只有1个白蚁科(Termitidae, 包括4个亚科, 即大白蚁亚科(Macrotermitinae)、尖白蚁亚科(Apicotermitinae)、白蚁亚科(Termitinae)、 象白蚁亚科Nasutitermitinae)[3], 其中低等白蚁占白蚁总数的25%, 高等白蚁占75%. 低等白蚁主要食木, 而高等白蚁食性复杂, 包括木材、腐殖质、土壤、真菌等[4].

白蚁消化道包括唾液腺(Salivary gland)、前肠(Foregut)、中肠(Midgut)和后肠(Hindgut). 后肠膨大, 不同的白蚁种类后肠结构不同. 高等木食性白蚁和土食性白

收稿日期 Received: 2016-09-14 接受日期 Accepted: 2016-10-13
*国家重点基础研究发展计划(973计划)子课题(2011CB707402)和国家自然科学基金项目(31272370、30870085)资助 Supported by the State Key Basic R & D Program of China (973 Program, 2011CB707402) and the National Natural Science Foundation of China (31272370, 30870085)
**通讯作者 Corresponding author (E-mail: jinfgni@sdu.edu.cn)

蚁后肠复杂，分为P1-P5 区，其中P3区显著膨大[5]，微生物大量聚集在后肠[6]. 尽管白蚁自身能够分泌木质纤维素酶（包括唾液腺或中肠分泌的纤维素酶）[7-8]，但是难降解的食物需要肠道微生物的协助. 白蚁肠道共生微生物包括原生生物、细菌、古菌和真菌. 在低等白蚁后肠栖息着大量的原生生物（鞭毛虫），而高等白蚁中不存在原生生物；低等和高等白蚁中均存在细菌和古菌[3]. 白蚁肠道有一百多种甚至更多种细菌，而且大多数只存在于白蚁后肠中[9]. 然而至今，仅有为数不多的微生物从白蚁肠道中得以分离并作了鉴定（表1），分离到的细菌主要为拟杆菌菌门（Bacteroidaceae）、厚壁菌门（Firmicutes）、变形菌门（Proteobacteria）、螺旋体门（Spirochaetes）、放线菌门（Actinobacteria）、迷踪菌门（Elusimicrobia）. 这一方面是由于白蚁肠道环境太复杂，不能用常规方法培养研究这些微生物；另一方面由于大多数微生物为不可培养微生物[9].

随着现代分子生物学技术的发展，一些以16S rRNA 基因序列分析为基础的分子技术被广泛用于微生物的鉴定、分类以及微生物之间进化关系的确定等，如变性梯度凝胶电泳（Denaturing Gradient Gel Electrophoresis, DGGE）、末端限制性片段长度多态性技术（Terminal Restriction Fragment Length Polymorphism, TRFLP）等常规分子生物学技术[10]，以及可精确定位细菌在肠道中分布位置的荧光原位杂交（Fluorescence *in situ* hybridization, FISH）方法[11]，这些技术主要用来鉴定或描述一些特殊菌群的特征. 随着科技的发展，用于研究复杂微生物群体的研究方法越来越多，并且随着高通量测序技术的迅速发展，以高通量测序和宏基因组测序技术为主的最新测序技术将逐渐取代常规的分子生物学方法. 一些在蛋白质组和代谢组学中应用的新技术和新方法也被逐渐运用到肠道微生物的研究中.

1 低等白蚁肠道微生物

低等白蚁肠道中存在丰富的原生生物，原生动物在降解纤维素和半纤维素中发挥着重要的作用[37]. 所有白蚁共生鞭毛虫均属于原生动物动鞭毛纲（Zoomastigophorea）中的3个目，即毛滴虫目（Trichomonadida）、超鞭虫目（Hypermastigida）和锐滴虫目（Oxymonadida）. 1877年，Leidy首先报道了在黄肢散白蚁*Reticulitermes flavipes*肠道内有鞭毛虫之后，人们从许多低等白蚁肠道内发现了多种鞭毛虫. Yamin（1981）首次从动原白蚁中分离到原白蚁毛丝虫（*Trichomitopsis termopsidis*）和球形披发虫（*Trichonympha sphaerica*）. 栖北散白蚁（*Reticulitermes speratus*）肠道内至少有11种鞭毛虫；台湾乳白蚁（*Coptotermes formosanus*）肠道内有3种鞭毛虫；在黄肢散白蚁后肠存在着至少12种鞭毛虫[38]. 低等白蚁共生鞭毛虫在木质纤维素降解中发挥重要的作用，这些鞭毛虫绝大部分都具有吞噬木质颗粒的能力，并产生大量丰富的纤维素酶，与白蚁自身的纤维素酶系统相互合作，可能形成相对独立的二元纤维素消化系统，也可能是紧密的一元消化系统，维持白蚁的营养[5]. 在低等白蚁中，后肠原生生物通过吞噬作用水解小木屑或碎木片的木质纤维素. 在原生生物细胞中小木屑通过木质颗粒的自身荧光可以被检测到. 由于原生生物很难培养，所以小木屑的最终去向不明确[3]. 上述研究结果揭示了以纤维素为食的原生动物和白蚁之间的营养关系，表明没有肠道内鞭毛虫的帮助，低等木食

表1 已分离和鉴定的白蚁肠道微生物
Table 1 The isolated and identified microbes from termite gut

宿主 Host species	门 Phylum	科 Family	种 Species
黑胸散白蚁 *Reticulitermes chinensis* [12]	放线菌门 Actinobacteria	微杆菌科 Microbacteriaceae	*Gryllotalpicola reticulitermitis*
达尔文澳白蚁 *Mastotermes darwiniensis* [13]	放线菌门 Actinobacteria	原小单胞菌科 Promicromonosporaceae	*Cellulosimicrobium variabile*
栖北散白蚁 *Reticulitermes speratus* [14]	拟杆菌菌门 Bacteroidetes	拟杆菌科 Bacteroidaceae	*Bacteroides reticulotermitis*
栖北散白蚁 *Reticulitermes speratus* [15]	拟杆菌菌门 Bacteroidetes	紫单胞菌科 Porphyromonadaceae	*Dysgonomonas termitidis*
黄翅大白蚁 *Macrotermes barneyi* [16]	拟杆菌门 Bacteroidetes	紫单胞菌科 Porphyromonadaceae	*Dysgonomonas macrotermitis*
内华达古白蚁 *Zootermopsis nevadensis* [17]	迷踪菌门 Elusimicrobia	Endomicrobiaceae	*Endomicrobium proavitum*
桑特散白蚁 *Reticulitermes santonensis* [18]	厚壁菌门 Firmicutes	芽孢杆菌科 Bacillaceae	*Bacillus subtilis*
Nasutitermes exitiosus [19]	厚壁菌门 Firmicutes	梭菌科 Clostridiaceae	*Clostridium beijerinckii* Ne1
Wood-feeding higher termite [20]	厚壁菌门 Firmicutes	类芽孢杆菌科 Paenibacillaceae	*Paenibacillus macerans* Ⅱ PSP3
Nasutitermes sp. [21]	厚壁菌门 Firmicutes	类芽孢杆菌科 Paenibacillaceae	*Paenibacillus nasutitermitis*
Nasutitermes hainanensis [22]	厚壁菌门 Firmicutes	链球菌科 Streptococcaceae	*Lactococcus nasutitermitis*
Termes comis [23]	厚壁菌门 Firmicutes	韦荣球菌科 Veillonellaceae	*Sporomusa intestinalis*
Thoracotermes macrothorax [23]	厚壁菌门 Firmicutes	韦荣球菌科 Veillonellaceae	*Sporotalea propionica*
达尔文澳白蚁 *Mastotermes darwiniensis* [24]	变形菌门 Proteobacteria	脱硫弧菌科 Desulfovibrionaceae	*Desulfovibrio intestinalis*
台湾乳白蚁 *Coptotermes formosanus* [25]	变形菌门 Proteobacteria	肠杆菌科 Enterobacteriaceae	*Citrobacter* sp. TM1522
台湾乳白蚁 *Coptotermes formosanus* [26]	变形菌门 Proteobacteria	肠杆菌科 Enterobacteriaceae	*Enterbacter agglommerans*
印巴结构木异白蚁 *Heterotermes indicola* [27]	变形菌门 Proteobacteria	肠杆菌科 Enterobacteriaceae	*Enterobacter cloacae*
台湾乳白蚁 *Coptotermes formosanus* [28]	变形菌门 Proteobacteria	肠杆菌科 Enterobacteriaceae	*Klebsiella pneumoniae*
纳塔尔大白蚁 *Macrotermes natalensis* [29]	变形菌门 Proteobacteria	肠杆菌科 Enterobacteriaceae	*Trabulsiella odontotermitis*
黄肢散白蚁 *Reticulitermes flavipes* [30]	变形菌门 Proteobacteria	奈瑟氏菌科 Neisseriaceae	*Stenoxybacter acetivorans*
Nasutitermes nigriceps [31]	变形菌门 Proteobacteria	根瘤菌科 Rhizobiaceae	*Ensifer adhaerens* strain M3A
达尔文澳白蚁 *Mastotermes darwiniensis* [32]	螺旋体门 Spirochaetes	螺旋体科 Spirochaetaceae	*Spirochaeta coccoides*
Isoptera [33]	螺旋体门 Spirochaetes	螺旋体科 Spirochaetaceae	*Spirochaeta* sp. strain JC202
Incisitermes tabogae [34]	螺旋体门 Spirochaetes	螺旋体科 Spirochaetaceae	*Treponema isoptericolens*
黄肢散白蚁 *Reticulitermes flavipes* [35]	疣微菌门 Verrucomicrobia	丰佑菌科 Opitutaceae	Opitutaceae bacterium strain TAV5
黄肢散白蚁 *Reticulitermes flavipes* [36]	广古菌门 Euryarchaeota	甲烷菌科 Methanobacteriaceae	*Methnobrevibacter cuticularis* and *Methnobrevibacter curvatus*

性白蚁就不能消化含木质纤维素的木质纤维素，一定时间内白蚁就会死亡[38]，所以后肠原生生物对于低等白蚁水解木质纤维素是必要的[39].

白蚁肠道内细菌十分丰富，且数量惊人，但是大多数为不可培养微生物[40]. 后肠细菌和古菌主要存在于鞭毛虫的细胞质和外表面[3, 41]，许多较小的细菌和古菌结合在后肠表面或后肠细胞壁[36-42]. 通过16S rRNA测序等方法对低等白蚁肠道细菌多样性研究表明，低等白蚁肠道共生细菌的主要类群为螺旋体菌门、拟杆菌菌门、厚壁菌门、迷踪菌门、变形菌门[43]；而古菌所占的比例很少，不到1%，主要是产甲烷的甲烷短杆菌.

2 高等白蚁肠道微生物

高等白蚁后肠不存在原生生物，大多数微生物为细菌和古菌[3]. 与低等白蚁相比，高等白蚁的食性呈现多样化，分为食木高等白蚁、食土和食腐殖质高等白蚁和培菌高等白蚁，如表2所示，不同食性的高等白蚁其肠道微生物组成不同[44].

2.1 食木高等白蚁的微生物组成

与食木的低等白蚁相比，食木高等白蚁肠道不含能够降解木质纤维素的鞭毛虫[51]. 由于不含鞭毛虫，食木高等白蚁中含有许多降解木质纤维素的细菌[52]. 在食木高等白蚁中，优势微生物为螺旋体菌门（Spirochaetes）、纤维杆菌菌门（Fibrobacteres）和TG3菌门（TG3 phylum），这3类微生物在其他食性的白蚁中较少[44, 47, 53-54]. 象白蚁属白蚁*Nasutitermes takasagoensis*和*N. walkeri*后肠具有很强的纤维素酶活[55]，另外一个象白蚁属的白蚁宏基因组分析发现许多的纤维素酶和半纤维素酶基因，并且通过生物系统进化分析说明这些基因来源于螺旋菌菌门和纤维杆菌菌门[56]. 此研究证明，高等白蚁肠道内含有极为丰富的木质纤维素降解基因，为开发利用新型高效的木质纤维素降解酶提供了理论支持.

2.2 食土和食腐殖质高等白蚁的微生物组成

食土高等白蚁肠道由于pH、氧化还原电势和其他物理参数不同具有明显的区分度，从而为肠道微生物提供了不同的环境[57]. 在P1区以厚壁菌门为主，随肠道向后，厚壁菌门逐渐减少[58]，优势菌群变为放线菌门[57]. 土食性白蚁的食物材料含有极为丰富的氮元素，对共生微生物的固氮能力没有过高的需求，所以土食性高等白蚁肠道微生物的固氮能力要低于木食性高等白蚁的固氮能力. 食土白蚁后肠含有许多耐碱性的梭菌，但其具体作用仍不明确，可能与其分泌碱性水解蛋白酶有关. 通过对食土高等白蚁（*Amitermes wheeleri*）后肠宏基因组和宏转录组分析发现梭菌中含有与碱性蛋白水解酶基因，而且梭菌中含有木聚糖酶基因，预测该菌具有降解木质纤维素的作用[59]. 土食性白蚁的食物中木质纤维素的含量较木食性白蚁食物材料低，所以其肠道微生物的纤维素降解能力比高等食木白蚁的降解能力要低.

2.3 培菌高等白蚁的微生物组成

高等白蚁中大白蚁亚科（Macrotermitinae）能够在菌圃中培养真菌，作为其营养来源，这类白蚁又称为培菌白蚁，培菌白蚁的食物包括真菌菌丝体和木头[60]. 培菌白蚁肠道微生物的优势菌以拟杆菌门和厚壁菌门微生物为主，这与食木白蚁的优势微生物有很大的差异，可能是培菌白蚁的食性引起的[61-62]. 培菌白蚁与杂食性的蟑螂肠道菌群结构相似，以拟杆菌菌门理研菌科的*Alistipes* clusters II、III 和 IV为优势菌群，但是*Alistipes*对白蚁的具体作用仍是未知的[63]. 培菌白蚁与其共生真菌关系的模式可以概括为：高龄工蚁收集木质屑带回巢内，低龄工蚁取食这些收集入巢的碎屑，初步消化并迅速排便，这些物质形成菌圃（Fungus garden或fungus comb）来培养真菌. 低龄工蚁还取食主要由无性孢子组成的菌丝节（Fungus nudoles）. 高龄工蚁主要取食菌圃较老的部

表2 不同食性的高等白蚁肠道优势微生物组成

Table 2 Abundance of dominant bacterial phyla in the gut microbiota of higher termites with different feeding diets

宿主 Host species	食性 Diet	微生物 Microbes
云南土白蚁 *Odontotermes yunnanensis* [45]	真菌 Fungus	Bacteroidetes (48.65%), Firmicutes (21.72%), Proteobacteria (14.55%)
Macrotermes michaelseni [46]	真菌 Fungus	Bacteroidetes (58.3%), Firmicutes（27.5%）
Odontotermes sp. [46]	真菌 Fungus	Bacteroidetes (53.8%), Firmicutes (23.7%), Spirochaetes (10.9%)
Odontotermes sp.	真菌 Fungus	Firmicutes (31.97%), Bacteroidetes (24.31%), Proteobacteria (10.48%)
Macrotermes sp. [47]	真菌 Fungus	Bacteroidetes (38.45%), Firmicutes (33.35%), Proteobacteria (12.28%)
Macrotermes subhyalinus [47]	真菌 Fungus	Firmicutes (34.36%), Bacteroidetes (33.32%), Proteobacteria (9.93%)
Microtermes sp. [46]	真菌 Fungus	Bacteroidetes (40.1%), Spirochaetes (36.5%), Firmicutes (17.5%)
自曝白蚁 *Neocapritermes taracua* [48]	腐殖质 Humus	Firmicutes (48.73%), Bacteroidetes (12.81%)
Termes hospes [48]	腐殖质 Humus	Firmicutes (45.5%), Bacteroidetes (14.18%)
Syntermes wheeleri [49]	凋落物 Litter	Firmicutes (76.65%)
Cornitermes sp. [47]	凋落物 Litter	Spirochaetes (39.88%), Firmicutes (23.25%)
Nasutitermes takasagoensis [47]	木材 Wood	Spirochaetes (69.33%), Firmicutes (12.85%)
Nasutitermes corniger [47]	木材 Wood	Spirochaetes (55.51%), TG3 (13.05%), Firmicutes (9.97%)
Microcerotermes sp. [46]	木材 Wood	Spirochaetes (70.9%), Fibrobacteres (13.4%)
Microcerotermes sp. [47]	木材 Wood	Spirochaetes (65.01%)
Microcerotermes parvus [48]	木材 Wood	Spirochaetes (41.75%), Firmicutes (18.84%)
Cubitermes ugandensis [48]	土壤 Soil	Firmicutes (52.31%), Bacteroidetes (21.21%), Spirochaetes (10.67%)
Alyscotermes trestus [47]	土壤 Soil	Firmicutes (44.36%), Bacteroidetes (21.16%)
Ophiotermes sp.	土壤 Soil	Firmicutes (68.65%)
澳大利亚磁石白蚁 *Amitermes meridionalis* [47]	NR	Firmicutes (56.56%), Spirochaetes (13.32%), Actinobacteria (10.94%)
Mironasutitermes shangchengensis [50]	NR	Proteobacteria (51.73%), Fibrobacteres (14.11%), Firmicutes (13.86%)

优势微生物：百分比大于10%的菌门；NR：未报道.

Dominant bacterial phyla: the percentage is more than 10% of the bacterial phyla; NR: Not reported.

分, 是被真菌降解的植物材料[64]. 研究报道培菌白蚁肠道中的放线菌和芽孢杆菌产生次级的代谢物具有抑制菌圃杂菌生长的作用[65].

3 白蚁肠道微生物的作用

白蚁肠道可以被看作一个微生物反应器, 它与哺乳动物的瘤胃类似, 但是物理化学环境和微生物的适应过程导致肠道微环境与瘤胃微环境具有本质的不同[6].

3.1 肠道微生物的发酵作用

肠道微生物将木质纤维素转化为短链的脂肪酸, 脂肪酸积累在后肠, 最后被宿主吸收. 在低等白蚁中, 发酵产物的产生归功于鞭毛虫. 肠道鞭毛虫在无氧条件下将纤维素转化为乙酸、H_2和CO_2, 乙酸作为主要能量和碳源被宿主吸收[3]. 鞭毛虫有可能产生乳酸, 这占呼吸电子流的10% [66-67]. 乳酸不累积, 肠道周围的细菌在有氧环境下将乳酸迅速转化为乙酸. 而且, 在许多白蚁的后肠产生甲酸. 根据白蚁物种不同, 甲酸被氧化为CO_2或者转化为乙酸. 高等白蚁细菌的发酵过程尚未明确, 在肠道体液中检测到的产物与低等白蚁基本相同[66-68], 而且报道过的白蚁均释放部分氢气[69-70]. 在高等白蚁后肠的密螺旋体属主要产氢气, 而在低等白蚁中主要是肠道原生生物产氢气[71].

3.2 乙酸化

Breznak和Switzer发现在白蚁肠道中H_2和CO_2产生乙酸($4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$)[72]. 在许多低等和高等白蚁肠道有乙酸的产生[72], 约四分之一的乙酸产生于后肠[67]. 几种产乙酸的细菌已经从白蚁肠道分离出来[73-74]. 通过Wood-Ljungdahl途径利用H_2和CO_2产生乙酰-COA和乙酸的微生物被称作同型产乙酸菌. 在这途径中的关键酶是甲酰四氢叶酸合成酶, 通过PCR技术已经在许多低等白蚁中检测到该酶基因, 估计大多数甲酰四氢叶酸合成酶基因起源于螺旋体菌门[75-76]. 通过对高等白蚁*N. ephratae*宏基因组分析发现, 除了乙酸脱氢酶, 与Wood-Ljungdahl途径有关的蛋白均得到[77]. 因此, 在食木的低等和高等白蚁肠道中乙酸的产生主要归功于螺旋体菌门.

3.3 产生甲烷

尽管在白蚁肠道中的细菌超过古菌两个数量级, 但是大多数白蚁释放甲烷. 在白蚁中分离出的所有产甲烷的菌都属于甲烷短杆菌属[6]. 产甲烷的古菌在低等和高等白蚁肠道普遍存在, 氢气和二氧化碳合成甲烷($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$). 产生乙酸和甲烷都需要氢气, 同型产乙酸菌和产甲烷菌竞争氢气. 然而, 食木白蚁中同型产乙酸菌超过产甲烷的古菌, 产生的甲烷仅是乙酸的10% [67]. 在低等白蚁体内甲烷菌分布在后肠的上皮细胞、特定原生生物的细胞或在肠液中自由生活. 从多种低等和高等白蚁肠道检测到甲烷, 从低等白蚁黄肢散白蚁中已经分离出来了3种产甲烷古菌[78-79].

3.4 固氮作用

木质纤维素含氮低而且仅包含少量的氨基酸和维生素. 肠道微生物可以有效利用含氮的产物, 而且促进氨转化为有价值的产物[6]. 在乙炔还原法实验中Benemann和Breznak首次证明了白蚁的固氮作用. 消化道内固氮菌的固氮能力与白蚁的食性有关, 培菌白蚁和食土白蚁消化道内共生菌一般不具有固氮能力. 食物中木质材料的比例与消化道内固氮菌活性大致呈正比, 纯粹以木质材料为食的白蚁, 消化道内共生菌的固氮能力最强[80]. 尽管不同白蚁固氮的效率不同但固氮作用在白蚁中普遍存在. 在原核生物中发生氮的固定需要大量能量: $N_2 + 8H^+ + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$. 通过PCR和RT-PCR方法在多种白蚁中已经得到编码固氮酶的固氮基因*nifH*. 在白蚁肠道已经分离出了少量固氮细菌, 但最重要的固氮细菌是未培养微生物[6]. 白蚁肠道固氮基因大多数来源于绝对厌氧细菌螺旋体菌门和梭状芽孢杆菌, 还有一些来源于拟杆菌门、变形菌门和产甲烷古菌, 然而只有少量的固氮基因得到表达[81].

4 展望

白蚁肠道具有一个复杂的、多层次的共生系统, 但是肠道微生物培养的复杂性和艰难性阻碍阐明该共生系统的分子机制. 白蚁肠道微生物的宏基因组分析、宏转录组分析等揭示白蚁肠道中存在糖苷水解酶. 宏基因组分析发现了与发酵、产乙酸、固氮作用有关的基因, 在共生系统中这些活动是细菌必须参与的活动. 传统的分离鉴定方法分离出的菌体纯度较高, 能得到单一菌株, 便于研究其对白蚁的具体功能. 但是由于白蚁肠道环境的复杂性, 仅有为数不多的微生物从白蚁肠道中得以分离并作了鉴定. 宏基因组学、宏转录组学和宏蛋白组学技术的应用揭示了白蚁肠道微生物群落的结构、系统发生、代谢功能、调控规律等. 目前很多学者已经从微生态学的角度研究了肠道内部菌群结构及其影响因素, 并取得一定的进展, 但是白蚁肠道微生物和白蚁之间具体作用机制还知之甚少. 随着宏基因组学技术与高通量测序技术的进一步发展, 相信这些技术不仅有助于人们从系统角度全面认识白蚁肠道微生物群落结构与其功能, 也对开辟木质纤维素资源生物转化新途径有着重要的实际应用意义.

参考文献 [References]

1 Inward DJ, Vogler AP, Eggleton P. A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology [J]. *Mol Phylogenet Evol*, 2007, **44** (3): 953-967

2 Ni JF, Tokuda G. Lignocellulose-degrading enzymes from termites and their symbiotic microbiota [J]. *Biotechnol Adv*, 2013, **31** (6): 838-850

3 Hongoh Y. Toward the functional analysis of uncultivable, symbiotic microorganisms in the termite gut [J]. *Cell Mol Life Sci*, 2011, **68** (8): 1311-1325

4 宁娜, 张倪, 吴燕, 倪金凤. 台湾乳白蚁和黄翅大白蚁消化道主要木质纤维素降解酶活性比较[J]. 应用与环境生物学报, 2015, **21** (4): 678-682 [Ning N, Zhang N, Wu Y, Ni JF. Comparison of lignocellulolytic enzyme activities of the fungus-growing termite *Macrotermes barneyi* and the lower termite *Coptotermes formosanus* [J]. *Chin J Appl Environ Biol*, 2015, **21** (4): 678-682]

5 相辉, 周志华. 白蚁及共生微生物木质纤维素水解酶的种类[J]. 应用昆虫学报, 2009, **46** (1): 32-40 [Xiang H, Zhou ZH. Lignocellulolytic enzymes in termite and its symbiotic microbes [J]. *Chin J Appl Entomol*, 2009, **46** (1): 32-40]

6 Brune A. Symbiotic digestion of lignocellulose in termite guts [J]. *Nat Rev Microbiol*, 2014, **12** (3): 168-180

7 Watanabe H, Tokuda G. Animal cellulases [J]. *Cell Mol Life Sci*, 2001, **58** (9): 1167-1178

8 Watanabe H, Tokuda G. Cellulolytic systems in insects [J]. *Annu Rev Entomol*, 2010, **55**: 609-632

9 Hongoh Y. Diversity and genomes of uncultured microbial symbionts in the termite gut [J]. *Biosci Biotechnol Biochem*, 2010, **74** (6): 1145-1151

10 Shi W, Syrenne R, Sun JZ, Yuan JS. Molecular approaches to study the insect gut symbiotic microbiota at the 'omics' age [J]. *Insect Sci*, 2010, **17** (17): 199-219

11 Ikeda - Ohtsubo W, Strassert JFH, Köhler T, Mikaelyan A, Gregor I, Mchardy AC, Tringe SG, Hugenholtz P, Radek R, Brune A. '*Candidatus Adiutrix intracellularis*', an endosymbiont of termite gut flagellates, is the first representative of a deep - branching clade of *Deltaproteobacteria* and a putative homoacetogen [J]. *Environ Microbiol*, 2016, **18** (8): 2548-2564

12 Fang H, Lü W, Huang Z, Liu SJ, Yang H. *Gryllotalpicola reticulitermitis* sp. nov. isolated from a termite gut [J]. *Int J Syst Evol Microbiol*, 2014, **65** (Pt 1): 78-83

13 Bakalidou A, Kampfer P, Berchtold M, Kuhnigk T, Wenzel M, Konig H. *Cellulosimicrobium variabile* sp. nov., a cellulolytic bacterium from the hindgut of the termite *Mastotermes darwiniensis* [J]. *Int J Syst Evol Microbiol*, 2002, **52** (Pt 4): 1185-1192

14 Sakamoto M, Ohkuma M. *Bacteroides reticulotermitis* sp. nov., isolated from the gut of a subterranean termite (*Reticulitermes speratus*) [J]. *Int J Syst Evol Microbiol*, 2013, **63** (Pt 2): 691-695

15 Pramono AK, Sakamoto M, Iino T, Hongoh Y, Ohkuma M. *Dysgonomonas termitidis* sp. nov., isolated from the gut of the subterranean termite *Reticulitermes speratus* [J]. *Int J Syst Evol Microbiol*, 2015, **65** (Pt 2): 681-685

16 Yang YJ, Zhang N, Ji SQ, Lan X, Zhang KD, Shen YL, Li FL, Ni JF. *Dysgonomonas macrotermitis* sp. nov., isolated from the hindgut of a fungus-growing termite [J]. *Int J Syst Evol Microbiol*, 2014, **64** (9): 2956-2961

17 Hao Z, Dietrich C, Radek R, Brune A. *Endomicrobium proavitum*, the first isolate of *Endomicrobia* class. nov. (phylum *Elusimicrobia*) - an ultramicrobacterium with an unusual cell cycle that fixes nitrogen with a Group IV nitrogenase [J]. *Environ Microbiol*, 2016, **18** (1): 191-204

18 Tarayre C, Brognaux A, Brasseur C, Bauwens J, Millet C, Mattéotti C, Destain J, Vandenbol M, Portetelle D, Pauw ED. Isolation and cultivation of a xylanolytic *Bacillus subtilis* extracted from the gut of the termite *Reticulitermes santonensis* [J]. *Appl Biochem Biotechnol*, 2013, **171** (1): 225-245

19 Wang H, Lin H, Trandinh N, Li D, Greenfield P, Midgley DJ. Draft genome sequence of *Clostridium* sp. Ne2, *Clostridia* from an enrichment culture obtained from Australian Subterranean termite, *Nasutitermes exitiosus* [J]. *Genome Announc*, 2014, **3** (2): 304-315

20 Dheeran P, Nandhagopal N, Kumar S, Jaiswal YK, Adhikari DK. A novel thermostable xylanase of *Paenibacillus macerans* IIPSP3 isolated from the termite gut [J]. *J Ind Microbiol Biotechnol*, 2012, **39** (6): 851-860

21 Wang XM, Ma SC, Yang SY, Peng R, Zheng Y, Yang H. *Paenibacillus nasutitermitis* sp. nov., isolated from a termite gut [J]. *Int J Syst Evol Microbiol*, 2015, **66** (2): 901-905

22 Yang SY, Zheng Y, Huang Z, Wang XM, Yang H. *Lactococcus nasutitermitis* sp. nov. isolated from a termite gut [J]. *Int J Syst Evol Microbiol*, 2015, **66** (1): 518-522

23 Hattori S, Hongoh Y, Itoh T, Deevong P, Trakulnaleamsai S, Noparatnaraporn N, Kudo T, Ohkuma M. *Sporomusa intestinalis* sp. nov., a homoacetogenic bacterium isolated from the gut of a higher termite, *Termes comis* (Termitinae) [J]. *J Gen Appl Microbiol*, 2013, **59** (4): 321-324

24 Fröhlich J, Sass H, Babenzien HD, Kuhnigk T, Varma A, Saxena S, Nalepa C, Pfeiffer P, König H. Isolation of *Desulfovibrio intestinalis* sp. nov. from the hindgut' of the lower termite Mastotermes darwiniensis [J]. *Can J Microbiol*, 1999, **45** (2): 145-152

25 Tikhe CV, Martin TM, Gissendanner CR, Husseneder C. Complete genome sequence of *Citrobacter* Phage CVT22 isolated from the gut of the Formosan Subterranean termite, *Coptotermes formosanus* Shiraki [J]. *Genome Announc*, 2015, **3** (4): e00408-15

26 Potrikus CJ, Breznak JA. Nitrogen-fixing *Enterobacter agglomerans* isolated from guts of wood-eating termites [J]. *Appl Environ Microbiol*, 1977, **33** (2): 392-399

27 Vasan PT, Piriya PS, Prabhu DIG, Vennison SJ. Cellulosic ethanol production by Zymomonasmobilis harboring an endoglucanase gene from *Enterobacter cloacae* [J]. *Bioresour Technol*, 2010, **102** (3): 2585-2589

28 Doolittle M, Raina A, Lax A, Boopathy R. Presence of nitrogen fixing *Klebsiella pneumoniae* in the gut of the Formosan subterranean termite (*Coptotermes formosanus*) [J]. *Bioresour Technol*, 2008, **99** (8): 3297-3300

29 Sapountzis P, Gruntjes T, Otani S, Estevez J, Da CR, Rd PG, Perna NT, Poulsen M. The Enterobacterium *Trabulsiella odontotermitis* presents novel adaptations related to its association with fungus-growing termites [J]. *Appl Environ Microbiol*, 2015, **81** (19): 6577-6588

30 Wertz JT, Breznak JA. *Stenoxybacter acetivorans* gen. nov., sp nov., an acetate-oxidizing obligate microaerophile among diverse O_2-consuming bacteria from termite guts [J]. *Appl Environ Microbiol*, 2007, **73** (21): 6819-6828

31 Fröhlich J, Koustiane C, Kämpfer P, Rosselló-Mora R, Valens M, Berchtold M, Kuhnigk T, Hertel H, Maheshwari DK, König H. Occurrence of rhizobia in the gut of the higher termite *Nasutitermes nigriceps* [J]. *Syst Appl Microbiol*, 2007, **30** (1): 68-74

32 Droge S, Frohlich J, Radek R, Konig H. *Spirochaeta coccoides* sp. nov., a novel coccoid spirochete from the hindgut of the termite Neotermes castaneus [J]. *Appl Environ Microbiol*, 2006, **72** (1): 392-397

33 Tushar L, Sravanthi T, Sasikala C, Ramana CV. Draft genome sequence of *Spirochaeta* sp. strain JC202, an endosymbiont of the termite (*Isoptera*) gut [J]. *Genome Announc*, 2014, **3** (1): e01481-14

34 Dröge S, Rachel R, Radek R, König H. *Treponema isoptericolens* sp. nov., a novel spirochaete from the hindgut of the termite *Incisitermes tabogae* [J]. *Int J Syst Evol Microbiol*, 2008, **58** (5): 1079-1083

35 Kotak M, Isanapong J, Goodwin L, Bruce D, Chen A, Han CS, Huntemann M, Ivanova N, Land ML, Nolan M. Complete genome sequence of the *Opitutaceae Bacterium* Strain TAV5, a potential facultative methylotroph of the wood-feeding termite *Reticulitermes flavipes* [J]. *Genome Announc*, 2015, **3** (2): e00060-15

36 Leadbetter JR, Breznak JA. Physiological ecology of *Methanobrevibacter cuticularis* sp. nov. and Methanobrevibacter curvatus sp.

nov., isolated from the hindgut of the termite *Reticulitermes flavipes* [J]. *Appl Environ Microbiol*, 1996, **62** (10): 3620-3631

37 Bignell DE, Roisin Y, Lo N. Biology of Termites: a Modern Synthesis[M]. Netherlands: Springer, 2011: 439-475

38 赵凯, 常志威, 张小燕, 郝妍, 吴桐, 平文祥, 周东坡. 白蚁肠道共生微生物多样性及其防治方法研究现状[J]. 应用与环境生物学报, 2012, **18** (2): 331-337 [Zhao K, Chang ZW, Zhang XY, Hao Y, Wu T, Ping WX, Zhou DP. Recent advances in diversity of symbiotic microbes in termite gut and termite control methods [J]. *Chin J Appl Environ Biol*, 2012, **18** (2): 331-337]

39 Ikeda-Ohtsubo W, Brune A. Cospeciation of termite gut flagellates and their bacterial endosymbionts: *Trichonympha* species and 'Candidatus *Endomicrobium trichonymphae*' [J]. *Mol Ecol*, 2009, **18** (2): 332-342

40 Ohkuma M. Termite symbiotic systems: efficient bio-recycling of lignocellulose [J]. *Appl Microbiol Biotechnol*, 2003, **61** (1): 1-9

41 Hackstein JHP. (Endo)symbiotic methanogenic Archaea [J]. *Microbiol Monogr*, 2010, **19**: 55-59

42 Thompson CL, Vier R, Mikaelyan A, Wienemann T, Brune A. '*Candidatus* Arthromitus' revised: segmented filamentous bacteria in arthropod guts are members of *Lachnospiraceae* [J]. *Environ Microbiol*, 2012, **14** (6): 1454-1465

43 Huang XF, Bakker MG, Judd TM, Reardon KF, Vivanco JM. Variations in diversity and richness of gut bacterial communities of termites (*Reticulitermes flavipes*) fed with grassy and woody plant substrates [J]. *Microb Ecol*, 2013, **65** (3): 531-536

44 Mikaelyan A, Dietrich C, Köhler T, Poulsen M, Sillam-Dussès D, Brune A. Diet is the primary determinant of bacterial community structure in the guts of higher termites [J]. *Mol Ecol*, 2015, **24** (20): 5284-5295

45 Liu N, Zhang L, Zhou H, Zhang M, Yan X, Wang Q, Long Y, Xie L, Wang S, Huang Y, Zhou Z. Metagenomic insights into metabolic capacities of the gut microbiota in a fungus-cultivating termite (*Odontotermes yunnanensis*) [J]. *PLoS ONE*, 2013, **8** (7): e69184

46 Makonde HM, Mwirichia R, Osiemo Z, Boga HI, Klenk HP. 454 Pyrosequencing-based assessment of bacterial diversity and community structure in termite guts, mounds and surrounding soils [J]. *Springerplus*, 2015, **4** (471): 1-11

47 Dietrich C, Köhler T, Brune A. The cockroach origin of the termite gut microbiota: patterns in bacterial community structure reflect major evolutionary events [J]. *Appl Environ Microbiol*, 2014, **80** (7): 2261-2269

48 Rossmassler K, Dietrich C, Thompson C, Mikaelyan A, Nonoh JO, Scheffrahn RH, Sillam-Dussès D, Brune A. Metagenomic analysis of the microbiota in the highly compartmented hindguts of six wood- or soil-feeding higher termites [J]. *Microbiome*, 2015, **3** (1): 1-6

49 Santana RH, Catao EC, Lopes FA, Constantino R, Barreto CC, Kruger RH. The gut microbiota of workers of the litter-feeding termite *Syntermes wheeleri* (Termitidae: Syntermitinae): archaeal, bacterial, and fungal communities [J]. *Microb Ecol*, 2015, **70** (2): 545-556

50 Su LJ, Liu YQ, Liu H, Wang Y, Li Y, Lin HM, Wang FQ, Song AD. Linking lignocellulosic dietary patterns with gut microbial Enterotypes of *Tsaitermes ampliceps* and comparison with *Mironasutitermes shangchengensis* [J]. *Genet Mol Res*, 2015, **14** (4): 13954-13967

51 Brugerolle G, Radek R. Symbiotic Protozoa of Termites [J]. *Soil Biol*, 2005, **6**: 243-269

52 Brune A, Dietrich C. The gut microbiota of termites: digesting the diversity in the light of ecology and evolution [J]. *Annu Rev Microbiol*, 2015, **69**: 145-166

53 Hongoh Y, Deevong P, Hattori S, Inoue T, Noda S, Noparatnaraporn N, Kudo T, Ohkuma M. Phylogenetic diversity, localization, and cell morphologies of members of the *Candidate* phylum TG3 and a subphylum in the phylum *Fibrobacteres*, recently discovered bacterial groups dominant in termite guts [J]. *Appl Environ Microbiol*, 2006, **72** (10): 6780-6788

54 Rahman NA, Parks DH, Willner DL, Engelbrektson AL, Goffredi SK, Warnecke F, Scheffrahn RH, Hugenholtz P. A molecular survey of Australian and North American termite genera indicates that vertical inheritance is the primary force shaping termite gut microbiomes [J]. *Microbiome*, 2015, **3** (1): 1-16

55 Tokuda G, Watanabe H. Hidden cellulases in termites: revision of an old hypothesis [J]. *Biol Lett*, 2007, **3** (3): 336-339

56 Warnecke F, Luginbühl P, Ivanova N, Ghassemian M, Richardson TH, Stege JT, Cayouette M, Mchardy AC, Djordjevic G, Aboushadi N. Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite [J]. *Nature*, 2007, **450** (7169): 560-565

57 Tim K, Ulrich S, Katja M, Andreas B. Novel lineages of Planctomycetes densely colonize the alkaline gut of soil-feeding termites (*Cubitermes* spp.) [J]. *Environ Microbiol*, 2008, **10** (5): 1260-1270

58 Schmitt-Wagner D, Friedrich MW, Wagner B, Brune A. Axial dynamics, stability, and interspecies similarity of bacterial community structure in the highly compartmentalized gut of soil-feeding termites (*Cubitermes* spp.) [J]. *Appl Environ Microbiol*, 2003, **69** (10): 6018-6024

59 He SM, Ivanova N, Kirton E, Allgaier M, Bergin C, Scheffrahn RH, Kyrpides NC, Warnecke F, Tringe SG, Hugenholtz P. Comparative metagenomic and metatranscriptomic analysis of hindgut paunch microbiota in wood- and dung-feeding higher termites [J]. *PLoS ONE*, 2013, **8** (4): e61126

60 Donovan SE, Eggleton P, Bignell DE. Gut content analysis and a new feeding group classification of termites [J]. *EcolEntomol*, 2001, **26** (4): 356-366

61 Hongoh Y, Ekpornprasit L, Inoue T, Moriya S, Trakulnaleamsai S, Ohkuma M, Noparatnaraporn N, Kudo T. Intracolony variation of bacterial gut microbiota among castes and ages in the fungus-growing termite *Macrotermes gilvus* [J]. *Mol Ecol*, 2006, **15** (2): 505–516

62 Liu N, Zhang L, Zhou H, Zhang M, Yan X, Wang Q, Long Y, Xie L, Wang S, Huang Y. Metagenomic insights into metabolic capacities of the gut microbiota in a fungus-cultivating termite (*Odontotermes yunnanensis*) [J]. *PLoS ONE*, 2013, **8** (7): e69184

63 Peterson BF, Scharf ME. Lower termite associations with microbes: synergy, protection, and interplay [J]. *Front Microbiol*, 2016, **7**: 00422

64 Poulsen M. Towards an integrated understanding of the consequences of fungus domestication on the fungus-growing termite gut microbiota [J]. *Environ Microbiol*, 2015, **17** (8): 2562-2572

65 Visser AA, Nobre T, Currie CR, Aanen DK, Poulsen M. Exploring the potential for actinobacteria as defensive symbionts in fungus-growing termites [J]. *Microb Ecol*, 2012, **63** (4): 975-985

66 Tholen A, Brune A. Impact of oxygen on metabolic fluxes and *in situ* rates of reductive acetogenesis in the hindgut of the wood-feeding termite *Reticulitermes flavipes*. [J]. *Environ Microbiol*, 2000, **2** (4): 436-449

67 Pester M, Brune A. Hydrogen is the central free intermediate during lignocellulose degradation by termite gut symbionts [J]. *ISME J*, 2007, **1**

(6): 551-565

68 Anklin-Mühlemann R, Bignell DE, Veivers PC, Leuthold RH, Slaytor M. Morphological, microbiological and biochemical studies of the gut flora in the fungus-growing termite *Macrotermes subhyalinus* [J]. *J Insect Physiol*, 1995, **41** (11): 929-940

69 Sugimoto A, Inoue T, Tayasu I, Miller L, Takeichi S, Abe T. Methane and hydrogen production in a termite-symbiont system [J]. *J Agr Sci*, 1998, **13** (2): 241–257

70 Baldi A, Bardelli S, Zucca E. Hydrogen profiles and localization of methanogenic activities in the highly compartmentalized hindgut of soil-feeding higher termites (*Cubitermes* spp.) [J]. *Appl Environ Microbiol*, 1999, **65** (10): 4490-4496

71 Inoue J, Kudo T, Ui S, Ohkuma M. Hydrogen production by termite gut protists: characterization of iron hydrogenases of parabasalian symbionts of the termite *Coptotermes formosanus* [J]. *Mater Construcc*, 2007, **6** (10): 1925-1932

72 Breznak JA, Switzer JM. Acetate synthesis from H_2 plus CO_2 by termite gut microbes [J]. *Appl Environ Microbiol*, 1986, **52** (4): 623-630

73 Breznak JA, Switzer JM, Seitz HJ. *Sporomusa termitida* sp. nov., an H_2/CO_2-utilizing acetogen isolated from termites [J]. *Arch Microbiol*, 1988, **150** (3): 282-288

74 Kane MD, Breznak JA. *Acetonema longum* gen.nov.sp.nov., an H_2/CO_2 acetogenic bacterium from the termite, *Pterotermes occidentis* [J]. *Arch Microbiol*, 1991, **156** (2): 91-98

75 Salmassi TM, Leadbetter JR. Analysis of genes of tetrahydrofolate-dependent metabolism from cultivated spirochaetes and the gut community of the termite *Zootermopsis angusticollis* [J]. *Microbiology*, 2003, **149** (Pt 9): 2529-2537

76 Pester M, Brune A. Expression profiles of *fhs* (FTHFS) genes support the hypothesis that spirochaetes dominate reductive acetogenesis in the hindgut of lower termites [J]. *Environ Microbiol*, 2006, **8**: 1261-1270

77 Warnecke F, Luginbuhl P, Ivanova N, Ghassemian M, Richardson TH, Stege JT, Cayouette M, McHardy AC, Djordjevic G, Aboushadi N, Sorek R, Tringe SG, Podar M, Martin HG, Kunin V, Dalevi D, Madejska J, Kirton E, Platt D, Szeto E, Salamov A, Barry K, Mikhailova N, Kyrpides NC, Matson EG, Ottesen EA, Zhang X, Hernandez M, Murillo C, Acosta LG, Rigoutsos I, Tamayo G, Green BD, Chang C, Rubin EM, Mathur EJ, Robertson DE, Hugenholtz P, Leadbetter JR. Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite [J]. *Nature*, 2007, **450** (7169): 560-565

78 Leadbetter JR, Breznak JA. Physiological ecology of *Methanobrevibacter cuticularis* sp. nov. and *Methanobrevibacter curvatus* sp. nov., isolated from the hindgut of the termite *Reticulitermes flavipes* [J]. *Appl Environ Microbiol*, 1996, **62** (10): 3620-3631

79 Leadbetter JR, Crosby LD, Breznak JA. *Methanobrevibacter filiformis* sp. nov., a filamentous methanogen from termite hindguts [J]. *Arch Microbiol*, 1998, **169** (4): 287-292

80 王亚召, 嵇保中, 刘曙雯, 丁芳. 白蚁共生菌固氮研究进展[J]. 应用与环境生物学报, 2016, **22** (2): 342-349 [Wang YZ, Ji BZ, Liu SW, Ding F. Research progress of nitrogen fixation of symbiotic bacteria in termites [J]. *Chin J Appl Environ Biol*, 2016, **22** (2): 342-349]

81 Du X, Li X, Wang Y, Peng J, Hong H, Yang H. Phylogenetic diversity of nitrogen fixation genes in the intestinal tract of *Reticulitermes chinensis* Snyder [J]. *Curr Microbiol*, 2012, **65** (5): 547-551