

三种稻飞虱成虫体内酵母类共生菌的形态差异

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摘要 试验采用冷冻切片技术结合显微摄像系统观察法研究了酵母类共生菌在3种稻飞虱成虫体内的存在状态、形态特性以及种类和组成。光学显微镜观察证实,3种稻飞虱成虫的头部和胸部均未观察到共生菌,在其腹部脂肪体中有大量酵母类共生菌,且以出芽进行无性繁殖,并伴有菌胞的出现。3种稻飞虱成虫体内共生菌的形态和组成明显不同。其中,褐飞虱体内的共生菌个体较大,以梭形、杆状和卵形为主,分别占共生菌总数的30.7%、53.5%和15.1%;灰飞虱和白背飞虱体内的共生菌个体较小,以卵形为主,分别占共生菌总数的93.4%和94.7%。此外,褐飞虱成虫体内的各类共生菌都显著大于灰飞虱和白背飞虱成虫体内的同种类型个体,且灰飞虱和白背飞虱体内的同种共生菌个体大小之间也存在一定差异。

关键词 褐飞虱, 灰飞虱, 白背飞虱, 酵母类共生菌, 形态特性。

中图分类号 Q964

褐飞虱 *Nilaparvata lugens* Stål、灰飞虱 *Laodphax striatellus* Fallén 和白背飞虱 *Sogatella furcifera* Horváth 均属迁飞性害虫,是我国及东南亚水稻产区的主要害虫,其造成的直接危害及因携带病毒所造成的间接损失一直是水稻生产面临的严重问题(李汝铎, 1996; 程遐年, 2003)。与多数刺吸危害的其它同翅目昆虫一样,3种稻飞虱体内也普遍存在共生菌,该类共生菌主要存在于虫体腹部脂肪内(Nasu & Suenaga, 1958; Nasu, 1963; Nasu et al., 1981; Noda, 1977; Chen et al., 1981a, 1981b; Cheng & Hou, 1996, 2001),即通常所讲的酵母类共生菌(Yeastlike endosymbiote, YLES)。国内外大量的研究证实,该类共生菌在褐飞虱体内参与醇类物质代谢(Wetzel et al., 1992)、氨基酸和维生素供给(傅强等, 2001),以及氮素循环(Sasaki et al., 1996; Hongoh & Ishikawa, 1997)中起着十分重要的作用;此外,还能生物合成褐飞虱胚胎发育和胚后发育所需的蛋白质,并对胚胎的腹节分化具有促进作用(Lee & Hou, 1987);最终影响到寄主褐飞虱的生长发育和繁殖(吕仲贤等, 2001c)。

国外早在20世纪60年代就有有关褐飞虱体内酵母类共生菌的形态描述(Nasu, 1963)。上世纪80年代开始,我国台湾地区也陆续报道了褐飞虱体内酵母类共生菌的显微组织观察结果(Chen et al.,

1981a, 1981b; Cheng & Hou, 1996, 2001)。我国大陆地区,褐飞虱体内共生菌的研究起步较晚,进入20世纪90年代才陆续开展,且该领域研究是结合抗性品种水稻防治褐飞虱危害的研究开展的(吕仲贤等, 2000, 2001a, 2001b, 2001c; Lee et al., 2004)。国内有关褐飞虱体内酵母类共生菌的形态描述很少(陈法军等, 2006),而3种稻飞虱体内该类共生菌的形态和种类组成等的报道至今未见。Nasu & Suenaga (1958)指出3种稻飞虱体内的酵母类共生菌存在明显的形态差异。其中,灰飞虱和白背飞虱体内的共生菌为卵形个体,长度为1.13 μm;褐飞虱体内的酵母类共生菌为长梭形和杆状类型,长度约为15 μm(Nasu & Suenaga, 1958)。Noda (1977), Nasu (1981), Chen et al. (1981)和Cheng & Hou (1996)的观察结果一致,即卵形共生菌为8~10 μm,鞘状个体为15~20 μm。

陈法军等(2006)系统观察了褐飞虱成虫体内酵母类共生菌的个体形态、菌胞有无、虫体内的存在状态及出芽繁殖等,指出褐飞虱体内的酵母类共生菌以长梭形和杆状个体最多、占绝大多数,卵形较少,球状个体更少见。本文采用冷冻切片技术结合显微摄像系统观察法开展褐飞虱、灰飞虱和白背飞虱体内酵母类共生菌的形态观察,以进一步明确3种稻飞虱体内该类共生菌的个体形态和种类组成

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等的差异。

1 材料和方法

111 供试虫源

2005年7月中旬, 供试褐飞虱、灰飞虱和白背飞虱分别采集于浙江省农科院实验稻田内, 饲养于盆栽的60日龄的感虫品种水稻TN1上, 温度: 26±1℃; 光周期: 14 L: 10 D; RH: 85±10% (DNP29272型光照培养箱; 上海精虹电子有限公司)。连续继代饲养2代后, 随机取3种未交配的稻飞虱成虫, 分别置于4℃冷藏柜中备用。

112 3种稻飞虱成虫体内酵母类共生菌的形态观察

从供试的3种稻飞虱成虫中随机各取短翅型成虫300头, 每次随机取10头成虫解剖镜(Leica S8 APO; German)下取头部、胸部和腹部分别进行冷冻切片(Leica CM1900; German)。冷冻切片机的参数设置、样品处理及结晶紫染色过程等详见陈法军等(2006)。处理后的样品置于载玻片上, 室内风干后在显微镜(Leica DMLS2; German)下观察、拍照(Leica DFC320; German)。

根据陈法军等(2006)的观察结果, 即褐飞虱成虫体内的酵母类共生菌存在形态多样性(梭形和杆状个体最多, 卵形其次, 球状共生菌极少见), 分别取褐飞虱、灰飞虱和白背飞虱组织的显微照片各10张, 20次重复(共计200张), 统计各种类型共生菌的数量及其所占比例。

113 3种稻飞虱成虫体内酵母类共生菌的大小测定

随机取以上拍摄的3种稻飞虱成虫体内的酵母

类共生菌的显微照片各200张, 利用IPP软件(Image2Pro Plus version 410; Media Cybernetics, Inc. Georgia Avenue Silver Spring, MD, USA)分别测定褐飞虱、灰飞虱和白背飞虱体内各种类型成熟共生菌(以出芽为准)的长(长径)、宽度(短径)等指标。

114 统计分析

采用单因子方差分析(SAS 6112)统计3种稻飞虱成虫体内同种酵母类共生菌的个体大小及所占比例的差异。统计分析前先将百分比数据进行反正弦平方根转换, 以符合数据的正态分布, 处理间的显著性检验采用Duncan检验(P<0.05)。

2 结果

211 共生菌在3种稻飞虱成虫体内的存在状态

3种稻飞虱成虫的头部和胸部均未发现酵母类共生菌的存在(表1), 在其腹部观察到大量共生菌(表1, 图1)。其个体形状包括梭形、杆状、卵形和球形个体, 且均以出芽方式进行无性繁殖(图3~4, 均以褐飞虱成虫体内共生菌形态及出芽繁殖方式示之)。

3种稻飞虱成虫体内的酵母类共生菌的显微照片显著不同(图1)。其中, 褐飞虱体内的酵母类共生菌个体较大, 且以梭形和杆状为主(图1A~B); 而白背飞虱和灰飞虱体内的共生菌个体较小, 以卵形为主(图1C~D)。此外, 显微镜下观察还发现, 褐飞虱(图2A~B)、灰飞虱(图2C)和白背飞虱(图2D)的成虫腹部脂肪体内均有菌胞的存在。

表1 3种稻飞虱成虫体内酵母类共生菌的冷冻切片观察

Table 1. Observation on the cryo-sliced slices of yeast-like endosymbiote (YLES) in brown planthopper (BPH), Nilaparvata lugens, smaller brown planthopper (SBPH), Laodelphax striatellus and white backed planthopper (WBPH), Sogatella furcifera.

稻飞虱 Rice planthoppers		酵母类共生菌 Yeast-like endosymbiote				菌胞 Mycelocytes	出芽繁殖 Proliferation	
		梭形 Fusiform	杆状 Bacilliform	卵形 Oval	球形 Globose		顶端芽殖 Cephalon budding	侧面芽殖 Lateral budding
		褐飞虱 N. lugens	头部 (Cephalosome)	-	-		-	-
	胸部 (Baenosome)	-	-	-	-	-	-	
	腹部 (Abdomen)	+	+	+	-	+	+	
灰飞虱 L. striatellus	头部 (Cephalosome)	-	-	-	-	-	-	
	胸部 (Baenosome)	-	-	-	-	-	-	
	腹部 (Abdomen)	+	+	+	-	+	+	
白背飞虱 S. furcifera	头部 (Cephalosome)	-	-	-	-	-	-	
	胸部 (Baenosome)	-	-	-	-	-	-	
	腹部 (Abdomen)	+	+	+	-	+	+	

注 (Note): - 表示不存在 (non-existing); + 表示存在 (existing)。

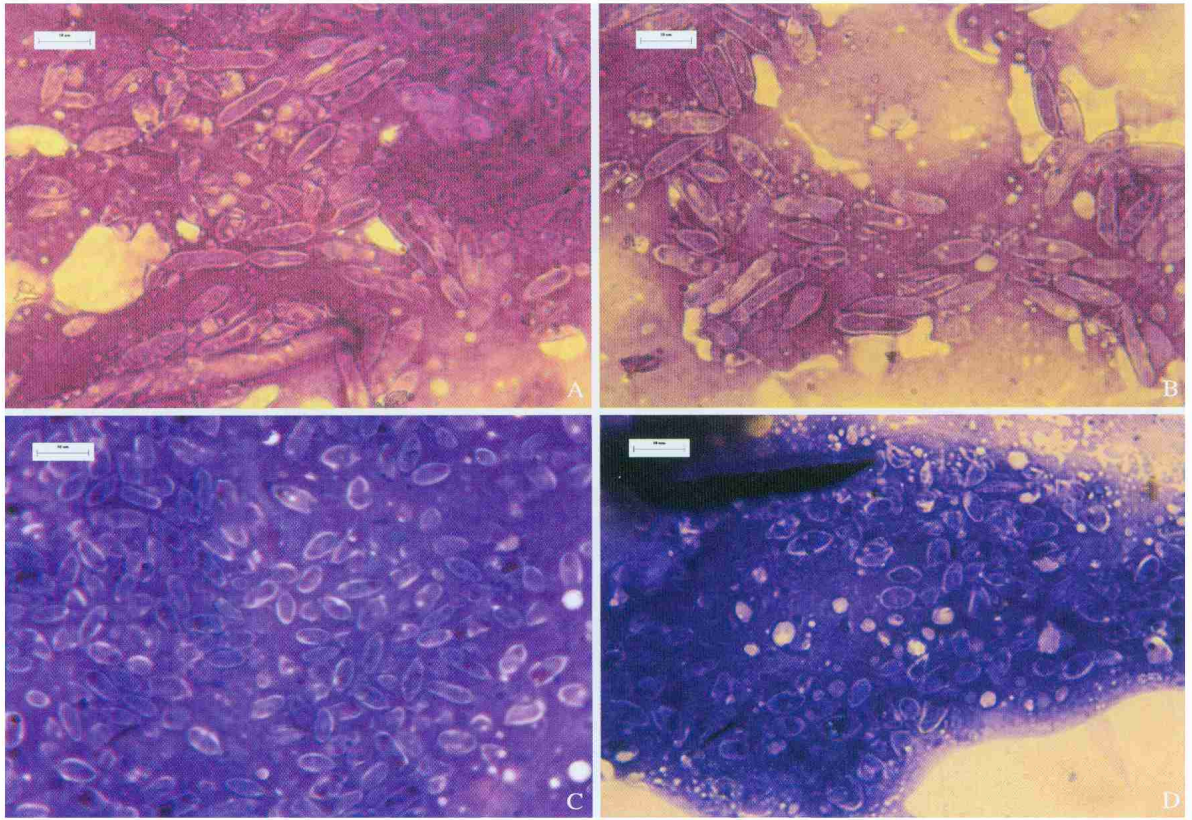


图 1 3 种稻飞虱成虫腹部脂肪体内的酵母类共生菌

Fig1. Yeas2like endosymbiote (YLES) excising in abdomen fat body of the adults of brown planthopper (BPH), Nilaparvata lugens, smaller brown planthopper (SBPH), Laodphax striatellus and white backed planthopper (WBPH), Sogatdla furcifera .

A~ B. 褐飞虱 *N. lugens* (BPH) C. 白背飞虱 *S. furcifera* (WBPH) D. 灰飞虱 *L. striatellus* (SBPH)
比例尺 (scale bars) = 10 Lm 100 @

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3 种稻飞虱成虫体内均发现 4 种类型的酵母类共生菌, 但不同种飞虱体内共生菌的组成存在明显差异 (表 2)。其中, 褐飞虱体内的共生菌以梭形和杆状为主, 分别占共生菌总数的 3017% 和 5315%, 都显著大于灰飞虱和白背飞虱体内同种类型的共生

菌 ($P < 0105$); 灰飞虱和白背飞虱体内的卵形共生菌所占比例分别为 9314% 和 9417%, 均显著大于褐飞虱体内的卵形个体所占比例 (1511%) ($P < 0105$; 表 2)。此外, 3 种稻飞虱成虫体内球形共生菌所占比例差异不显著, 均低于 017% ($P > 0105$; 表 2)。

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表 2 3 种稻飞虱成虫腹部脂肪体内酵母类共生菌的种类和组成

Table 2. Species and constitutions of YLES in abdomen fat body of the adults of BPH, SBPH and WBPH.

稻飞虱 Rice planthoppers	酵母类共生菌 Yeas2like endosymbiote (Mean? SD)			
	梭形 Fusiform	杆状 Bacillifor m	卵形 Oval	球形 Globose
褐飞虱 <i>N11ugens</i>	3017? 1219 a	5315? 1413 a	1511? 812 b	017? 119 a
灰飞虱 <i>L1 striatellus</i>	518? 618 b	012? 017 c	9314? 710 a	017? 210 a
白背飞虱 <i>S1 furcifera</i>	318? 316 b	114? 118 b	9417? 416 a	012? 014 a

不同的小写字母表示不同稻飞虱处理间差异显著 (Different lowercases indicate significant difference in percentage data among three species of rice planthopper by Duncan test at $P < 0105$)

3 种稻飞虱成虫体内的共生菌个体大小差异显著 (表 3)。其中, 褐飞虱体内的共生菌显著大于灰

飞虱和白背飞虱体内的同种类型个体 ($P < 0105$; 表 3)。此外, 灰飞虱体内的梭形和卵状个体的宽度

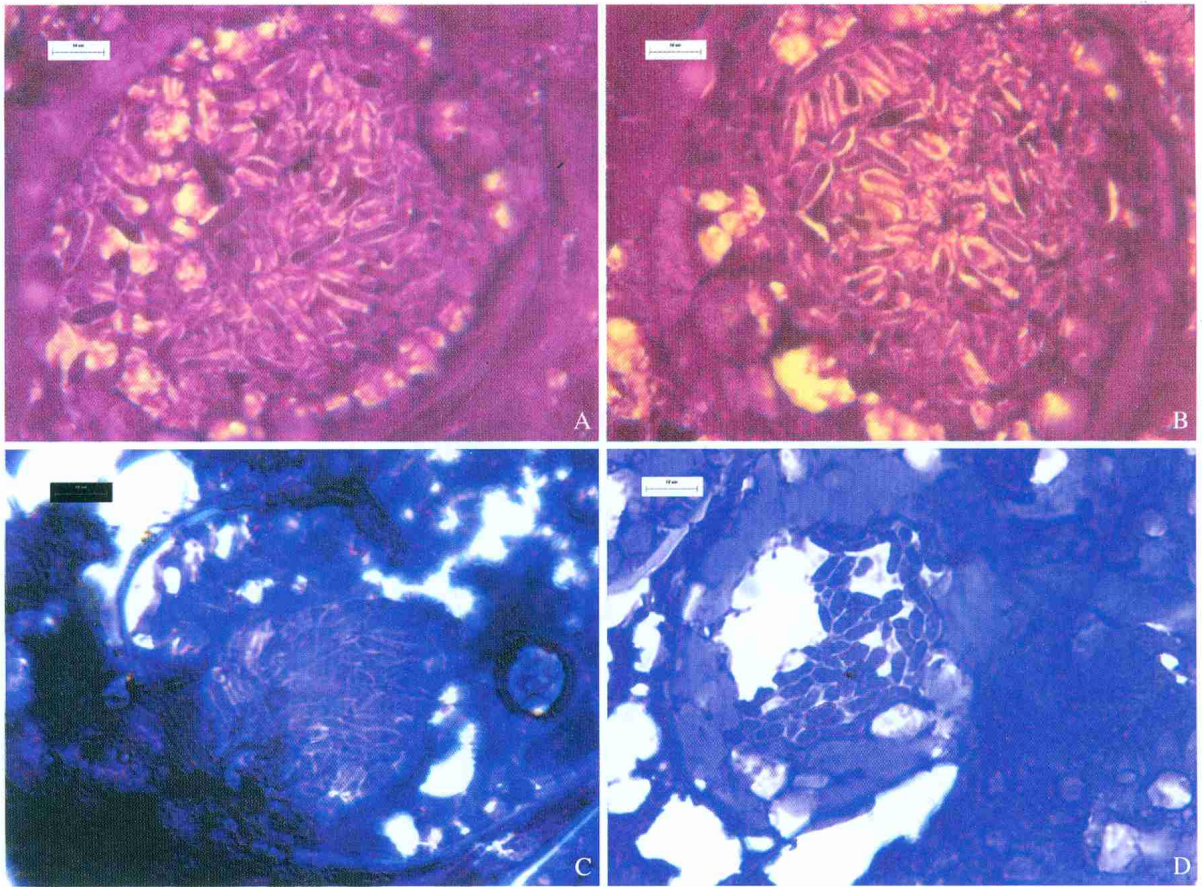


图2 3种稻飞虱成虫腹部脂肪体内的菌胞

Fig2. Mycetocytes of YLES excising in abdomen fat body of the adults of BPH, SBPH and WBPH.

A~ B. 褐飞虱 *N. lugens* (BPH) C. 灰飞虱 *L. striatellus* (SBPH) D. 白背飞虱 *S. furcifera* (WBPH)
比例尺 (scale bars) = 10 Lm 100@

表3 3种稻飞虱成虫腹部脂肪体内酵母类共生菌的个体大小

Table 3. Individual size (Mean; cm) of YLES in abdomen fat body of the adults of BPH, SBPH and WBPH.

稻飞虱 Rice planthoppers	酵母类共生菌 Yeastlike endosymbiote										
	梭形 Fusiform			杆状 Bacilliform			卵形 Oval			球形 Globose	
	N	长 (L)	宽 (W)	N	长 (L)	宽 (W)	N	长径 (LD)	短径 (WD)	N	直径 (D)
褐飞虱 <i>N. lugens</i>	62	131.1 a	41.0 a	80	141.0 a	31.4 a	25	81.6 a	31.9 a		
灰飞虱 <i>L. striatellus</i>	23	81.8 b	31.4 b				92	71.5 c	31.6 b	2	51.5
白背飞虱 <i>S. furcifera</i>	42	81.6 b	31.0 c	16	81.7 b	21.9 b	134	71.8 b	31.4 c	1	51.9

注 (note): N 样本大小 (sampling size); L 长度 (length), W 宽度 (width), LD 长径 (long diameter), WD 短径 (short diameter), D 直径 (diameter). 不同的小写字母表示不同稻飞虱处理间差异显著 (Different lowercases indicate significant difference in percentage data among three species of rice planthopper by Duncan test at $P < 0.05$)

(短径) 显著大于白背飞虱体内同类共生菌的宽度 (短径) ($P < 0.05$), 而白背飞虱体内的卵形个体的长径长度又显著大于灰飞虱体内该类共生菌的长径 ($P < 0.05$; 表 3)。

3 讨论

大量的光学显微镜和电子显微镜观察的结果表明, 褐飞虱体内共生菌主要存在于虫体腹部脂肪体

内, 在头、胸部没有共生菌 (Chen et al., 1981; Cheng & Hou, 1996)。本研究证实, 同褐飞虱一样 (陈法军等, 2006), 灰飞虱和白背飞虱成虫的头部和胸部均不存在共生菌, 该类共生菌主要存在于虫体腹部脂肪体内。从长期的协同进化的角度看, 虫体腹部脂肪体距离生殖系统近, 有利于寄主世代间的卵巢垂直传递; 同时, 虫体脂肪体主要存在于虫体腹部。可见, 共生菌在寄主体内的存在状态对共

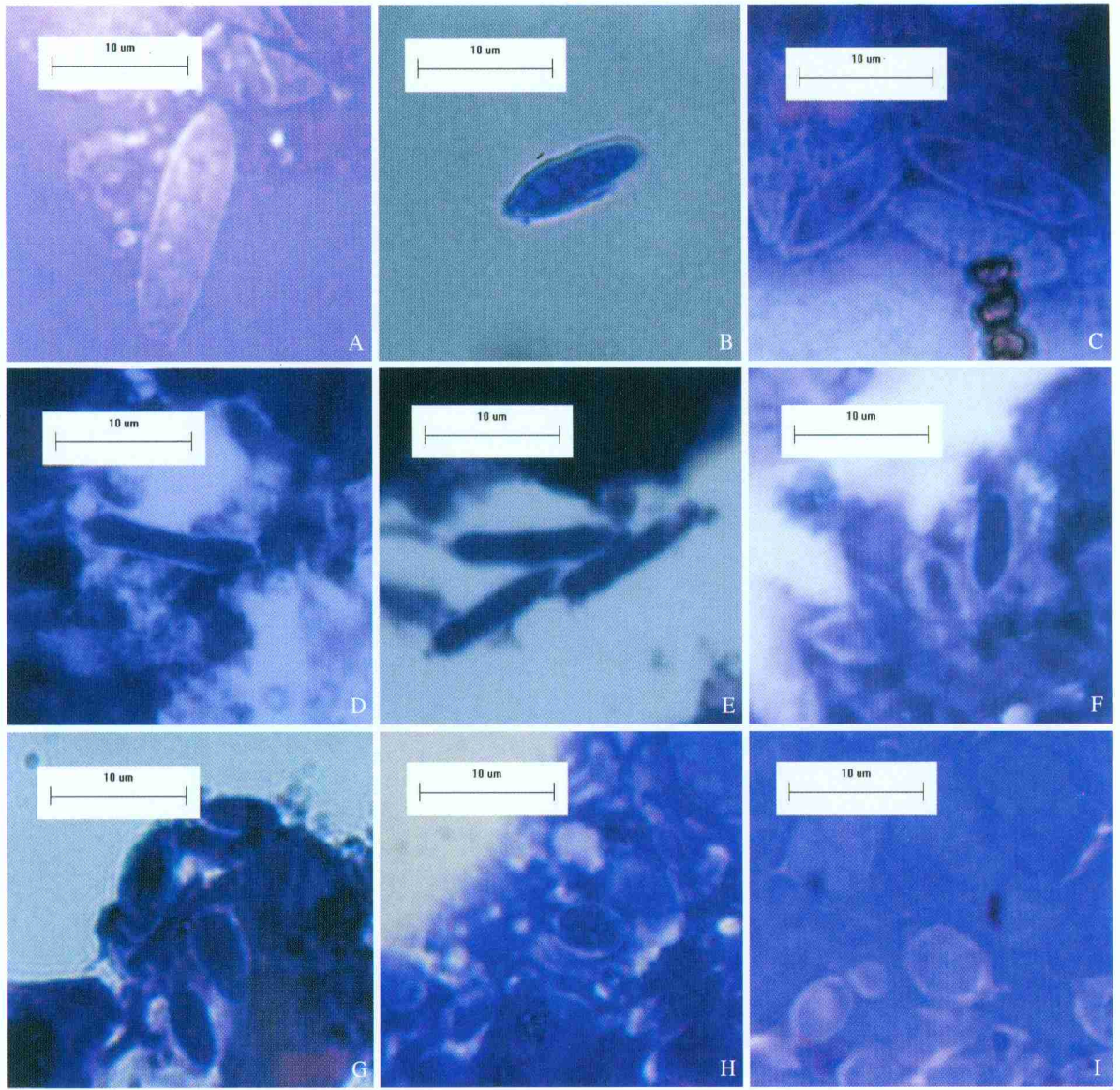


图3 褐飞虱成虫腹部脂肪体内酵母类共生菌的形态多样性

Fig13. Morphological variety of YLES excising in abdomen fat body of the adults of BPH.

A~ C. 梭形 (fusiform) D~ E. 杆状 (bacilliform) F~ G. 卵形 (oval) H~ I. 球形 (globose) 比例尺 (scale bars) = 10Lm 100@

生关系的双方都有利。

3种稻飞虱成虫体内酵母类共生菌的显微照片明显不同。与前人的观察结果一致(如 Nasu & Suenaga, 1958; Noda, 1977; Nasu, 1981; Chen et al., 1981a, 1981b; Cheng & Hou, 1996, 2001), 即褐飞虱体内的酵母类共生菌主要包含梭形、杆状和卵形, 分别占共生菌总量的 3017%、5315% 和 1511%; 而灰飞虱和白背飞虱体内的共生菌则以卵形为主, 其含量分别高达 9314% 和 9417%。可见, 褐飞虱体内酵母类共生菌的形态(种类)多样性组成较灰飞虱和白背飞虱体内的复杂。此外, 褐飞虱体内的共生菌个体显著大于灰飞虱和白背飞虱体内

的共生菌。根据其食性(李汝铎, 1996; 程遐年, 2003), 褐飞虱是专食性害虫, 其唯一危害寄主是水稻; 白背飞虱为寡食性害虫, 其寄主除水稻外, 还有禾本科杂草; 而灰飞虱则是广食性害虫, 其天然寄主除水稻和禾本科杂草外还有小麦、大麦等(丁锦华, 2002)。可见, 食谱越广, 稻飞虱体内酵母类共生菌的形态越单一; 而食谱越窄, 虫体内共生菌的组成越复杂。体内酵母类共生菌的形态和组成多样性和复杂性是否导致了褐飞虱较灰飞虱和白背飞虱对水稻具有更强的致害性, 及对抗性品种水稻的快速适应性, 这还有待于进一步的试验研究来验证。此外, 探明稻飞虱食谱变化与其体内共生菌的种类

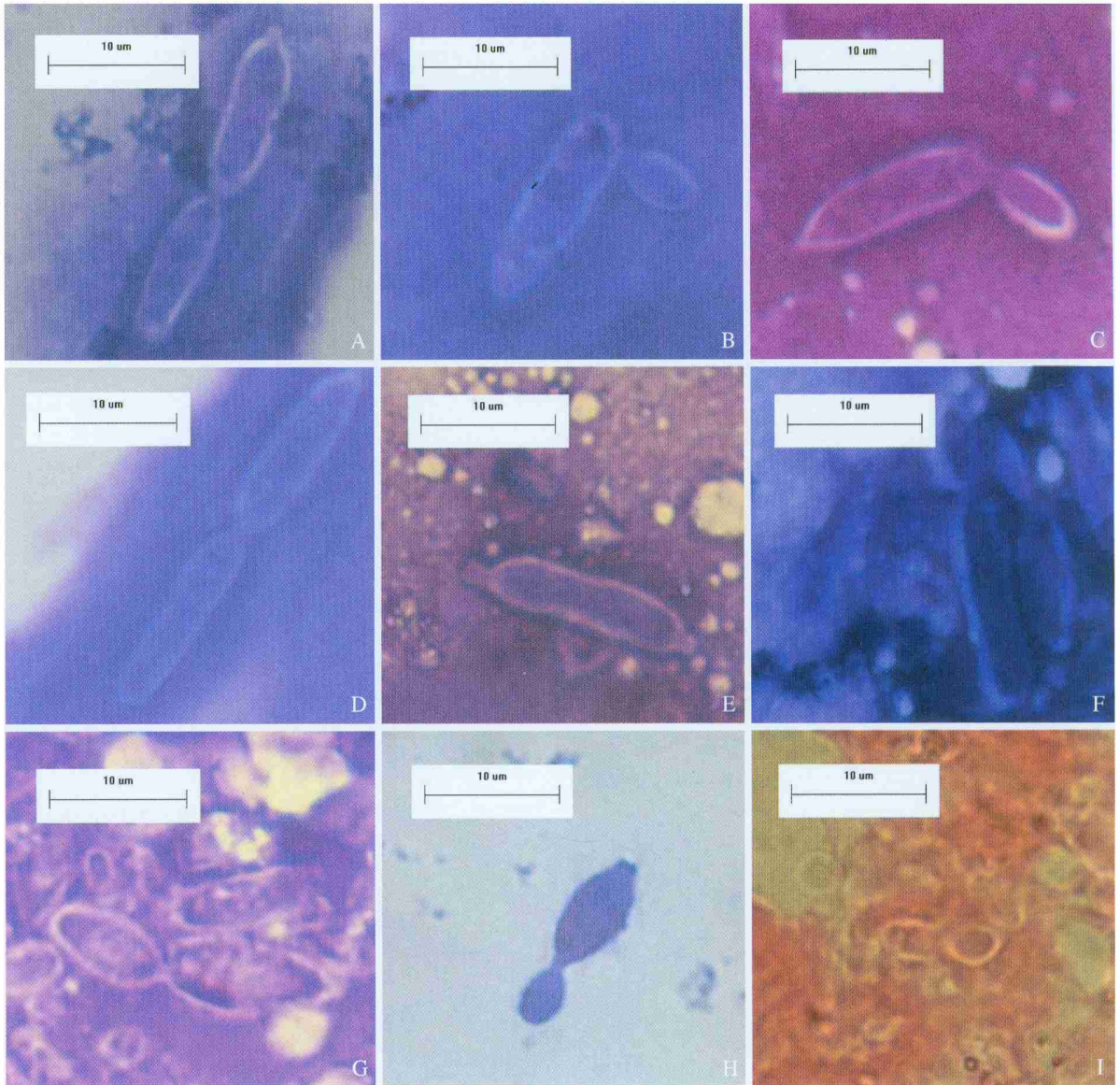


图4 褐飞虱成虫腹部脂肪体内酵母类共生菌的出芽繁殖

Fig4. Budding proliferation of YLES excising in abdomen fat body of the adults of BPH.

A~ C. 梭形 (fusiiform) D~ F. 杆状 (bacilliform) G~ H. 卵形 (oval) I. 球形 (globose) A, D, F, G~ H. 顶端芽殖 (budding on one cephalon) E. 两端芽殖 (budding on two cephalons simultaneously) B~ C, I. 侧面芽殖 (lateral budding)

和组成变化之间的相关关系将有助于进一步明确 / 稻飞虱2酵母类共生菌0 这一营养共生关系。

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MORPHOLOGICAL DIFFERENCE OF THE YEAST LIKE ENDOSYMBIOTES IN ADULT PLANTHOOPERS OF *NILAPARVATA LUGENS* (STÅL), *LAODELPHAX STRIATELLUS* (FALLÉN) AND *SOGATELLA FURCIFERA* (HORVATH)

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Abstract Distribution, morphological characteristics, species constitute of yeast like endosymbionts (YLES) in the adult planthoppers of brown planthopper (BPH) *Nilaparvata lugens* (Stål), smaller brown planthopper (SBPH) *Laodelphax striatellus* (Fallén), and white backed planthopper (WBPH) *Sogatella furcifera* (Horvath)

(Homoptera, Delphacidae) were studied by the cryostat microtome method in combination with the photomicrography. Light micrographs confirmed that there were no YLES in neither of cephalosome and baenosome of the adults of BPH, SBPH or WBPH. However, mass YLES and some mycetocytes were found existing in abdomen fat body of the adults of

these three species of rice planthoppers, and YLES reproduces by budding proliferation. There was obvious difference in morphological characteristics and species constitute of YLES in adult planthoppers among *N. lugens*, *L. striatellus* and *S. furcifera*. It was mainly fusiform (30.17%), bacilliform (53.15%) and oval (15.11%) forms to constitute the YLES in BPH,

Key words *Nilaparvata lugens*, *Laodelphax striatellus*, *Sogatella furcifera*, yeast-like endosymbiote, morphological characteristics.

and oval forms in SBPH (93.14%) and WBPH (94.17%). Moreover, the YLES in adults of BPH are all larger than those respective forms of YLES in adults of SBPH and WBPH, and there was some difference in YLES size between SBPH and WBPH for same type of YLES.