海湾扇贝衣原体样生物超微结构观察

刘英杰[1,2] 吴信忠[2] 王崇明[3] 潘金培[2]
(1. 中国水产科学院资源环境中心, 北京 100039; 2. 中国科学院南海海洋研究所, 广州 510301;
3. 中国水产科学院黄海水产研究所, 青岛 266003)

摘要: 对海湾扇贝生长不同阶段的样品进行了透射电镜观察, 在样品消化腺上皮细胞内发现类衣原体感染。该类衣原体具有原体、网状体和中间体等典型发育阶段, 原体个体小而致密, 网状体大而疏松, 以等体积分裂进行繁殖。在细胞质内形成单层膜包被的包涵体, 包涵体发育晚期破裂, 宿主细胞崩解, 衣原体逸出。衣原体感染可导致细胞核变形, 染色质凝聚, 内质网核糖体脱落, 线粒体内嵴消失。

关键词: 海湾扇贝; 类衣原体; 包涵体; 超微结构
中图分类号: S944.3 文献标识码: A 文章编号: 1000-3207(2003)05-0492-004

海湾扇贝是我国海水养殖的重要品种, 具有重要的经济价值。近年来, 在海湾扇贝养殖生产的不同阶段(幼体培育、稚贝中间暂养、海上成体养殖) 均出现过不同程度的死亡现象[1]。2000—2001 年在进行流行病学调查时, 通过取样透射电镜观察, 在消化腺细胞质内发现一种衣原体样生物, 其形态特点及发生过程与已报道的种类明显不同。

1 材料与方法

海湾扇贝幼体取山东省莱州市金城镇扇贝育苗场的育苗池及人工感染试验水槽, 稚贝取自金城养殖场中间暂养池, 成体取自金城养殖场。成体取样后用过滤海水冲洗干净, 用解剖剪和解剖镊取出扇贝的外套膜、鳃、消化腺、肾、肠等组织, 用刀片切成约 1mm^3 的小块, 稚贝将软体部整体取出, 幼体过滤收集。样品用 2.5% 的戊二醛磷酸缓冲液(pH: 7.2) 前固定(4℃保存), 1% 的锇酸溶液后固定(4℃, 1h), 系列乙醇脱水, Epon-812 包埋。经 LKB 超薄切片机切片, 醋酸铀和柠檬酸铅双重染色, 日立 H-7000 型投射电镜观察并拍照。

2 结果

对不同时期样品的不同组织进行透射电镜观察, 在幼体、稚贝和成贝的消化腺上皮细胞内发现了原核生物。该原核生物具有大小不同的三种个体形态: 大个体呈圆形、椭圆形或不规则形状, 大小为 890.5±164.6×623.6±129.3nm(n=20)。细胞外有完整的包膜, 外层为细胞壁, 内层为细胞膜。细胞膜内周边区域为高电子密度的核糖体样颗粒, 细胞中央区域空间较大, 电子密度较低, 具有丝状结构(衣原体的 DNA) 并与细胞的胞浆膜连接, 中心区为拟核(图版Ⅰ: 1, 2, 3)。小个体呈较为整齐的短棒状或纺锤形, 大小为 317.5±40.1×180.3±40.0nm(n=18)。细胞外具两层细胞壁和一层细胞膜, 细胞中央区域致密, 在中央区和细胞膜之间有明显的空白区域(图版Ⅰ: 1, 2, 4)。另一种个体数量较少, 大小介于上述两种个体之间, 可能为二者的过渡阶段(图版Ⅰ: 1)。在上述三种个体之间, 还散布有一些小的空泡, 其内部无具体结构(图版Ⅰ: 3)。根据该原核生物细胞质内寄生、具有明显大小不同的个体及不同发育阶段等特点, 判断应与衣原体接近, 由于具体分类地位未定, 暂称为衣原体样生物(Chlamydia-like organism, CLO)。大个体应为其始体或网状体(Reticular body), 小个体为原体(Elementary body), 另一种类型为中间体(Intermediate body)。网状体为营养体阶段, 以二分裂或复分裂进行增殖(图版Ⅰ: 2, 3)。原体由于个体小, 细胞壁厚, 应具有体外存活的能力和侵染能力。

收稿日期: 2002-07-12; 修订日期: 2003-03-21
基金项目: 国家重点基础研究"973"专项经费资助(G1999012001)
作者简介: 刘英杰(1963—) 男, 河北省涿州市人; 博士; 从事海洋生物病害学研究。本工作得到了青岛海洋大学测试中心姜明、中国科学院海洋研究所谢嘉琳、国家海洋局第一研究所王文兴和军事医学科学院微生物流行病研究所陈万荣等教授的大力支持和帮助, 特此致谢!

该衣原体样原核生物(CLO)在消化腺上皮细胞的细胞质内形成包涵体(图版Ⅰ:5),包涵体呈圆形或椭圆形,大小约为9×7μm。包涵体有一单层膜包被。由于包涵体内CLO的增殖,其体积不断扩大,占据细胞内大部分空间,细胞器被挤向细胞一侧或四周(图版Ⅰ:2,3,5)。CLO发育中早期,其个体包围于包涵体内,对线粒体、内质网等细胞器损伤不大,对细胞核可造成一定损伤,使之变形,形成异染色质区域。CLO发育晚期,包涵体破裂,CLO逸出,部分线粒体内嵴消失,粗面内质网核糖体脱落,宿主细胞内部结构紊乱,处于崩解状态(图版Ⅰ:6)。

电镜检查人工感染海湾扇贝幼体试验样品时,还观察到了CLO侵入幼体的过程。细胞对CLO的侵入产生明显的反应,细胞膜向内弯曲、凹陷,此时为侵入过程的早期阶段。

3 讨论

衣原体是一类专性细胞内寄生、可对宿主细胞造成一定损害的微生物,通常具有大小两种个体。小的个体为原体,具细胞外生存能力,侵染性。大的个体为始体或网状体,是衣原体的繁殖阶段,不具感染性。原体侵入细胞后,被吞噬溶酶体吞噬,形成吞噬体(Phagosome)。原体在吞噬体内发育成网状体,网状体不断进行二分裂或等体积分裂,形成多个个体,这些个体最后发育成具有感染性的原体,随着包涵体和宿主细胞的破裂,被释放出来[2]。本文报道的原核生物具有原体、网状体和中间体等不同发育阶段以及不同发育时期的包涵体,这些方面均体现了衣原体的典型特点。有关海洋贝类衣原体的感染已有一些文献报道,Harshbarger[3]等首次描述了美国Chesapeake湾硬壳蛤*Mercenaria mercenaria*体内类衣原体(CLO)的轻微感染,随后Meyers[4,5]以及Otto等[6]也证实了这一结果。Cajaraville[7]报道了西班牙Basque沿海贻贝*Mytilus gallprovincialis*消化管上皮细胞类衣原体的寄生并发现其具有原体、网状体和中间体等不同阶段。Morrison等[8]和Leibovitz[9]分别报道了海湾扇贝*Argopecten irridians*成体与幼体的类衣原体感染,但其形态特征与本文报道的明显不一样。国内也曾有过这方面的初步报道[10,11],但未进行进一步的系统的描述。本文在国内首次系统地报道了海湾扇贝类衣原体(Chlamydiar like Organism,CLO)的形态发生、人工感染时对宿主细胞的吸附、侵入等过程以及其包涵体发育的不同阶段,为今后这方面的研究提供了较为可靠的参考依据。

一般认为,衣原体侵入体内后经36—48h完成其发育过程,最后宿主细胞破裂,衣原体释放到外界[2]。类衣原体感染贝类后其发育周期是否与此相近尚不清楚,有待于进一步的观察。在海湾扇贝幼体、稚贝和成贝体内均发现有类衣原体的感染,本文仅限于其超微形态学方面的描述,今后有必要对其不同阶段的致病性、血清型以及核酸等方面进行深一步的研究。

参考文献:

[1] Guan H S. Research on Immunology, Cell Culture and Disease in Maricultural Animals[M]. Jinan: Shandong Science and Technology Press, 1991, 114—130. [管华诗. 海水养殖动物的免疫、细胞培养和病害研究. 济南: 山东科技出版社, 1999, 114—130]

[2] Wei X. Medical Rickettsia[M]. Shanghai: Shanghai Science and Technology Press, 1983, 380—387. [魏曦. 医用立克次体学. 上海: 上海科学技术出版社, 1983, 380—387]

[3] Harshbarger J C, Chang S C, Otto S V. Chlamdiae (with phage), mycoplasmas, and rickettsiae in Chesapeake Bay bivalves[J]. *Science*, 1977, **196**: 666—668

[4] Meyers T R. Preliminary studies on a chlamydial agent in the digestive diverticular epithelium of hard clams *Mercenaria mercenaria* (L.) from Great South Bay, New York[J]. *Journal of Fish Diseases*, 1979, **2**: 179—189

[5] Meyers T R. Endemic diseases of cultured shellfish of Long Island, New York: adult and juvenile American oysters *(Crassostrea virginica)* and hard clams *(Mercenaria mercenaria)*[J]. *Aquaculture*, 1981, **22**: 305—330

[6] Otto S V, Harshbarger J C, Chang S C. Status of selected unicellular eucaryote pathogens, and prevalence and histopathology of inclusions containing obligate pocaryote parasites, in commercial bivalve mollusks from Maryland estuaries[J]. *Haliotis*, 1979, **8**: 285—295

[7] Cajaraville M P, Angulo E. Chlamydia-like organisms in digestive and duct cells of mussels from the Basque Coast[J]. *Journal of Invertebrate Pathology*, 1991, **58**: 381—386

[8] Morrison C, Shum G. Chlamydia-like organisms in the digestive diverticular of the bay scallop, *Argopecten irridians* (Lmk)[J]. *Journal of Fish Diseases*, 1982, **5**: 173—184

[9] Leibovitz L. Chlamydiosis: a newly reported serious disease of larva and postmetamorphic bay scallop, *Argopecten irridians* (Lamarck)[J]. *Journal of Fish Diseases*, 1989, **12**: 126—136

[10] Wang W X, Luo W T, Xue Q G, et al. Pathological research on Chlamydia-like organisms in the hepatopancreatic gland of the bay scallop Argopecten irridians (Lmk)[J]. *Marine Science*, 1998, (3): 23—25. [王文兴, 罗挽涛, 薛清刚, 等. 海湾扇贝消化盲囊衣原体样生物的病理学研究. 海洋科学, 1998, **3**: 23—25]

[11] Wang Y T, Xiang J H. Studies on causation of the mass mortality of *Chlamys farreri*[J]. *Oceanol et Limnol Sin*, 1999, **30**(6): 770—774. [王运涛, 相建海. 栉孔扇贝大规模死亡的原因探讨. 海洋与湖沼, 1999, **30**(6): 770—774]

CHLAMYDIA-LIKE ORGANISMS IN THE DIGESTIVE DIVERTICULAR OF CULTURED BAY SCALLOP *ARGOPECTEN IRRIDIANS* LAMARCK

LIU Ying-Jie[1,2], WU Xing-Zhong[2], WANG Chong-Ming[3] and PAN Jin-Pei[2]

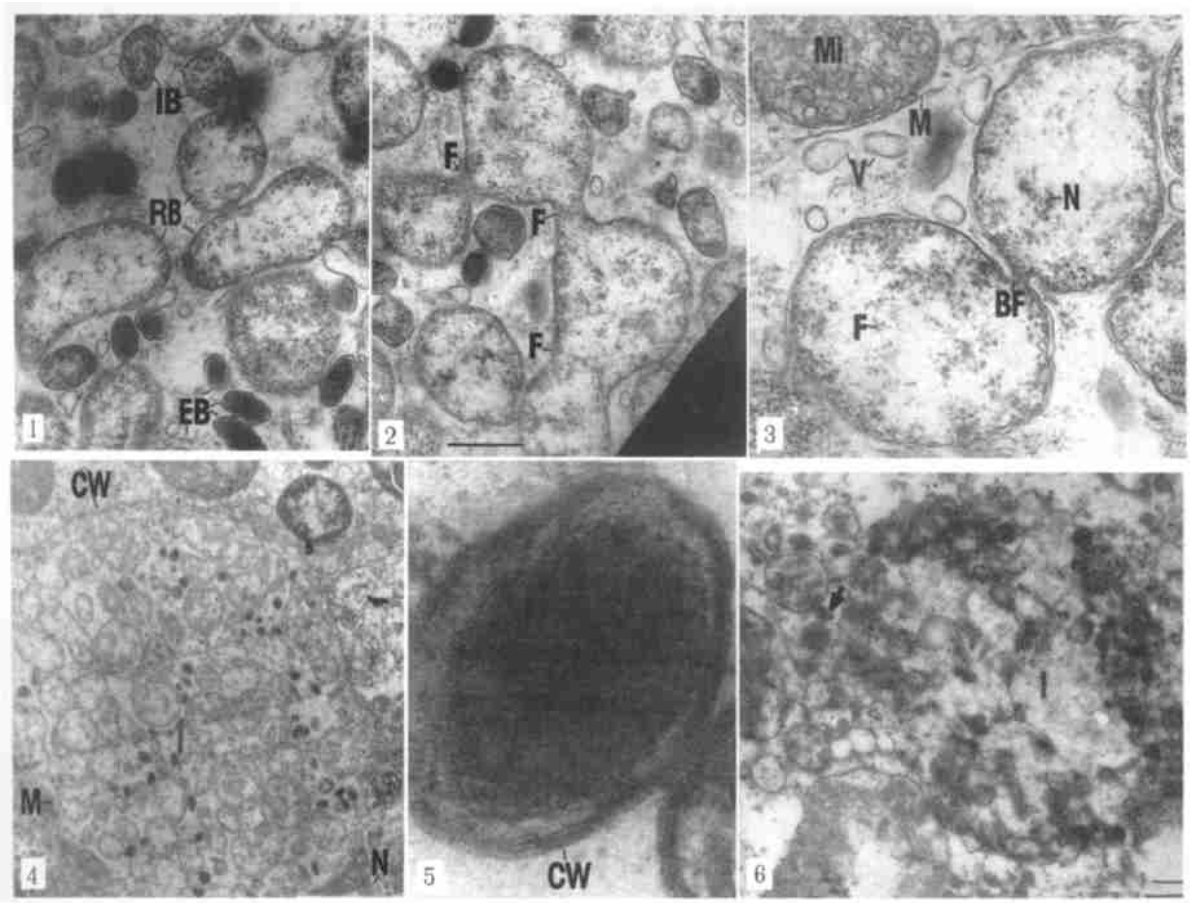
(1. *Center of Resource and Environment, China Academy of Fisheries Science, Beijing* 100039; 2. *South China Sea Institute of Oceanology The Chinese Academy of Science, Guanzhou* 510301; 3. *Yellow Sea Fisheries Research Institute, The Chinese Academy of Fisheries Science. Qingdao* 266003)

Abstract: The bay scallop *Argopecten irridians* was introduced into China in 1982 and has been the important cultured scallop from then. The epizootiological investigation was carried out from November, 1999 to April of 2001 and a chlamydiar-like organism was found in the epithelium of digestive diverticular. Samples of *A. irridians* in different stage were collected from Jincheng culture facility on Laizhou Bay, Laizhou County, Shandong, China. The sampling interval was 1 month. For light microscopy, the tissues including mantle, gill, digestive gland, intestine and kidney were excised and fixed with Bouin's fixative. All tissues were dehydrated through an ascending ethanol series, embedded in paraffin, sectioned at 5μm and stained with haematoxylin and eosin (H&E). For electron microscopy, fresh tissues were cut at 0.5—1mm^3, fixed in 2.5% glutaradehyde in phosphate buffer system (pH7.4) at 4℃ and post fixed in 1% Osmium tetraoxide for 1 hour, dehydrated in an ethanol series, embedded in Eporr-812. Ultrathin section were cut on LKB ultramicrotome, stained with uranyl acetate and lead citrate and examined with H-7000 transmission electron microscope at 75kV.

The light examination on histological sections of different tissues showed that basophilic intracellular inclusions were only present in the epithelium of the digestive diverticular. The inclusions take the shape of round or ellipsoid with the size of 9μm × 7μm. TEM examination of ultrathin section of tissues revealed that the intracellular basophilic inclusions were the clone of prokaryotes, which was membrane limited in vacuoles. The prokaryote was of three morphologies of different sizes and structures. The larger body was reticulate body which was round, ellipsoid or irregular shaped, and the size was 890.5(±164.6) nm × 623.6(±129.3) nm(n=20). The double membrane-bound structure can be clearly seen. The outer membrane was rippled with a slime layer around it. In the central area it was electrorr-lucent with fine filament structure, in the peripheral area it was ribosome-like granular electrorr-dense material. The binary fission can be observed. The smaller body was elementary body that was short-rod or spindle shaped with the size of 317.5 (±40.1) nm × 180.3(±40.0) nm(n=18). The cell wall of double trilamina structure was very clear. The central area was electrorr-dense. There was an evident space area between cell wall and central area. The middle-sized body was intermediate body, the size of which was between that of reticulate body and elementary body, the cell wall was similar to that of elementary body and the inner structure was similar to that of reticulate body. Intermediate body may be the transitional stage from reticulate body to elementary body. Besides three morphologies, there were also some small no structure blebs in the vacuole. The host cell response was not evident besides of hypertrophy. With the vacuole growing up, it took up the most space of host cell and the organelles were pressed to the side. The characteristics of the prokaryotes revealed that it should be categorize into chlamydiar-like organism (CLO) and the size and morphology suggested that it was not the same species as reported in other marine bivalves.

The present study was the first report of chlamydiar-like organism in cultured bay scallop *A. irridians* in China. Although there was not obvious observed damages at present, the potential effect of chlamydiar-like organism on the host need to be paid more attention to because of the importance of bay scallop culture to Chinese mariculture.

Key words: *Argopecten irridians* Lamarck; Chlamydiar-like organism (CLO); Inclusion; Ultrastructure



图版 I

1. 海湾扇贝衣原体样生物(CLO)透射电镜照片, 示原体(EB)、网状体(RB)和中间体(IB), Bar= 400nm; 2. CLO 电镜照片示网状体复分裂(F)情况, Bar= 400nm; 3. CLO 网状体的拟核(N)、丝状结构(F)、二分裂(BF)及包涵体内的空泡结构(V), M: 包涵体膜, Mi: 宿主细胞的线粒体, Bar= 280nm; 4. CLO 电镜照片示原体形态及细胞壁(CW)结构, Bar= 55nm; 5. 海湾扇贝衣原体样生物(CLO)透射电镜照片, 示包涵体(I)、包涵体膜(M)、宿主细胞细胞核(N)及细胞壁(CW), Bar= 1.5μm; 6. 电镜照片示发育晚期的包涵体(I), 原体散落(箭头), 宿主细胞处于崩解状态, Bar= 0.8μm

1. Transmission electron microscopy of Chlamydiar like organism in *Argopecten irridians* showing elementary body (EB), reticulate body (RB) and intermediate body (IB). Bar= 400nm; 2. Electron microscopy of CLO showing the multiple fission (F) of reticular body. Bar= 400nm; 3. Electron microscopy of CLO showing the nucleiod (N), filament structure(F), binary fission (BF) of reticulate body and the vesicle structure(V). M: membrane of CLO inclusion, Mi: mitochondrion of host cell. Bar= 280nm; 4. Higher magnification of CLO showing the cell structure and cell wall (CW) of elementary body, Bar= 55nm; 5. Transmission electron micrograph of CLO in *Argopecten irridians* showing inclusion (I), membrane (M) of inclusion, nucleus (N) and cell wall (CW) of host cell, Bar= 1.5μm; 6. Electron micrograph of CLO inclusion in late development stage show the scattered elementary body and the collapsed host cell, Bar= 0.8μm