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First Record of Mycoplasma-like Organism in Pacific Oyster (*Crassostrea gigas*) in Korea

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During routine survey of Pacific oyster (*Crassostrea gigas*) collected from Tongyoung area in southern coast of Korea, histological examination revealed that a intracellular microorganisms infected the digestive gland of the oyster. They infected hepatopancreatic cells extensively. The size of intracellular microorganism was of 45 to 86 nm in diameter and 200 nm to more than 500 nm in length. They were pleomorphic. The morphological characteristic of intracellular microorganisms lacked cell wall and was bounded by the plasma membrane. They contained typical prokaryotic ribosomes and fibrillar DNA-like strands. No additional internal structure has been observed. Based on the lack of cell wall and the cellular localization, the intracellular microorganism is considered as a Mycoplasma-like organism.

Key word: Pacific oyster, *Crassostrea gigas*, Intracellular bacteria, Mycoplasma-like organism

Introduction

Mycoplasmas are very small prokaryotic parasites totally devoid of a cell wall, and are bounded only by a plasma membrane (Razin and Freundt, 1983). Some Mycoplasmas have been reported in marine invertebrates. Harshbarger et al. (1977) first reported a Mycoplasma-like organism (MLO) within the epithelial cells of gut of the American oyster (*Crassostrea virginica*). A filamentous MLO was reported in the hepatopancreatic cells of shrimps, *Litopenaeus vannamei* (Krol et al., 1991) and *Penaeus japonicus* (Choi et al., 1996). And a new type of Mycoplasma was reported from *Urastoma cyprinae* (Comps and Tigé, 1999).

Tongyoung (previously known as Chungmu) is an important area for oyster culture production in Korea. The annual production of Pacific oyster (*Crassostrea gigas*) from this area is about 40% of total oyster production in Korea. During the disease monitoring of *C. gigas* from this area, we found a MLO in the digestive gland of Pacific oyster (*C. gigas*) by the histological examinations. We report herein for the first time the morphological features of the MLO found in the oyster, *C. gigas* cultured in Tongyoung area.

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Material and Methods

Pacific oyster (*C. gigas*) 1 to 2 years old, were collected from Tongyoung area (southern coast of Korea) in 2002. Specimens were removed from the shell and sectioned sagittally; then half of each specimen was fixed in Davidson's fixative for light microscopy and the other half in Carson's fixative for transmission electron microscopy. After 24 hr fixation in Davidson's fixative, samples were dehydrated using an ascending ethanol series, cleared in xylene and infiltrated with paraffin and sectioned at 4 μm thickness. Sections were stained with hematoxylin and eosin. For transmission electron microscopy, hepatopancreatic tissues previously fixed with Carson's fixative were transferred in cold 2.5% glutaraldehyde in 0.2 M cacodylate buffer at pH 7.2 for 1 hr. The tissues were rinsed for 48 hr in 0.2 M cacodylate buffer at 4℃ before fixation in glutaraldehyde. Then samples were post-fixed in 1% osmium tetroxide in the same buffer at 4℃ and embedded in Epon resin. Sections were stained in 5% uranyl acetate in 50% ethanol followed by lead citrate. They were examined in a JEOL 1200 EX-2 transmission electron microscope at 80 kV.

Results

Sections of MLO parasitizing hepatopancreatic tubules of the oyster showed normal morphology commonly found in *C. gigas* (Fig. 1). During careful observation of the tissues with the light microscopy, we could not find the presence of MLO. MLOs were observed clearly within hepatopancreatic epithelial cells of *C. gigas* by transmission electronmicroscopy (Fig. 2). Appeared intracellular microorganisms were highly pleomorphic as obligate intracellular parasite. The shape of MLOs wasa occurred as short-rods, with 45 to 86 nm in diameter and from 200 to more than 500 nm in length. Other type of MLOs, round structure, of 70 to 120 nm diameter also was observed (Fig. 3). Occasionally, typical MLOs containing spherical dilations were observed (Fig. 4). These microorganisms were only bounded by a plasma membrane (Fig. 5). They contained typical prokaryotic ribosomes and fibrillar DNA-like strands. No additional internal structure was observed. These prokaryotic organisms were found

Fig. 1. Section through digestive gland of *Crassostrea gigas*. Hematoxylin-eosin. Scale bar = 1 μm.

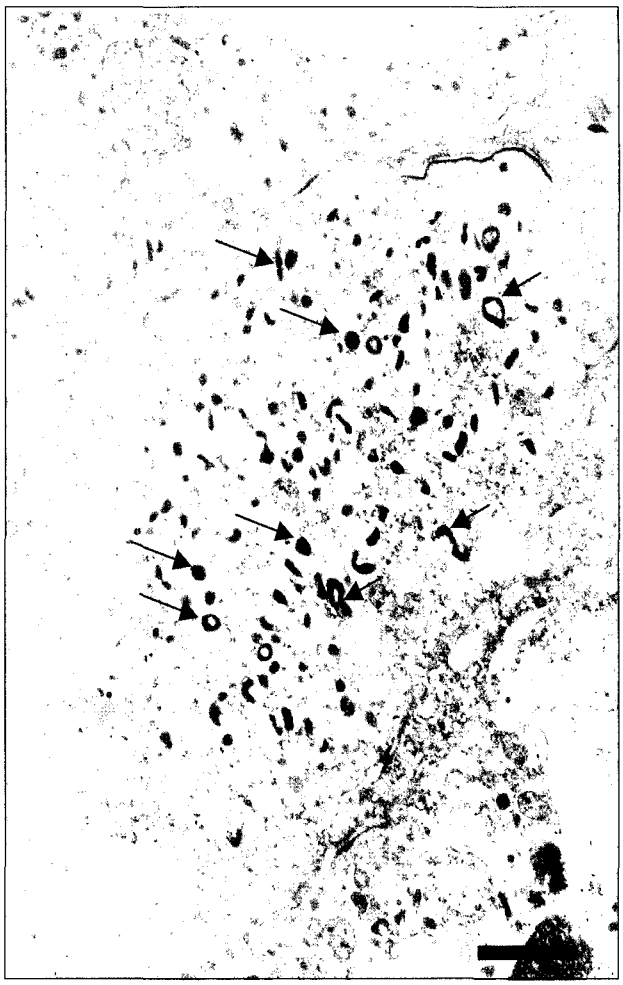

Fig. 2. MLO in hepatopancreatic cell of *Crassostrea gigas*. The MLOs (arrows) were found free in the cytoplasm. Scale bar = 1 μm.

in the cytoplasm of infected cells. In heavily infected cells, only mitochondria and ribosomes were the organelles that appeared normally distributed in the cytoplasm (Fig. 6). Although different levels of the MLO infection were found in the oyster, often nearly all hepatopancreatic cells of the diseased oyster were infected with the MLO. It was not observed any host response in affected areas.

Discussion

Mollicutes have no intracellular membrane or membrane-bound structure and thus they can be described as living membrane vesicles (Davis and Lee, 1982; Razin and Freundt, 1983). Thus demonstration of single membrane in properly fixed and

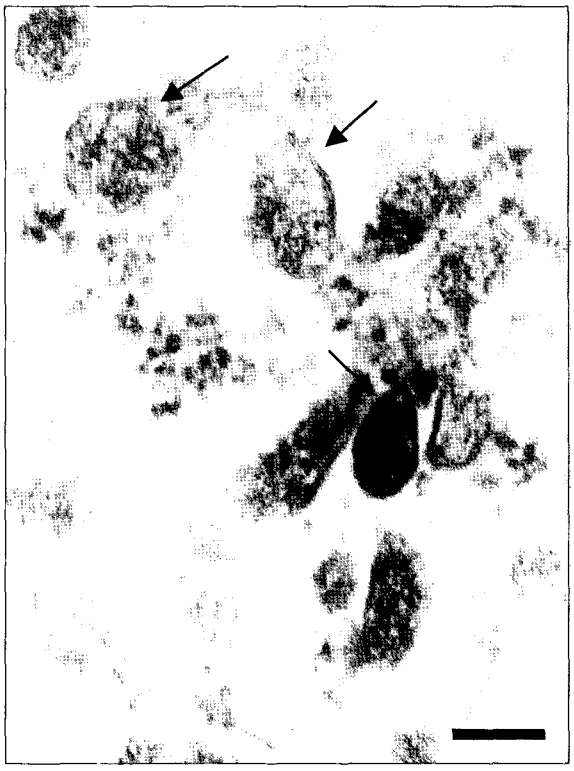

Fig. 3. Electron micrograph of round MLOs. Scale bar = 100 nm.

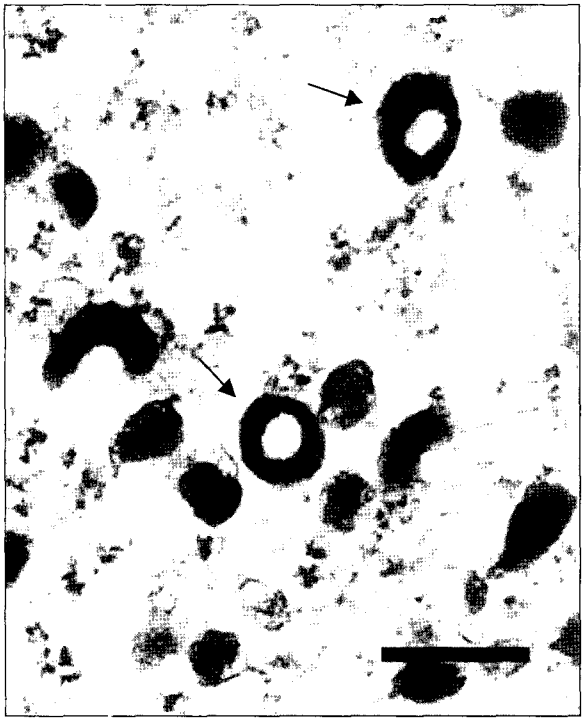

Fig. 4. Electron micrograph of MLOs with spherical dilations (arrows). Scale bar = 250 nm.

Fig. 5. Electron micrograph of a MLO showing fine structure of the cell membrane. Scale bar = 50 nm.

sectioned cells is an essential requirement for defining a new isolate as a Mycoplasma. The intracellular microorganism in hepatopancreatic cells of oyster (*C. gigas*) lacks a cell wall and is bounded only by the plasma membrane which suggest that these microorganisms are Mycoplasma. These microorganisms occur free in the cytoplasm of digestive gland which distinguishes them from the small intracellular rickettsia-like bacteria of oyster (Renault and Cochennec, 1994) that typically occur in membrane-bound cytoplasmic inclusions in infected cells. The intracellular microorganism was a highly pleomorphic. Theses facts are indicative of mollicutes. We suggest that these microorganisms are MLOs.

Filamentous mollicutes have been reported in shrimps, *L. vannamei* cultured in Texas (Krol et al., 1991) and *P. japonicus* (Choi et al., 1996). Structure of these mollicutes was filamentous and branched with terminal blebs. And MLO from *U. cyprinae* was rod shaped (Comps and Tigé, 1999), but the MLO in this study was short rod or round type. Cell shape of mycoplasmas appears to depend upon the nutritional qualities and the osmotic pressure of

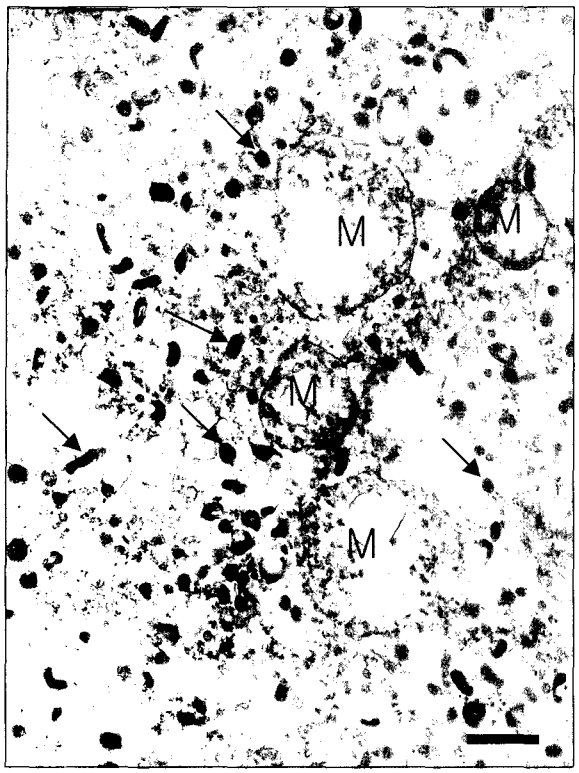

Fig. 6. Electron micrograph of MLOs (arrows) associated with mitochondrias (M). Scale bar = 100 nm.

the growth medium as well as the growth phase of the culture. Filamentous growth is usually associated with young logarithmic cultures growth under optimum conditions (Razin and Freundt, 1983). However, the filamentous phase is transitory and the filaments transform into chains of cocci which later break (Razin and Freundt, 1983). The MLO in oyster was often associated with mitochondria. It explains why the mycoplasmas have limited biosynthetic abilities, probably reflecting their small genome and parasitic mode of life (Razin and Freundt, 1983).

Mycoplasmas have been reported in a range of marine animals (Tully and Whitcomb, 1979; Lauckner, 1983; Sparks, 1985). Among them, some MLOs were described associated with mortalities of different bivalves (Azevedo, 1993; Bower and Meyer, 1991, 1995; Hine and Diggles, 2002). There was no mortality despite of high prevalence of MLO in *C. gigas* from Tongyoung in 2002; therefore the virulence of the MLO in *C. gigas* remains uncertain. It is obvious that more experimental date is needed to determine the exact relationship between the MLO and the oyster.

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References

Azevedo, C. 1993. Occurrence of an unusual branchial mycoplasma-like infection in cockle *Cerastoderma edule* (Mollusca, Bivalvia). Dis. Aqua. Org.. 16, 55-59.

Bower, S.M. and G. Meyer. 1991. Diseases of Japanese scallops (*Patinopecten yessoensis*) caused by an intracellular bacterium. J. Shellfish Res., 10, 531.

Bower, S.M. and G. Meyer. 1995. Causes of mortalities among cultured Japanese scallops (*Patinopecten yessoensis*) in British Columbia. J. Shellfish Res., 14, 227.

Choi, D.L., S.G. Sohn, M.A. Park, M.S. Heo and T. Renault. 1996. Detection of a mollicute-like organism in kuruma shrimp, *Penaeus japonicus*. J. Fish Pathol., 9, 33-40.

Comps, M. and G. Tigé. 1999. Procaryotic infections in the mussel *Mytilus galloprovinciallis* and its parasite the turbellarian *Urastoma cyprinae*. Dis. Aquat. Org., 38, 211-217.

Davis, R.E. and I.M. Lee. 1982. Pathogenicity of Spiroplasmas, Mycoplasma-like organisms, and vascular-limited fastidious walled bacteria. In: Phytopathogenic Prokaryotes, Vol. 1. Academic Press, NY, pp. 499-504.

Harshbarger, J.C., S.C. Chang and S.V. Otto. 1977. Chlamydiae (with phages), mycoplasmas, and rickettsiae in Chesapeake Bay bivalves. Science, 196, 666-668.

Hine, P.M. and B.K. Diggles. 2002. Prokaryote infections in New Zealand scallops *Pecten novaezealandiae* and *Chlamys delicatula*. Dis. Aquat. Org., 50, 145-152.

Krol, R.M., W.E. Hawkins and R.M. Overstreet. 1991. Rickettsial and mollicute infections in hepatopancreatic cells of cultured Pacific white shrimp (*Penaeus vannamei*). J. Invertebr. Pathol., 57, 362-370.

Lauckner, G. 1983. Diseases of Mollusca: Bivalvia. In: Diseases of Marine Animals. Vol. II, O. Kinne ed. Biologische Anstalt Helgoland, Hamburg, pp. 477-961.

Razin, S. and E.A. Freundt. 1983. Class Mollicutes. In: Bergey's manual of systematic bacteriology, Vol. 1. Williams & Wilkins, Baltimore, MD, pp. 740-757.

Renault, T. and N. Cochennec. 1994. Rickettsia-like organisms in the cytoplasm of gill epithelial cells of the Pacific oyster *Crassostrea gigas*. J. Invertebr. Pathol. 64, 160-162.

Sparks, A.K. 1985. Rickettsiae, Clamydiae, and mycoplasmas of invertebrate. In: Synopsis of Invertebrate Pathology. A.K. Sparks ed. Elsevier, Amsterdam, pp. 165-180.

Tully, J.G. and R.F. Whitcomb. 1979. Human and animal mycoplasmas. In: The Mycoplasmas, Vol. II. J.G. Tully and R.F. Whitcomb, eds. Academic Press, NY

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