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Short communication

Bacterial pathogens and flora isolated from farm-cultured eels (*Anguilla japonica*) and their environmental waters in Korean eel farms

Seong-Joon Joh [a,*], Eun-Hye Ahn [b], Hye-Jin Lee [a], Gee-Wook Shin [c], Jun-Hun Kwon [a], Choi-Gyu Park [d]

[a] *Animal, Plant and Fisheries Quarantine and Inspection Agency, Republic of Korea*
[b] *Komipharm International Co. Ltd., Republic of Korea*
[c] *College of Veterinary Medicine, Chonbuk National University, Republic of Korea*
[d] *College of Veterinary Medicine, Kyungpook National University, Republic of Korea*

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ABSTRACT

The purpose of this study was to investigate bacterial pathogens and flora in both sick and clinically healthy eels, *Anguilla japonica*, and the environmental rearing waters of Korean eel farms. Between 2003 and 2010, a total of 621 sick eels were submitted for diagnosis, while 216 healthy eels and 87 environmental water samples were collected during a survey of 26 eel farms in Korea. Seven different bacterial species were obtained from 183 isolates, which were recovered from the internal organs of the 621 sick eels. The most frequently isolated bacterium was *Edwardsiella tarda* (71.0%), followed by *Aeromonas hydrophila* (9.3%), *Citrobacter freundii* (7.7%), *Aeromonas veronii* (6.0%), *Listonella anguillarum* (2.7%), *Plesiomonas shigelloides* (2.2%), and *Pseudomonas anguilliseptica* (1.1%). From the eel and water samples of the survey, a total of 472 isolates from 34 different species belonging to 15 genera of bacteria were isolated. The most prevalent genus of bacteria was *Aeromonas* spp. (141/472, 29.8%). Among the 34 types of bacterial species, *C. freundii* (20.1%) and *A. hydrophila* (19.9%) were the most frequently isolated. The results of this study indicate that a wide range of bacterial species, which can act as primary or opportunistic pathogens, may be recovered from clinically healthy eels and rearing waters. This study provides baseline information about bacterial pathogens and floral contamination for the control and treatment of bacteria in Korean eel farms.

1. Introduction

Eels are one of the most important freshwater fish in the aquaculture industry in the Republic of Korea (referred to as Korea hereafter), with Japanese eel, *Anguilla japonica*, being the main culture species. The recent development of this industry has led to a significant growth in domestic demand for edible eels. According to the Fishery Products Statistics System of Korea, eel production in Korea increased from 5775 tons in 2005 to 10,557 tons in 2007; however, there has been a subsequent decline and stabilization at 6500–8000 tons since 2008 (Standard Manual of Eel [*A. japonica*] Aquaculture by the National Fisheries Research and Development Institute in the Republic of Korea, 2009, http://portal.nfrdi.re.kr/upload/farm/farm_04.pdf). One factor that might influence variation in eel production might be the mass death of eels as a result of disease.

Disease is a major cause of economic loss in the aquaculture industry. In particular, bacterial diseases are the most frequent and major cause of mass death in fish.

* Corresponding author at: Laboratory of Aquatic Animal Diseases, Department of Animal Health, Animal, Plant and Fisheries Quarantine and Inspection Agency, 175 Jungang-Ro, Manan-Gu, Anyang-Si, Kyonggi-Do 430-757, Republic of Korea. Tel.: +82 31 467 1805, fax: +82 31 467 1814; mobile: +82 010 3955 9739.
E-mail address: johsj0901@korea.kr (S.-J. Joh).

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Important bacteria that cause diseases in anguilliculture include *Edwardsiella tarda* (Wakabayashi and Egusa, 1973; Hah et al., 1984; Chen and Kou, 1992; Joh et al., 2011), *Listonella anguillarum* (Austin and Austin, 1999), *Vibrio vulnificus* serovar E (Hoi et al., 1998; Austin and Austin, 1999), *Aeromonas hydrophila* (Hah et al., 1984; Yoo et al., 1990), *Pseudomonas anguilliseptica* (Wakabayashi and Egusa, 1972; Michel et al., 1992; Haenen and Dvvidse, 2001; Berthe et al., 1995). There have been several reports of bacterial infections in eels in Korea that were caused by *E. tarda*, *A. hydrophila*, and *Vibrio* spp. (Hah et al., 1984; Yoo et al., 1990; Kim et al., 2011; Joh et al., 2011).

Prevention is the best treatment for disease, with a combination of good husbandry and management techniques ensuring that stocks remain relatively disease-free. Like most fish, eels are particularly susceptible to infection at times of high stress. Since the health of fish is mostly dependent on water quality, this parameter should be maintained at a certain level to relieve stress. Although the bacterial load in water *per se* does not present a significant health hazard, the presence of some obligate pathogens, such as *Aeromonas salmonicida*, in water is an indication of the presence of fish disease on farms (Caldreich and Clarke, 1966). The contamination of fishes by fecal coliforms in polluted waters is also a concern in fisheries (Caldreich and Clarke, 1966; Buller, 2004). To establish control and prevention measures against bacterial diseases in eel culture farms, basic information about bacterial pathogens and floral contamination in eels and their environment is needed. However, there are limited studies on bacterial pathogens and flora in eels, such as *A. japonica*, and the environmental waters of eel farms in Korea. Therefore, in this study, we aimed to investigate bacterial pathogens and flora in both sick and healthy eels (*A. japonica*) and the environmental waters inhabited by eels at eel farms in Korea.

2. Materials and methods

2.1. Fish and environmental water samples

During 2003–2010, a total of 117 cases (621 eels) were submitted to the diagnostic laboratory of the Quarantine and Inspection Agency from 26 eel farms located in the Jeonnam and Jeonbuk provinces of Korea. All eel samples were packaged in vinyl containing cool water with saturated oxygen and were delivered to the laboratory within 24 h, as described by Whitman (2004). A separate random survey of eels was also performed to evaluate the health status of eels at 21 eel farms during 2009–2010. Each farm was visited twice during different seasons, and 2 to 3 eel samples were collected from 2 or 3 ponds at each farm. All samples were stocked in a box containing ice and were transferred to the laboratory within 5 or 6 h. In total, 216 eels (22 cases) were autopsied, and the internal organs were examined for bacterial growth (Table 1). In addition, we conducted a bacterial examination of 87 environmental water samples of eel habitats, including underground water, reservoir tank water, and rearing water.

Table 1
Number of cases reported and eel samples collected for pathological diagnosis through survey during 2003–2010.

Year	Area (province)	Number of cases (eel samples)	
		Pathological diagnosis	Survey
2003	Jeonnam	7 (26)	–
	Jeonbuk	21 (95)	–
2004	Jeonnam	12 (64)	–
	Jeonbuk	32 (199)	–
2005	Jeonnam	4 (41)	–
	Jeonbuk	11 (67)	–
2006	Jeonnam	13 (67)	–
	Jeonbuk	9 (49)	–
2007	Jeonnam	3 (23)	–
	Jeonbuk	–	–
2008	Jeonnam	2 (4)	–
	Jeonbuk	–	–
2009	Jeonnam	–	–
	Jeonbuk	1 (2)	3 (27)
2010	Jeonnam	2 (4)	14 (155)
	Jeonbuk	–	5 (34)
Total		117 (621)	22 (216)[a]

[a] Eighty-seven environmental samples (including underground water, reservoir tank water, and rearing water) examined in this survey were excluded.

2.2. Isolation of bacteria

All eels submitted for diagnosis were autopsied, and any organs exhibiting pathological gross lesions and ascites samples were subjected to routine microbiological examination. For the survey, 4 types of internal organs were obtained from each eel (specifically, the gills, liver, kidney, and spleen) and were examined for bacterial growth. All samples of internal organs and ascites obtained aseptically were directly streaked onto blood agar plates (CoMed Co., Korea). One liter of each environmental water sample (specifically, underground water, reservoir water, and rearing water) was collected aseptically from each eel farm. Then, each water sample was passed through a membrane filter system with a pore size of 0.45 μm (Whatman, USA). Next, the filtrate on the membrane was transferred to blood agar plates. The plates were incubated at 28 °C (similar to field conditions) for 24–48 h, similar to that described by Whitman (2004). Each single representative colony was collected and sub-cultured for identification. The reference strains used in this study were as follows: *Edwardsiella* sp. (ATCC 23685, 15947, 33379, 33828, and 33202), *Aeromonas* sp. (ATCC 51108, 33658, 7966, and 35623), *Citrobacter freundii* (ATCC 8090), *Lactococcus* sp. (ATCC 700018 and 49156), *L. anguillarum* (ATCC 43308 and KCTC 2711), *Pseudomonas anguiliseptica* (ATCC 33660), *Streptococcus iniae* (ATCC 29177), *Tenacibaculum maritimum* (ATCC 43397), *Yersinia ruckeri* (ATCC 29473), *Vibrio alginolyticus* (ATCC 17750), *Vibrio harveyi* (ATCC 35084), *Vibrio ichthyoenteri* (ATCC 700023), and *Photobacterium damselae* (ATCC 33539 and 51736).

2.3. Identification of bacterial isolates

Standard morphological and biochemical tests were performed for the preliminary identification of each bacterial isolate. To confirm the identification of each