日本および米国株Photobacterium damselae subsp. piscicida由来プラスミドDNAの構造比較解析

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Short communication

Comparative Analysis of Plasmid DNAs from Two Strains of *Photobacterium damselae* subsp. *piscicida* Isolated from Japan and the United States

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ABSTRACT—Pseudotuberculosis pathogen, *Photobacterium damselae* subsp. *piscicida* (*Pdp*) shows some differences in virulence between Japanese and US strains. In this study, we analyzed plasmid DNAs derived from Japanese and US strains of *Pdp*, and found two homologous regions in these plasmids, pOT-51443-4/p91-197-1 and pOT-51443-1/p91-197-2. All genes in the p91-197-1 from US strain were observed in the pOT-51443-4 from Japanese strain. This event might have occurred during transmission of the US plasmid to Japanese strain. Inhibitor of vertebrate lysozyme (*ivy*), a known pathogenicity factor was encoded in the homologous region of pOT-51443-1/p91-197-2. Thus, the genomic region containing *ivy* may have been horizontally transmitted between the two *Pdp* strains.

Key words: *Photobacterium damselae* subsp. *piscicida*, plasmid, inhibitor of vertebrate lysozyme (*ivy*)

Among fish species, there is more yellowtail (*Seriola quinqueradiata*) produced by aquaculture in Japan than any other species. Export of yellowtail, especially to the United States, has increased in recent years. However, since the development of aquaculture, various kinds of bacterial infections have been observed in marine fish with increasing frequency, which has caused severe economic damage over the years. One disease of yellowtail that has a relatively high incidence is pseudotuberculosis, caused by the pathogen *Photobacterium damselae* subsp. *piscicida* (*Pdp*). Affected fish appear dark-colored, descaled, and are marked with dark spots. They feed poorly, leave the flock, and can often be seen lying dead at the bottom of the fish culture enclosure (Nagano *et al.*, 2011; Kanai, 2017). Pseudotuberculosis has been reported not only in Japan but also in the United States and Europe, and the strains responsible contain some transferable resistance plasmids (R plasmids) encoding drag resistance and virulence factor genes, including a class A β-lactamase gene for ampicillin resistance (Morii *et al.*, 2004), *catI* and *catII* genes for chloramphenicol resistance (Morii *et al.*, 2003), *ppflo* and *floR* genes for florfenicol resistance, the *aphA7* gene for kanamycin resistance, the *sul2* gene for sulfonamide resistance, the *erm* gene for erythromycin resistance (Morii *et al.*, 2012) and a gene cluster (*i.e.*, high-pathogenicity island) producing piscibactin as a siderophore (Osorio *et al.*, 2015). These drug resistance and virulence factor genes are often flanked by transposons or insertion sequences (Kim *et al.*, 1994; Kim *et al.*, 2008; Osorio *et al.*, 2015). However, the detailed transmission mechanism of these genes remains unclear. Therefore, it is important to improve knowledge on the genetic basis for pathogenicity of this bacterium (Balado *et al.*, 2013; Del Castillo *et al.*, 2013; Osorio *et al.*, 2015). Previously, we determined the whole-genomic sequence of *Pdp* strains from Japan (OT-51443) and the US (91-197) and performed a comparative structural analysis of their chromosomal genomes. The genomes of strains OT-51443 and 91-197 consist of two circular chromosomes each, and 5 and 2 plasmids, respectively. Comparative structural analysis of the chromosomal genomes of strains OT-51443 and 91-197 shows that each strain carries two circular chromosomes of approximately 3 Mb and 1 Mb, which show a high degree of homology (Teru *et al.*, 2017; Aoki *et al.*, 2017). However, the structure of the plasmid DNAs remains unknown despite the need for a detailed analysis.

Most drug-resistance and virulence factor genes are incorporated into the chromosomal and plasmid DNA of resistant bacteria. Plasmid DNAs can be transmitted, not only within the same species but also across bacterial species by conjugation. Therefore, it is important to understand how the genetic information encoded by a plasmid was acquired (Callewaert *et al.*, 2005; Vanderkelen *et al.*, 2012; Osorio *et al.*, 2015). In this study, we focused on plasmid DNA derived from Japanese and US strains, OT-51443 and 91-197. We compared the genetic structures of these strains and searched for similar plasmid DNAs and genetic regions between Japanese and US strains.

Materials and Methods

Whole genome sequencing of the Japanese (OT-51443) and US strains (91-197) of *Pdp* was previously performed using the Pacific Biosciences (PacBio) RS II sequencing platform (Macrogen Japan, http://www.macrogen-japan.co.jp)(Aoki *et al.*, 2017; Teru *et al.*, 2017), and the data obtained from the GenBank database (accession numbers are shown in Table 1). The Japanese (OT-51443) and US strains (91-197) were isolated from *Pdp*-infected yellowtail or striped seabass, and both the strains contained multiple plasmid DNAs. Only plasmid DNA sequence information was used for this analysis. All plasmids were subjected to BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to find a homologous plasmid which was deposited in the GenBank database.

Comparative and structural analyses of plasmids from the Japanese and US strains

Dot plot analysis for comparing plasmid DNA structures was performed with Rapid Annotations using Subsystems Technology (RAST) version 2.0 (http://rast.nmpdr.org/rast.cgi)

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Table 1. Deposited information on plasmid DNAs from *Photobacterium damselae* subsp. *damselae* (*Pdp*)

Strain	Host fish	Plasmid	Size (bp)	No. of genes (CDSs)	GenBank Acc. No.	Homologous plasmid (BLAST search)		
						Homologous plasmid (isolate country)	GenBank Acc. No.	Identity % (Coverage %)
OT-51443	Yellowtail (*Seriola quinqueradiata*)	pOT-51443-1	145,279	190	BDMQ01000003	p91-197-2 (USA)	AP081048	99.70 (9)
		pOT-51443-2	138,806	178	BDMQ01000004	pP99-018 (Japan)	AB277723	99.99 (100)
						pP0855 (Japan)	LC225353	100.00 (99)
						pP91278 (USA)	AB277724	99.99 (91)
		pOT-51443-3	55,613	59	BDMQ01000005	pP9014 (Japan)	AB453229	99.89 (100)
		pOT-51443-4	39,759	52	BDMQ01000006	p91-197-1 (USA)	AP081047	99.88 (96)
		pOT-51443-5	25,053	35	BDMQ01000007	None	None	None
91-197	Striped bass (*Morone* sp.)	p91-197-1	37,140	47	AP081047	pOT-51443-4 (Japan)	BDMQ01000006	99.88 (100)
		p91-197-2	29,328	30	AP081048	pOT-51443-1 (Japan)	BDMQ01000003	99.70 (29)

(Aziz *et al.*, 2008). The genetic structure of homologous regions was evaluated by comparing the structure of the plasmid DNAs. Structural analysis was performed with drawGeneArrows3 (http://www.ige.tohoku.ac.jp/joho/index.html).

*Characterization of the inhibitor of vertebrate lysozyme (*ivy*) gene*

For characterization of the *ivy* genes encoded by *Pdp* pOT-51443-4 and p91-197-1, the genetic sequences were rearranged. Alignment and comparative analysis with known *ivy* genes from pathogenic bacteria were performed using ClustalW ver. 2.1 (http://clustalw.ddbj.nig.ac.jp/) and BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). A maximum likelihood phylogenetic tree of aligned sequences was constructed using MEGA7 software (http://www.megasoftware.net/).

Results

Comparative analysis of plasmids from the Japanese and US strains

The genome of Japanese strain OT-51443 consists of two circular chromosomes and five circular plasmid DNAs. Two of the plasmid DNAs (pOT-51443-1 and pOT-51443-2) were 145,279 bp and 138,806 bp long and encoded 190 and 178 coding sequences (CDSs), respectively. The other three plasmid DNAs (pOT-51443-3, pOT-51443-4, and pOT-51443-5) were 55,613 bp, 39,759 bp and 25,053 bp long and encoded 59, 52, and 35 CDSs, respectively (Table 1).

The genome of US strain 91-197 consists of two circular chromosomes and two plasmid DNAs (p91-197-1 and p91-197-2), which were 37,140 bp and 29,328 bp long, and encoded 47 and 30 CDSs, respectively (Table 1). Results from the dot plot analyses using RAST showed two homologous regions between the plasmids of the two strains (Fig. 1). Homologous region 1 showed high similarity between all the regions of pOT-51443-4 and p91-197-1. Thus, the two plasmids were considered homologous. Homologous region 2 showed only partial homology between pOT-51443-1 and p91-197-2. The homologous region 2 possessed 10 CDSs in p91-197-2 (located from 109,869 to 122,495 bp) identical to the region including 15 CDSs in p91-197-2 (91 to 8,829 bp), however there was no identical region in their plasmids. No homology existed between pOT-51443-3 and the two plasmids of strain 91-197, however, pOT-51443-3 was highly homologous to pP9014 with 99.89% identity and 100% coverage (Table 1). Furthermore, pOT-51443-2 was highly homologous to two Japanese (pP99-018 and pP0855) and one US (pP91278) plasmids with 99.99-100% identities (Table 1), however, no typical homologous plasmid was found in pOT-51443-5 (Table 1). In other

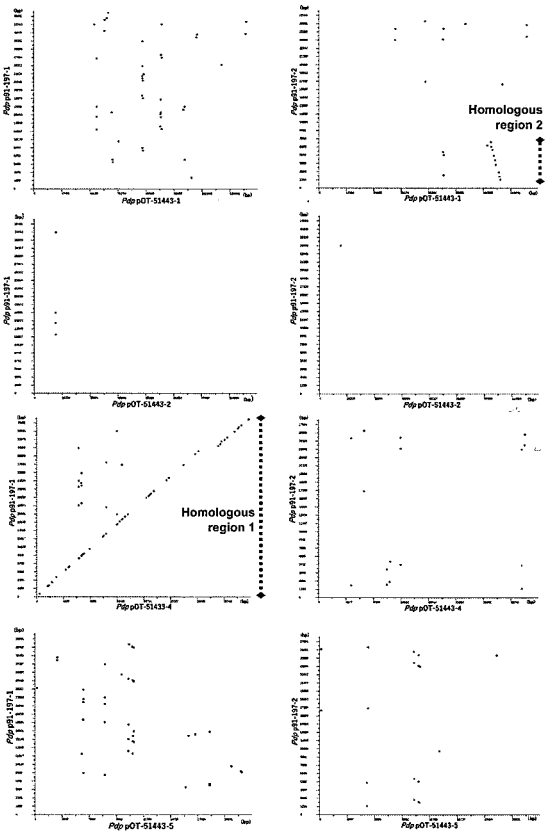

Fig. 1. Comparison of plasmid DNA structure from two *Pdp* strains by dot plot analysis. The vertical axis shows two plasmid DNAs derived from *Pdp* strain 91-197 and the horizontal axis shows the sequences of four plasmid DNAs derived from *Pdp* strain OT-51443. One of the plasmid DNAs, pOT-51443-3, was excluded because it contained no regions of homology with the two plasmids p91-197-1 and -2. The positions of homologous genes are indicated by dots.

combinations of plasmids, we found scattered homologous genes, but no continuous regions of homology were observed. DNA structures were different between Japanese and US strains except for these homologue regions. The two homologous regions were also not found in any *Pdp*-derived plasmids [*i.e.*, pP99-018 (Acc. No. AB277723) and pP9014 (AB453229) in Japanese strains NP9014 and PT99-018, pP91278 (AB277724) in the US strain USA91278], and in any of the whole genome sequences (WSG) of *Pdp* strains [*i.e.*, European

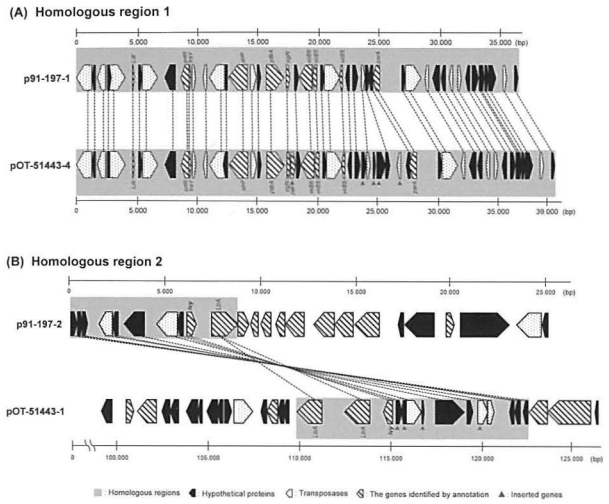

Fig. 2. (A) Comparison of the gene structure in homologous region 1 between strains p91-197-1 and pOT-51443-4. (B) Comparison of gene structure in identical region 2 in strains p91-197-2 and pOT-51443-1. Identical genes are connected by lines.

strains MT1415 (Acc. No. SUMH01000001~SUMH01000474), SNW-8.1 (SRHD01000001~SRHD01000641), DI21 (AKYG 01000001~AKYG01001377), L091106-03H (MCFX 02000001~MCFX02000016), and Japanese strain PP3 (SRHT 01000001~SRHT01000520)] deposited in the GenBank database (Data not shown).

Structural analysis of the identical gene loci between Japanese and US plasmids

All genes encoded by p91-197-1 were also present in pOT-51443-4, along with an insertion of five genes observed in homologous region 1 (Fig. 2A). In contrast, within homologous region 2, all genes encoded by the homologous region of p91-197-2 were in reverse orientation, and two genes were replicated in pOT-51443-1 (Fig. 2B). Moreover, a partial sequence coding for a transposase was observed in pOT-51443-1. Homologous region 2 contained the sequence for the inhibitor of vertebrate lysozyme (*ivy*) gene, a known virulence factor, in both plasmids. Furthermore, the *ivy* gene was not found in any of the *Pdp*-derived plasmids and also in whole genome sequences of *Pdp* strains deposited in the GenBank database by BLAST search (Data not shown).

Characterization of the ivy *gene*

The *ivy* genes encoded by pOT-51443-1 and p91-197-2 were 100% identical and showed the functional motif CKPHDC, which is characteristic of Ivy. A comparison of the amino acid sequences of Ivy from various pathogenic bacteria showed conservation of this functional motif. The similarity between the Ivy proteins derived from the two *Pdp* strains and those isolated from other pathogenic bacteria was approximately 13.1 to 26.9%. The highest similarity (26.9%) was observed with *Serratia marcescens* (Fig. 3A). According to phylogenetic tree analysis, the *ivy* genes of *Pdp* are conserved, and clustered in the same genus (Fig. 3B).

Discussion

In this study, we investigated differences between two

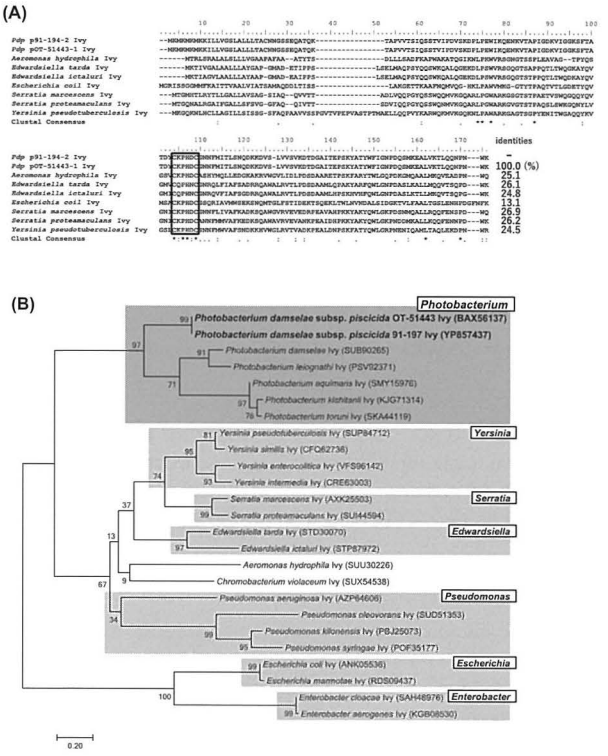

Fig. 3. (A) Comparison of Ivy amino acid sequences from *Pdp* and known pathogenic bacteria. The section enclosed by a box indicates a functional motif that is essential for inhibition of lysozyme activity. The percent similarities of Ivy sequences between *Pdp* 91-197 and other species are shown on the right. (B) Phylogenetic tree constructed using the aligned sequences of Ivy from *Pdp* and other species. Phylogenetic results were tested by bootstrapping 1,000 times.

strains of *Pdp*, OT-51443 and 91-197, to better understand their genetic structure and identify virulence factors. High similarity and gaps were observed in the homologous region 1 of the two strains. Thus, the homologous plasmid DNA was possibly shared between the two strains, and genes not present in p91-197-1 were inserted into pOT-51443-4. The homologous region 2 in p91-197-2 and pOT-51443-1 displayed high sequence identity and had 4 genes inserted in pOT-51443-1, including a transposase gene, which suggests that it could be related to transmission of the inserted genes in homologous region 2. Furthermore, since the two homologous regions were not found in any of the plasmids and WGSs of *Pdp* strains, the finding of homologous region 2 was a unique case to suggest transmission possibility of the mobile gene between countries. In contrast, no homology existed between pOT-51443-3 and the two plasmids of strain 91-197, but the former was almost completely identical to pP9014. Since, both pOT-51443-3 and pP9014 were derived from Japanese strains, these are same plasmids. pOT-51443-2 was also highly homologous to Japanese (pP99-018 and pP0855) and the US (pP91278) plasmids. These plasmids were probably transferred between each other within Japan or between the US and Japan.

All genes encoded by p91-197-1 were also present in pOT-51443-4. In addition, the insertion of a transposase, a hypothetical protein, and a DNA binding protein was observed in

pOT-51443-4. These were considered newly acquired by strain OT-51443. The homologous region was also conserved in p91-197-1 and pOT-51443-4. However, partial deletion of a gene encoding a transposase was observed in pOT-51443-4. This event might have occurred during the transmission of the plasmid DNA to strain OT-51443.

The presence of *ivy*, which acts as a virulence factor (Kyomuhendo *et al.*, 2008; Liu *et al.*, 2015), was observed in both plasmids. Ivy has a characteristic functional "CKPHDC" motif to increase the bacterial infectivity, with a histidine residue in the motif forming a hydrogen bond with two of the three amino acid residues in the active site of lysozyme. The loop structure closes the active site of the lysozyme, thereby inhibiting lytic activity (Abergel *et al.*, 2007; Wang *et al.*, 2013). In our analysis, we found that the functional motif in Ivy derived from *Pdp* was highly conserved, suggesting that the *ivy* gene conserved in pOT-51443-4 and p91-197-1 is a virulence factor. This also suggests that the *Pdp* strains OT-51443 and 91-197 may have increased infectivity and pathogenicity.

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References

Abergel, C., V. Monchois, D. Byrne, S. Chenivesse, F. Lembo, J. C. Lazzaroni and J. M. Claverie (2007): Structure and evolution of the Ivy protein family, unexpected lysozyme inhibitors in Gram-negative bacteria. *Proc. Natl. Acad. Sci. U. S. A.*, **104**, 6394–6399.

Aoki, T., Y. Teru, N. Morimoto, T. Kono, M. Sakai, T. Takano, J. P. Hawke, Y. Fukuda, H. Takeyama and J. Hikima (2017): Complete genome sequence of *Photobacterium damselae* subsp. *piscicida* strain OT-51443 isolated from yellowtail (*Seriola quinqueradiata*) in Japan. *Genome Announc.*, **5**, e00404–17.

Aziz, K. R., D. Bartels, A. A. Best, M. DeJongh, T. Disz, R. A. Edwards, K. Formsma, S. Gerdes, E. M. Glass, M. Kubal, F. Meyer, G. J. Olsen, R. Olson, A. L. Osterman, R. A. Overbeek, L. K. McNeil, D. Paarmann, T. Paczian, B. Parrello, G. D. Pusch, C. Reich, R. Stevens, O. Vassieva, V. Vonstein, A. Wilke and O. Zagnitko (2008): The RAST Server: Rapid Annotations using Subsystems Technology. *BMC genomics*, **9**, 75.

Balado, M., M. L. Lemos and C. R. Osorio (2013): Genetic characterization of pPHDP60, a novel conjugative plasmid from the marine fish pathogen *Photobacterium damselae* subsp. *piscicida*. *Plasmid*, **70**, 154–159.

Balado, M., H. Benzekri, A. M. Labella, M. G. Claros, M. Manchado, J. J. Borrego, C. R. Osorio and M. L. Lemosa (2017): Genomic analysis of the marine fish pathogen *Photobacterium damselae* subsp. *piscicida*: Insertion sequences proliferation is associated with chromosomal reorganisations and rampant gene decay. *Infect. Genet Evol*, **54**, 221–229.

Callewaert, L., B. Masschalck, D. Deckers, D. Nakimbugwe, M. Atanassova, A. Aertsen and C. W. Michiels (2005): Purification of Ivy, a lysozyme inhibitor from *Escherichia coli*, and characterization of its specificity for various lysozymes. *Enzyme Microb. Technol.*, **37**, 205–211.

Debris, A., F. Pierre, M. Merchez, E. Pradel, S. Laouami, I. Ricard, J. Sirard, J. Fritz, N. Lemaître, H. Akinbi, G. I. Boneca and F. Sebbane (2013): Inheritance of the lysozyme inhibitor Ivy was an important evolutionary step by *Yersinia pestis* to avoid the host innate immune response. *J. Infect. Dis.*, **207**, 1535–1543.

Deckers, D., D. Vanlint, L. Callewaert, A. Aertsen and C. W. Michiels (2008): Role of the lysozyme inhibitor ivy in growth or survival of *Escherichia coli* and *Pseudomonas aeruginosa* bacteria in hen egg white and in human saliva and breast milk. *Appl. Environ. Microbiol.*, **74**, 4434–4439.

Del Castillo, C. S., H. B. Jang, J. Hikima, T. S. Jung, H. Morii, I. Hirono, H. Kondo, C. Kurosaka and T. Aoki (2013): Comparative analysis and distribution of pP9014, a novel drug resistance IncP-1 plasmid from *Photobacterium damselae* subsp. *piscicida*. *Int. J. Antimicrob. Agents*, **42**, 10–18.

Del Castillo, C. S., J. Hikima, H. B. Jang, S. W. Nho, T. S. Jung, J. Wongtavatchai, H. Kondo, I. Hirono, H. Takeyama and T. Aoki (2013): Comparative sequence analysis of a multidrug-resistant plasmid from *Aeromonas hydrophila*. *Antimicrob. Agents Chemother.*, **57**, 120–129.

Kanai, K. (2017): Pseudotuberculosis. *Fish Pathol.*, **52**, 53–56.

Kim, E. H. and T. Aoki (1994): The transposon-like structure of IS26-tetracycline, and kanamycin resistance determinant derived from transferable R plasmid of fish pathogen, *Pasteurella piscicida*. *Microbiol. Immunol.*, **38**, 31–38.

Kim, M. J., I. Hirono, K. Kurokawa, T. Maki, J. Hawke, H. Kondo, M. D. Santos and T. Aoki (2008): Complete DNA sequence and analysis of the transferable multiple-drug resistance plasmids (R plasmids) from *Photobacterium damselae subsp. piscicida* isolates collected in Japan and the United States. *Antimicrob. Agents. Chemother.*, **52**, 606–611.

Kyomuhendo, P., I. W. Nilsen, B. O. Brandsdal and A. O. Smalas (2008): Structural evidence for lack of inhibition of fish goose-type lysozymes by a bacterial inhibitor of lysozyme. *J. Mol. Model.*, **14**, 777–788.

Li, M. F., C. Wang and L. Sun (2015): *Edwardsiella tarda* MliC, a lysozyme inhibitor that participates in pathogenesis in a manner that parallels Ivy. *Infect. Immun.*, **83**, 583–590.

Liu, Z., B. Garcia-Diaz, B. Catacchio, E. Chiancone and H. J. Vogal (2015): Protecting gram-negative bacteria cell envelopes from human lysozyme: Interactions with ivy inhibitor proteins from *Escherichia coli* and *Pseudomonas aeruginosa*. *Biochim. Biophys. Acta-Biomembr.*, **1848**, 3032–3046.

Monchois, V., C. Abergel, J. Sturgis, S. Jeudy and J. M. Claverie (2001): *Escherichia coli* ykfE ORFan Gene Encodes a Potent Inhibitor of C-type Lysozyme. *J. Biol. Chem.*, **276**, 18437–18441.

Morii, H. and Y. Ishikawa (2012): Cloning and nucleotide sequence analysis of the chloramphenicol and erythromycin resistance genes on a transferable R plasmid from the fish pathogen *Photobacterium damselae* subsp. *piscicida*. *Bull. Fac. Fish. Nagasaki Univ*, **93**, 41–50.

Morii, H., N. Hayashi and K. Uramoto (2003): Cloning and nucleotide sequence analysis of the chloramphenicol resistance gene on conjugative R plasmids from the fish pathogen *Photobacterium damselae* subsp. *piscicida*. *Dis. Aquat. Organ*, **53**, 107–113.

Morii, H., M. S. Bharadwaj and N. Eto (2004): Cloning and nucleotide sequence analysis of the ampicillin resistance gene on a conjugative R plasmid from the fish pathogen *Photobacterium damselae* subsp. *piscicida*. *J. Aquat. Anim. Health*, **16**, 197–207.

Nagano, I., S. Oshima and K. Kawai (2011): In vivo analysis on the adherence and infection route of *Photobacterium damselae* subsp. *piscicida* in yellowtail. *Fish Pathol.*, **46**, 45–50.

Osorio, C. R., A. J. Rivas, M. Balado, J. C. Fuentes-Monteverde, J. Rodríguez, C. Jiménez, M. L. Lemos and M. K. Waldor (2015): A transmissible plasmid-borne pathogenicity island confers piscibactin biosynthesis in the fish pathogen *Photobacterium damselae* subsp. *piscicida*. *Appl. Environ. Microbiol.*, **81**, 5867–5879.

Teru, Y., J. Hikima, T. Kono, M. Sakai, T. Takano, J. P. Hawke, H. Takeyama and T. Aoki (2017): Whole-genome sequence of *Photobacterium damselae* subsp. *piscicida* strain 91-197, isolated from hybrid striped bass (*Morone* sp.) in the United States. *Genome Announc.*, **5**, e00600–17.

Vanderkelen, L., E. Ons, J. M. V. Herreweghe, L. Callewaert, M. B. Goddeeris and C. W. Michiels (2012): Role of lysozyme inhibitors in the virulence of avian pathogenic *Escherichia coli*. *PLoS One*, **7**, e45954.

Wang, C., Y. H. Hu, B. G. Sun, J. Li and L. Sun (2013): *Edwardsiella tarda* Ivy, lysozyme inhibitor that blocks the lytic effect of lysozyme and facilitates host infection in a manner that is dependent on the conserved cysteine residue. *Infect. Immun.*, **81**, 3527–3533.

日本および米国株 *Photobacterium damselae* subsp. *piscicida* 由来プラスミド DNA の構造比較解析

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類結節症の原因菌である *P.damselae* subsp. *piscicida*（*Pdp*）は，日本および米国株における病原性の違いが知られている。本研究では日本および米国株の *Pdp* 由来プラスミド DNA の構造解析を行った。その結果，pOT-51443-4 と p91-197-1，pOT-51443-1 と p91-197-2 にそれぞれ相同性の高い領域が検出された。米国由来 p91-197-1 上の全ての遺伝子が日本株由来 pOT-51443-4 に認められたことから，米国株のプラスミドが日本株に伝播したものと考察された。また，pOT-51443-1 と p91-197-2 の相同領域に病原性因子であるリゾチーム阻害遺伝子（*ivy*）が存在していた。このことから，日本－米国株間におけるプラスミドを介しての病原性因子伝達の可能性が示唆された。

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