Latin American Journal of Aquatic Research
E-ISSN: 0718-560X
lajar@ucv.cl
Pontificia Universidad Católica de Valparaíso
Chile

Salas-Leiva, Joan; Opazo, Rafael; Remond, Camille; Uribe, Eduardo; Velez, Antonio; Romero, Jaime

Characterization of the intestinal microbiota of wild-caught and farmed fine flounder (Paralichthys adspersus)

Latin American Journal of Aquatic Research, vol. 45, núm. 2, mayo, 2017, pp. 370-378
Pontificia Universidad Católica de Valparaíso
Valparaíso, Chile

Lat. Am. J. Aquat. Res., 45(2): 370-378, 2017
DOI: 10.3856/vol45-issue2-fulltext-12

Research Article

Characterization of the intestinal microbiota of wild-caught and farmed fine flounder (*Paralichthys adspersus*)

Joan Salas-Leiva[1,2], Rafael Opazo[1,5], Camille Remond[1], Eduardo Uribe[3,5] Antonio Velez[4] & Jaime Romero[1,5]

[1]Laboratorio de Biotecnología de los Alimentos, Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago, Chile
[2]Doctorado en Acuicultura, Programa Cooperativo Universidad Católica del Norte Pontificia Universidad Católica de Valparaíso, Universidad de Chile
[3]Departamento de Acuicultura, Universidad Católica del Norte, Coquimbo, Chile
[4]Fundación Chile, Cultivos Marinos Tongoy, Coquimbo, Chile
[5]Aquapacífico, Coquimbo, Chile
Corresponding author: Jaime Romero (jromero@inta.uchile.cl)

ABSTRACT. A comparative analysis of the cultivable intestinal microbiota of farmed (AF) and wild-caught (WF) *Paralichthys adspersus* was performed. The 16S rRNA gene was used for taxa identification, and the ITS region for strain differentiation. We detected the presence of *Vibrio*, *Bacillus*, *Photobacterium*, *Staphylococcus* and *Carnobacterium* in AF, and *Exiguobacterium*, *Klebsiella*, *Arthrobacter*, *Raoultella*, *Kluyvera*, *Myroides*, *Streptococcus*, *Vagococcus*, *Staphylococcus*, *Acinetobacter*, *Psychrobacter*, *Lactobacillus*, *Weissella* and *Lactococcus* in WF. The microbial community was more diverse in WF than in AF. Some bacterial groups were only found in wild-caught fish and may be studied as potential beneficial agents for improving production traits in farmed fish. As the first study of microbiota of *P. adspersus*, it provides significant information that can potentially help improve farming practices by using strains as species-specific probiotics.

Keywords: microbiota, intestinal microbiology, fine flounder, lactic acid bacteria, 16SrRNA.

INTRODUCTION

The flatfish *Paralichthys adspersus* has great potential in Chilean aquaculture. However, its farming at a commercial scale is limited by low growth rates (Silva, 2010). Since few studies on digestion and nutrient metabolism are available, new information regarding the intestinal microbiota that can facilitate or promote these functions is needed to improve farming practices of this species. Microbiota plays a major role in nutrition, growth, health, and survival of the host fish because some bacteria supply exogenous nutrients, produce extracellular enzymes, vitamins and fatty acids (Dhanasiri *et al.*, 2011). For such reason, there is interest in understanding how bacterial populations in the gastrointestinal tract are structured, and the role they play on the fitness of their hosts.

Studies in mammals reveal associations among the composition of the microbiota and host diet, anatomy and phylogeny (Ley *et al.*, 2008). In fish, the variation in the composition of the microbiota is strongly associated with the habitat, trophic level, and phylogenetic relationships of the species (Sullam *et al.*, 2012). Comparative analyses using data from fish intestines and other environments revealed that the microbiota of fish is not a simple reflection of the organisms in their local habitat, but also the result of the host-specific selective pressures within the intestine (Bevins & Salzman, 2011; Navarrete *et al.*, 2012).

The effect of the diet on microbiota is one of the most documented aspects in farmed fish. Wong *et al.* (2013) report changes in the composition of the microbiota of *Oncorhynchus mykiss* when fishmeal is replaced for soybean meal. The fine flounder feeds on fish, crustaceans and mollusks in the wild, but is raised with artificial diets and kept captive in different habitats than its natural ones, similarly as other farmed organisms. In general, such conditions increase the interest in knowing what microbiota changes are experienced by organisms in captivity with respect to those on the wild. There are diversity studies regarding the microbiota in *S. salar* (Holben *et al.*, 2002), *Danio rerio* (Roeselers *et al.*, 2011), *Gadus morhua* (Dhanasiri *et al.*, 2011), *Peneaus monodon* (Rungrassamee *et al.*,

Corresponding editor: Sandra Bravo

2014). However, in the genus *Paralichthys*, such information is restricted to *P. olivaceus* (Kim & Kim, 2013). Colston & Jackson (2016) argue whether work on captive animals can be used to predict the gut microbiomes of animals in the wild. This problem has been suggested before (Amato, 2013), yet there is still a substantial lack of studies that have attempted to characterize the enteric microbial communities in hosts within a natural environment.

Populations of lactic acid bacteria (LAB) are highly variable in the intestines of fish and change as the aquatic environment does, *i.e.*, farming or wilderness (Hagi *et al.*, 2004). LAB is a group widely investigated in animals because it plays an important role on the health and nutrition of the host (Vázquez *et al.*, 2005; Lauzon & Ringø, 2012). Korean researchers observed that species richness of LAB in *P. olivaceus* was significantly higher in the intestines of wild fish than in farmed specimens (Kim & Kim, 2013). Our study aimed to characterize cultivable bacterial populations of microbiota of *P. adspersus* farmed (AF) and wild-caught (WF) emphasizing on the detection of LAB.

MATERIALS AND METHODS

Fish

Farmed fine flounder were obtained from aquaculture facilities of Cultivos Marinos Tongoy (AF: n = 15, average weight = 100 g). Wild fine flounder (WF: n = 7, weight = ~300 g) were captured in the Region of Coquimbo. AF came from the same cohort fed with commercial pellets, without addition of probiotics, immunostimulants or inhibitors. AF and WF showed no apparent deformities or diseases.

Isolation and counts of intestinal microbiota

The intestinal contents of wild-caught and farmed fish were collected and treated as previously described (Navarrete *et al.*, 2010). Serial dilutions of intestinal contents were spread over plates of Trypticase soy agar (TSA) for screening heterotrophic bacteria and Man, Rogosa and Sharpe Agar (MRS) for screening of lactic acid bacteria (LAB). Incubation was carried out at 17°C for 10 days and colonies were isolated in fresh medium for evaluation. The total bacterial counts were assessed by epifluorescence microscopy using acridine orange (Romero & Espejo, 2001). Briefly, serial dilution of intestinal content were filtered (0,2 µm) and then stained. Total counts were calculated after counting 10 fields for each sample, using 100x of a epifluorescence microscope. Analysis of feed was done following (Romero & Navarrete, 2006); briefly, pellets were weighed and an equal weight of sterile TE buffer (Tris 0.1M, EDTA 0.01M, NaCl 0.15M, pH 7.8) was added. The mixture was homogenized in vortex and then serial dilutions were spread and incubated as described above. Water samples were obtained directly from the farm's water source (water influent); total counts and viable count were performed from serial dilutions as described above. All the samples were analyzed in triplicate.

PCR amplification, sequencing and analysis of bacteria

The isolates were grown overnight in Tryptic soy broth (TSB) at 37°C and harvested by centrifugation. Extraction of bacterial genomic DNA was carried out as previously reported (Navarrete *et al.*, 2012). In order to select the strains that were going to be sequenced, we amplified a section of 16S rRNA for all isolates (positions 341 to 907) which was subjected to PCR-RFLP, using PCR conditions described in (Navarrete *et al.*, 2012). PCR products were digested with EcoRI-HF ™ and Hpa II (New England BioLabs Inc.), and visualized as (Romero *et al.*, 2002). Bacterial strains corresponding to different PCR-RFLP patterns were selected for sequencing (Macrogen, USA). For sequencing, the region 27 to 1492 of the 16S rRNA was amplified as described (Romero *et al.*, 2002). Isolates with identical sequences were subjected to ITS analysis (González *et al.*, 2003). This information allowed the comparison of microbiota diversity between WF and AF. In both cases, PCR-RFLP and ITS profiles were assessed with cluster analysis. The sequences were edited and cleaned up with BIOEDIT software (Hall, 1999) and compared to those of the public RDP database for identification.

RESULTS

The bacterial counts in the intestinal contents of wild flounder (WF) and aquaculture flounder (AF) and) were 5.14 ± 0.71 and 5.49 ± 0.59 (Log_{10} Colony Forming Units, CFU g^{-1}), respectively when TSA was used. These levels were according to reported bacterial load in other flatfish (MacDonald *et al.*, 1986; Eddy & Jones, 2002; Verner-Jeffreys *et al.*, 2003; Martin-Antonio *et al.*, 2007; Fig. 1). Total counts under epifluorescence microscope averaged 7.25 ± 0.81 and 7.30 ± 1.06 (Log_{10} bacteria g^{-1}) of intestinal content AF and WF respectively; hence, the cultivability in AF and WF was roughly near to 1%. The total bacterial counts in the water of the farm showed an average of 3.3 ± 0.47 (Log_{10} bacteria mL^{-1}), whereas the viable count in TSA showed an average of 1.67 ± 0.35 (Log_{10} CFU mL^{-1}). Simultaneously, feed samples were examined, showing a viable count of 2.99 ± 0.55 (Log_{10} CFU g^{-1}). Lactic

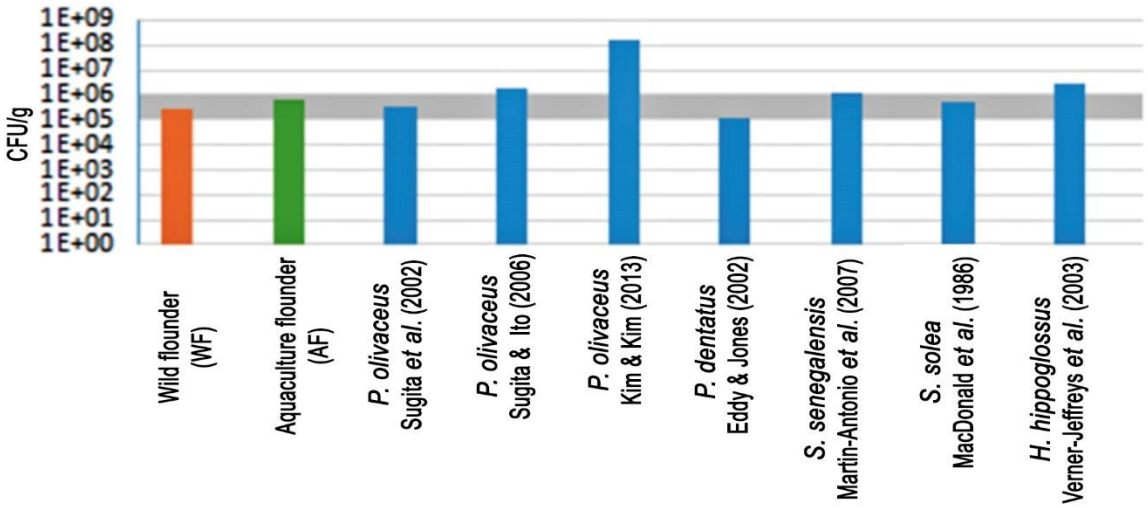

Figure 1. Viable counts retrieved in the intestinal contents collected from aquaculture (AF) and wild flounder (WF), obtained in this study and compared with the data available for other flatfish of aquaculture origin, in scientific literature.

acid bacteria were examined using MRS, however, only samples from intestinal contents of WF showed colonies at level of 3.51 ± 0.60 (Log_{10} CFU g^{-1}).

A total of 100 bacterial isolates were included in this study. A set of 73 isolates were analyzed by 16SrRNA sequencing and 16SrRNA RFLP, and 47 sequences of partial 16SrRNA were deposited in GenBak under the Accession Numbers: KP453988-KP453997; KP731 550-KP731586. Examination of these sequences in RDPii database allowed the taxonomic identification of the isolates.

The phylum *Proteobacteria* was the dominant bacterial group in WF, and ranked second after *Firmicutes* in AF (Fig. 2). *Proteobacteria* is the dominant phylum in the microbiota of other flatfish, such as, *Solea senegalensis* (Martin-Antonio *et al.*, 2007; Tapia-Paniagua *et al.*, 2010), *P. olivaceus* (Sugita *et al.*, 2002; Kim & Kim, 2013) and *Scophthalmus maximus* (Xing *et al.*, 2013). This bacterial group may contribute to the digestive process by providing a variety of enzymes (Neulinger *et al.*, 2008; Smriga *et al.*, 2010). In our research, the lower representation of *Proteobacteria* in AF with respect to WF requires further study to establish if this is affecting the nutrient metabolism in the fish resulting in low growth rates. The two most representative *Gammaproteobacteria* in AF were *Vibrio* and *Photobacterium* both belonging to *Vibrionaceae*, whereas in WF, *Psychrobacter* and *Acinetobacter* were the most common isolates (Fig. 2).

Isolates from the phylum *Firmicutes* correspond to 60% of bacteria in AF. In flatfish, they have been reported as the second most abundant phylum (Sugita *et al.*, 2002; Martin-Antonio *et al.*, 2007; Tapia-Paniagua *et al.*, 2010; Kim & Kim, 2013; Xing *et al.*, 2013). The most representative *Firmicutes* were *Bacillus* and *Carnobacterium* in AF, and *Staphylococcus* and *Lactobacillus* in WF (Fig. 2). *Bacillus* is frequently isolated from the microbiota of several marine species including flatfish (Hovda *et al.*, 2007; Sun *et al.*, 2010; Kim & Kim, 2013).

The examination of the microbiota at the genus level reveals that WF showed 11 genera, representing a higher diversity than AF with only 5 genera. In WF, *Psychrobacter* and *Acinetobacter* were the dominant bacteria summing a 52% of relative abundance. In contrast, in AF, *Vibrio* and *Bacillus* were the dominant genera (Fig. 2). Interestingly, *Bacillus* was the most abundant genus in the water of the farm and in the feed, 23% y 50%, respectively. Furthermore, the composition of the water of the farm showed two bacterial genera (*Staphylococcus* and *Bacillus*) in coincidence to the microbiota of AF and the genus *Bacillus* was detected in the feed and in the microbiota of AF (Figs. 2-3). *Bacillus*, *Lysinibacillus* and *Pseudomonas* were common component between water and feed (Fig. 3). Interesting bacterial groups such as lactic acid bacteria (LAB) showed also differential distribution, *Carnobacterium* was isolated from AF, whereas *Lactobacillus*, *Weisella*, *Lactococcus*, *Streptococcus* and *Vagococcus* were retrieved from WF. Figure 4 represents the clustering and molecular diversity of the bacterial isolates including LAB retrieved from the different samples examined, based on RFLP of 16S rRNA gene and some of them by sequencing. In this figure, the RFLP profile of isolates from different sources can be compared; in a roughly distribution, *Gammaproteobacteria* grouped separatedly from *Bacilli*, independently of the origin.

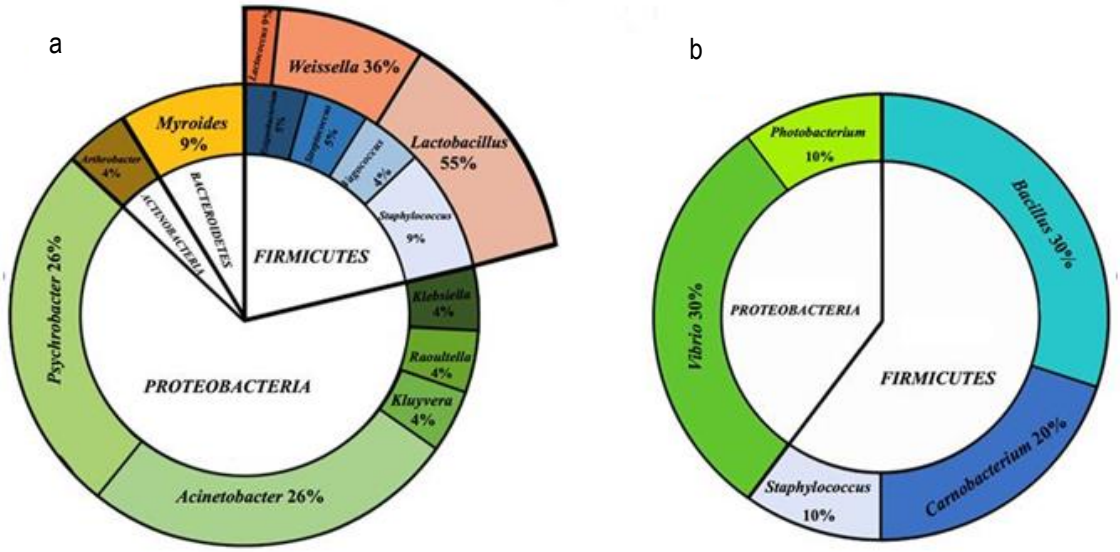

Figure 2. Composition of the intestinal microbiota. a) Wild-caught flounder, b) farmed flounder. Inner circle correspond to phylum, first ring correspond to genus of bacteria isolated in Tripticase Soy Agar (TSA); second ring correspond to Lactic acid bacteria (LAB) isolated in MRS; only wild-caught, showed this outer ring. All percentages are presented in relative abundances.

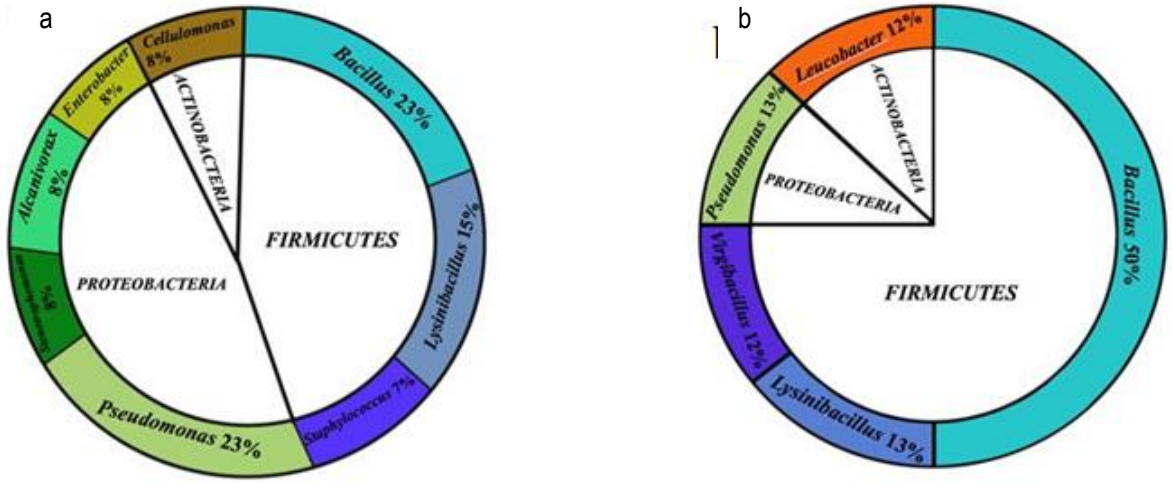

Figure 3. Bacterial populations present in a) water (aquaculture facility) and b) feed. Inner circle correspond to phylum, ring correspond to genus of bacteria isolated in TSA. All percentages are presented in relative abundances.

DISCUSSION

Low levels of cultivability observed in this work are attributed to the lack of knowledge of suitable conditions for culture to recover certain bacterial populations. However, this is not a limitation to study microbiota composition, because some abundant genus obtained by culture methods can be also retrieved from DNA analysis (Romero & Navarrete, 2006; Navarrrete *et al.*, 2010). Reduced cultivability *P. adspersus* may be due to the fact that microbiota is formed of species capable of forming colonies on agar plates but with low efficiency, or is composed of unknown species that do not grow on common microbiological media.

Vibrio has been reported as a typical genus in the intestine of the farmed *P. olivaceus* (Tanasomwang & Muraga, 1988; Sugita & Ito, 2006), *P. dentatus* (Eddy & Jones, 2002), *S. senegalensis* (Tapia-Paniagua *et al.*, 2010), *H. hippoglossus* (Verner-Jeffreys *et al.*, 2003) and *S. maximus* (Montes *et al.*, 2003). Intriguingly, in our study, *Vibrio* was isolated from AF but not from WF. One explanation is based on the use of artificial diets that may increase the load of *Vibrio* in *P. adspersus*, as it was previously observed in *S. senega-*

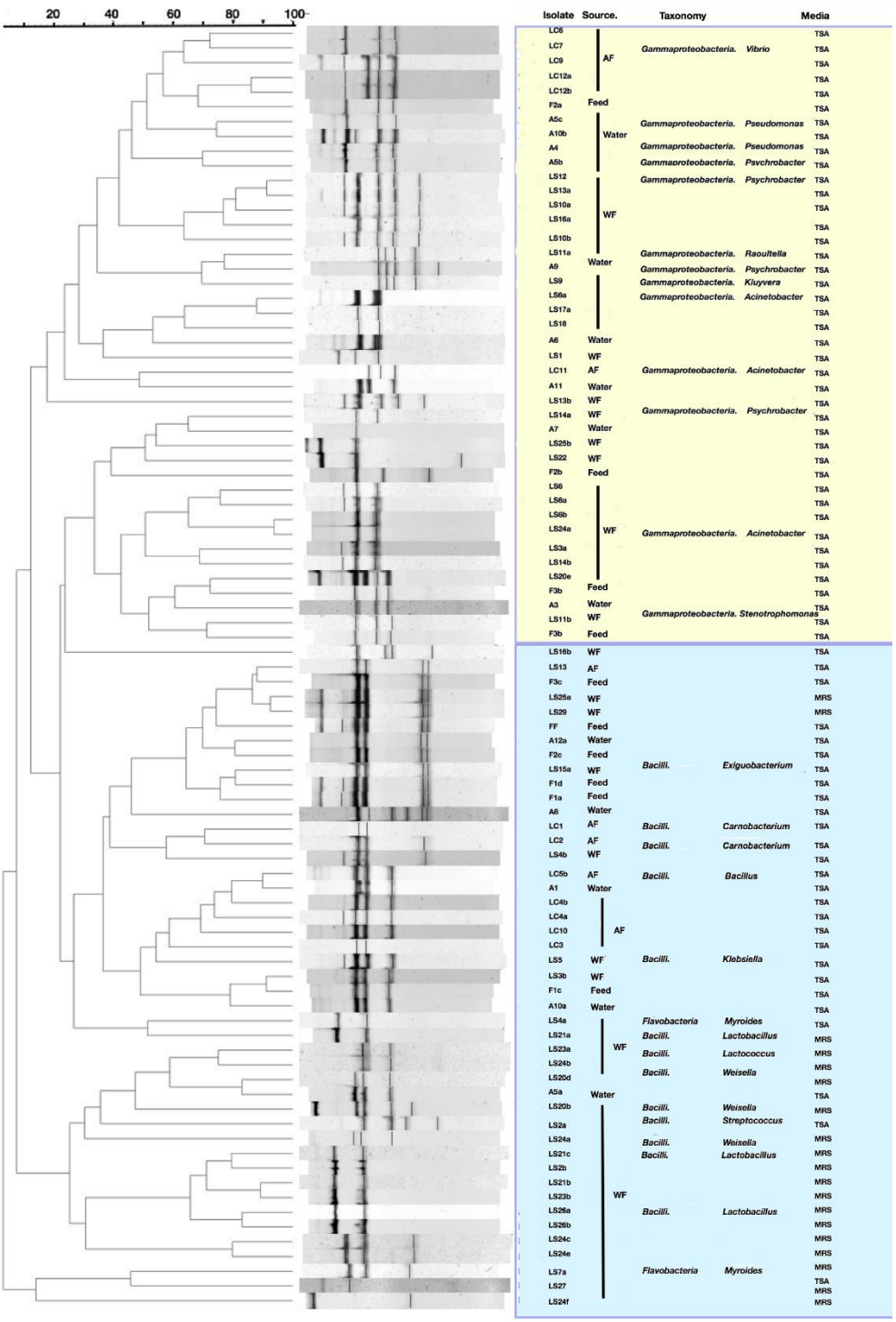

Figure 4. Dendrogram of 16S rRNA gene RFLP of bacterial isolates retrieved from microbiota of fine flounder. For some of the isolates the 16S rRNA gene was sequenced and the identification was included as phylum and genus.

lensis and *P. olivaceus* (Tanasomwang & Muroga, 1988; Martin-Antonio *et al.*, 2007). However, it is necessary to consider that the absence of *Vibrio* in WF may be due to (*i*) low dominance of this genus in the intestinal community, caused by the presence of bacteria with antagonistic effect against vibrios (Sun *et al.*, 2009) or (*ii*) *Vibrio* spp. are viable but non-cultivable on media (Silva *et al.*, 2011). Some species

of *Vibrio* and *Photobacterium* have been classified as opportunistic pathogens in farmed flatfish including *P. adspersus* (Miranda & Rojas, 1996; Sugita *et al.*, 2002; Villamil *et al.*, 2003; Vázquez *et al.*, 2005; Martin-Antonio *et al.*, 2007) and in some species like *Epinephelus coioides*, *Vibrio* spp. are part of the microbiota of fish with low growth rates (Sun *et al.*, 2009). Despite its negative connotation, *Vibrio* spp. has a wide enzymatic activity (*i.e.*, amylase, protease, lipase, chitinase) (MacDonald *et al.*, 1986; Sugita & Ito, 2006); and further studies are needed to establish its positive or negative role in AF.

Psychrobacter and *Acinetobacter* have been isolated from marine fish (MacDonald *et al.*, 1986; Tanasomwang & Muroga, 1988; Eddy & Jones, 2002; Sugita *et al.*, 2002; Ringø *et al.*, 2006; Sun *et al.*, 2009). Our research constitutes the first report of *Psychrobacter* in *Paralichthys* species. The occurrence of *Psychrobacter* in WF is remarkable because it is associated with positive physiological effects on growth rates and improved health of the host (Sun *et al.*, 2009, 2011). Sun *et al.* (2009) found that *Psychrobacter* only occurred and predominated the microbiota of *E. coioides* with fast growth rates. It has also been suggested that dietary management of *E. coioides* with *Psychrobacter* sp. inhibits the growth of pathogenic bacteria (*Vibrio* spp.), and set conditions in the gut bacteria promoting the establishment and colonization of other types of bacteria in the intestinal tract of fish.

In our study, *Firmicutes* corresponded to a abundant bacterial population in AF. In flatfish, they have been reported as the second most abundant phylum (Sugita *et al.*, 2002; Martin-Antonio *et al.*, 2007; Tapia-Paniagua *et al.*, 2010; Xing *et al.*, 2013; Kim & Kim, 2013). LAB, part of this phylum, are interesting bacteria because they have been used as a probiotic to improve health and growth rates of several flatfish (Villamil *et al.*, 2002). The diversity and load of LAB of fish is affected by nutritional and environmental factors. According to Kim & Kim (2013) the food commonly used in aquaculture of *P. olivaceus* is an unsuitable substrate for the colonization of LAB in the gut of this fish. For this reason, LAB populations are low or non-present in farmed *P. olivaceus.* The presence of a single genus of LAB in AF (*Carnobacterium*) is consistent with expectations in fish fed with artificial diets. The presence of this genus was detected in TSA instead of MRS, because it has been previously described that *Carnobacterium* is inhibited by components of this medium such as acetate (Leisner *et al.*, 2007). Other interesting genus is *Weissella*, it has been isolated from a broad variety of animals and specific *Weissella* strains are also receiving attention as potential probiotics, acting by inhibition of pathogens, and also some strains are known to produce copius amounts of non-digestible polysaccharides, with potential application as prebiotics (Fusco *et al.*, 2015). Recently, the species named *Weissella ceti* species was associated with diseased fish (Figueiredo *et al.*, 2014). Natural diets provide various nutritional factors such as amino acids, B vitamins, among others, that facilitate growth and colonization of LAB in the intestinal tract (Madigan *et al.*, 2010). The feed supplied to AF did not have any B vitamins supplemented, whereas small amounts of anchovy eaten by WF may contain tiamin (B_1) and riboflavin (B_2) that could help support LAB in WF (Reyes *et al.*, 2009).

Probiotic bacteria isolated from a particular host or their environment are more beneficial to the host itself or related species than bacteria isolated from other sources. This is due in large part because there is specificity in colonization by the strain-host complex or vice versa. Ying *et al.* (2007) observed that *Lactobacillus* adhesion to the surface of the intestinal tract of *P. olivaceus* depends on the specificity of the host strain. Given the above, we suggest that the microbial community of wild *P. adspersus* provides a suitable environment for the adhesion and colonization of this type of bacteria, and that the LAB isolated in this study could be studied as a specific probiotic to improve production traits in AF.

The phyla *Actinobacteria* and *Bacteroidetes* were underrepresented in WF and absent in AF (Fig. 1) similarly to the observed in farmed and wild *P. olivaceus* (Kim & Kim, 2013). Sullam *et al.* (2012) showed that in *S. senegalensis* these phyla are approximately 10% of the total microbiota. In our study, 13% of the microbiota of WF were represented by these phyla. *Actinobacteria* represents one of the largest taxonomic units among the 18 major lineages currently recognized within the domain Bacteria; members of this phylum exhibit diverse physiological and metabolic properties, such as the production of extracellular enzymes and the formation of a wide variety of secondary metabolites, however, its role in the microbiota of fish is not well documented (Ventura *et al.*, 2007).

Studies in mammals report that certain *Firmicutes* are linked to the intake of nutrients, and extraction and regulation of energy homeostasis from their diet (Krajmalnik-Brown *et al.*, 2012). However, in rodents an increase of *Firmicutes* may be caused by the increase in the intake of carbohydrates and/or polysaccharides (Turnbaugh *et al.*, 2008). The artificial food of AF containing an 11% of carbohydrates could explain the higher proportion of *Firmicutes* in AF with respect to WF. WF fed mainly on anchovy *Engraulis ringens*, a carbohydrate-free food when eaten fresh (Reyes *et al.*, 2009).

The load and diversity of fish microbiota are influenced by many intrinsic and extrinsic factors (Nayak, 2010). As it has been illustrated in Fig. 1, the load of bacterial microbiota in the intestinal contents of flatfish is roughly similar, in different species and different origin (wild or reared). Food strongly influences the composition of the microbiota in fish. As example, Dhanasiri *et al.* (2011) evidenced a reduction of microbiota diversity when wild fish was fed with artificial diets (*i.e.*, microbiota diversity was reduced more than 60% and *Vibrionaceae* and *Clostridiaceae* increased over *Ignavibacteriaceae* and *Erysipelotrichaceae*). Similar results are reported for *S. senegalensis* (Martin-Antonio *et al.*, 2007) and *P. olivaceus* (Kim & Kim, 2013). Our results for WF also showed greater bacterial diversity than AF (Fig. 2). However, our findings are not consistent with those for wild salmon (*Salmo salar*) and sturgeon (*Acipenser ruthenus*) indicating lower diversity of the microbiota in wild than in farmed fish (Holben *et al.*, 2002; Bacanu & Oprea, 2013).

ACKNOWLEDGEMENTS

J. Salas-Leiva received a scholarship from CONICYT-Chile. This work was funded by Fondecyt 1140734/1171129 from CONICYT and Centro Aquapacífico 15PCTI-46284 from Corfo. The authors thank Victoria Urzúa and Mauricio Valdés for technical assistance. Authors declare no conflicts of interest regarding the publication of this manuscript.

REFERENCES

Amato K.R. 2013. Co-evolution in context: the importance of studying gut microbiomes in wild animals. Microbiome Sci. Med., 1: 10-29.

Bacanu, G.M. & L. Oprea. 2013. Differences in the gut microbiota between wild and domestic *Acipenser ruthenus* evaluated by denaturing gradient gel electrophoresis. Rom. Biotech. Lett., 18: 8069-8076.

Bevins, C.L. & N.H. Salzman. 2011. The potter's wheel: the host's role in sculpting its microbiota. Cell. Mol. Life Sci., 68: 3675-3685.

Colston, T.J. & C.R. Jackson 2016. Invited review: microbiome evolution along divergent branches of the vertebrate tree of life: What's known and unknown. Mol. Ecol., pp. 3776-3800.

Dhanasiri, A.K., L. Brunvold, M.F. Brinchmann, K. Korsnes, O. Bergh & V. Kiron. 2011. Changes in the intestinal microbiota of wild Atlantic cod *Gadus morhua L.* upon captive rearing. Microb. Ecol., 61: 20-30.

Eddy, S.D. & S.H. Jones. 2002. Microbiology of summer flounder *Paralichthys dentatus* fingerling production at a marine fish hatchery. Aquaculture, 211: 9-28.

Figueiredo, H.C.P., C.A.G. Leal, F.A Dorella, A.F. Carvalho, S.C. Soares, F.L. Pereira & V.A.C. Azevedo. 2014. Complete Genome sequences of fish pathogenic weissella ceti strains WS74 and WS105. Genome Announcements, 2(5): e01014-14.

Fusco, V., G.M. Quero, G.S. Cho, J. Kabisch, D. Meske, H. Neve, W. Bockelmann & C.A.M.P. Franz. 2015. The genus Weissella: taxonomy, ecology and biotechnological potential. Front. Microbiol., 6: 155 pp.

González, N., J. Romero & R.T. Espejo. 2003. Comprehensive detection of bacterial populations by PCR amplification of the 16S-23S rRNA spacer region. J. Microbiol. Meth., 5: 91-97.

Hagi, T., D. Tanaka, Y. Iwamura & T. Hoshino. 2004. Diversity and seasonal changes in lactic acid bacteria in the intestinal tract of cultured freshwater fish. Aquaculture, 234: 335-346.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids, S41: 95-98.

Holben, W.E., P. Williams, M. Saarinen, L.K. Särkilahti & J.H. Apajalahti. 2002. Phylogenetic analysis of intestinal microflora indicates a novel *Mycoplasma* phylotype in farmed and wild salmon. Microb. Ecol., 44:1 75-185.

Hovda, M.B., B.T. Lunestad, R. Fontanillas & J.T. Rosnes. 2007. Molecular characterisation of the intestinal microbiota of farmed Atlantic salmon (*Salmo salar L.*). Aquaculture, 272: 581-588.

Kim, D.H. & D.Y. Kim. 2013. Microbial diversity in the intestine of olive flounder (*Paralichthys olivaceus*). Aquaculture, 414: 103-108.

Krajmalnik-Brown, R., Z.E. Ilhan, D.W. Kang & J.K. DiBaise. 2012. Effects of gut microbes on nutrient absorption and energy regulation. Nutr. Clin. Pract., 27: 201-214.

Lauzon, H. & E. Ringø. 2012. Prevalence and application of lactic acid bacteria in aquatic environments. In: S. Lahtinen, A. Ouwehand, S. Salminen & A. Von Wright (eds.). Lactic acid bacteria: microbiological and functional aspects. CRC Press, Boca Raton, pp. 593-631.

Leisner, J.J., B.G. Laursen, H. Prévost, D. Drider & P. Dalgaard. 2007. *Carnobacterium*: positive and negative effects in the environment and in foods. FEMS Microbiol. Rev., 31: 592-613.

Ley, R.E., C.A. Lozupone, M. Hamady, R. Knight & J.I. Gordon. 2008. Worlds within worlds: Evolution of the vertebrate gut microbiota. Nat. Rev. Microbiol., 6: 776-788.

MacDonald, N.L., J.R. Stark & B. Austin. 1986. Bacterial microflora in the gastro-intestinal tract of Dover sole (*Solea solea L.*), with emphasis on the possible role of bacteria in the nutrition of the host. FEMS Microbiol. Lett., 35: 107-111.

Madigan, M.T., J.M. Martinko, P.V. Dunlap & C.P. Clark. 2010. Brock biology of microorganisms. Pearson-Benjamin Cummings, San Francisco, 1061 pp.

Martin-Antonio, B., M. Manchado, C. Infante, R. Zerolo, A. Labella, C. Alonso & J.J. Borrego. 2007. Intestinal microbiota variation in Senegalese sole (*Solea senegalensis*) under different feeding regimes. Aquacult. Res., 38: 1213-1222.

Miranda, C.D. & R. Rojas. 1996. Vibriosis in the flounder *Paralichthys adspersus* (Steindachner, 1867) in captivity. Rev. Biol. Mar. Oceanogr., 31: 1-9.

Montes, M., R. Farto, M.J. Pérez, T.P. Nieto, J.L. Larsen & H. Christensen. 2003. Characterization of Vibrio strains isolated from turbot (*Scophthalmus maximus*) culture by phenotypic analysis, ribotyping and 16S rRNA gene sequence comparison. J. Appl. Microbiol., 95: 693-703.

Navarrete, P., F. Magne, C. Araneda, P. Fuentes, L. Barros, R. Opazo, R. Espejo & J. Romero. 2012. PCR-TTGE analysis of 16S rRNA from rainbow trout (*Oncorhynchus mykiss*) gut microbiota reveals host-specific communities of active bacteria. PLoS ONE, 7: e31335.

Navarrete, P., F. Magne, P. Mardones, M. Riveros, R. Opazo, A. Suau, P. Pochart & J. Romero. 2010. Molecular analysis of intestinal microbiota of rainbow trout (*Oncorhynchus mykiss*). FEMS Microb. Ecol., 71: 148-156.

Nayak, S.K. 2010. Role of gastrointestinal microbiota in fish. Aquacult. Res. 41: 1553-1573.

Neulinger, S.C., J. Järnegren, M. Ludvigsen, K. Lochte & W.C. Dullo. 2008. Phenotype-specific bacterial communities in the cold-water coral *Lophelia pertusa* (Scleractinia) and their implications for the coral's nutrition, health, and distribution. Appl. Environ. Microbiol., 74: 7272-7285.

Reyes, M., I. Gómez-Sánchez, C. Espinoza, F. Bravo & L. Ganoza. 2009. Tablas peruanas de composición de alimentos. Instituto Nacional de Salud, Ministerio de Salud, Lima, 64 pp.

Ringø, E., S. Sperstad, R. Myklebust, T.M. Mayhew & R.E Olsen. 2006. The effect of dietary inulin on aerobic bacteria associated with hindgut of Arctic charr (*Salvelinus alpinus* L.). Aquacult. Res., 37: 891-897.

Roeselers, G., E.K. Mittge, W.Z. Stephens, D.M. Parichy, C.M. Cavanaugh, K. Guillemin & J.F. Rawls. 2011. Evidence for a core gut microbiota in the zebrafish. ISME J., 5: 1595-1608.

Romero, J. & R.T. Espejo. 2001. The prevalence of noncultivable bacteria in oysters (*Tiostrea Chilensis*, Philippi, 1845). J. Shellfish Res., 20: 1235-1240.

Romero, J. & P. Navarrete. 2006. 16S rDNA-based analysis of dominant bacterial populations associated with early life stages of coho salmon (*Oncorhynchus kisutch*). Microb. Ecol. 51: 422-430.

Romero, J., M. García-Varela, J.P. Laclette & R.T. Espejo. 2002. Bacterial 16S rRNA gene analysis revealed that bacteria related to *Arcobacter* spp. constitute an abundant and common component of the oyster microbiota (*Tiostrea chilensis*). Microb. Ecol., 44: 365-371.

Rungrassamee, W., A. Klanchui, S. Maibunkaew, S. Chaiyapechara, P. Jiravanichpaisal & N. Karoonuthaisiri. 2014. Characterization of intestinal bacteria in wild and domesticated adult black tiger shrimp (*Penaeus monodon*). PLoS ONE, 9: e91853.

Silva, A. 2010. Culture of chilean flounder. In: H.V. Daniels & W.O. Watanabe (eds.). Practical flatfish culture and stock enhancement. Wiley-Blackwell, Oxford, pp. 30-45.

Silva, F.C., J.R. Nicoli, J.L. Zambonino-Infante, S. Kaushik & F.J. Gatesoupe. 2011. Influence of the diet on the microbial diversity of faecal and gastrointestinal contents in gilthead sea bream (*Sparus aurata*) and intestinal contents in goldfish (*Carassius auratus*). FEMS Microbiol. Ecol., 78: 285-296.

Smriga, S., S.A. Sandin & F. Azam. 2010. Abundance, diversity, and activity of microbial assemblages associated with coral reef fish guts and feces. FEMS Microbiol. Ecol., 73: 31-42.

Sugita, H. & Y Ito. 2006. Identification of intestinal bacteria from Japanese flounder (*Paralichthys olivaceus*) and their ability to digest chitin. Lett. Appl. Microbiol., 43: 336-342.

Sugita, H., R. Okano, Y. Suzuki, D. Iwai, M. Mizukami, N. Akiyama & S. Matsuura. 2002. Antibacterial abilities of intestinal bacteria from larval and juvenile Japanese flounder against fish pathogens. Fish. Sci., 68: 1004-1011.

Sullam, K.E., S.D. Essinger, C.A. Lozupone, M.P. O'Connor, G.L. Rosen, R. Knight, S.S. Kilham & J.A. Russell. 2012. Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. Mol. Ecol., 21: 3363-3378.

Sun, Y.Z., H.L.Yang, R.L. Ma & W.Y. Lin. 2010. Probiotic applications of two dominant gut *Bacillus* strains with antagonistic activity improved the growth performance and immune responses of grouper *Epinephelus coioides*. Fish Shellfish Immunol., 29: 803-809.

Sun, Y., H. Yang, Z. Ling, J. Chang & J. Ye. 2009. Gut microbiota of fast and slow growing grouper

Epinephelus coioides. Afr. J. Microbiol. Res., 3: 713-720.

Sun, Y.Z., H.L.Yang, R.L. Ma, C.X. Zhang & W.Y. Lin. 2011. Effect of dietary administration of *Psychrobacter* sp. on the growth, feed utilization, digestive enzymes and immune responses of grouper *Epinephelus coioides*. Aquacult. Nutr., 17: 733-e740.

Tanasomwang, V. & K. Muroga. 1988. Intestinal microflora of larval and juvenile stages in japanese flounder (*Paralichthys olivaceus*). Fish Pathol., 23: 77-83.

Tapia-Paniagua, S.T., M. Chabrillón, P. Díaz-Rosales, I.G. de la Banda, C. Lobo, M.C. Balebona & M.A. Moriñigo. 2010. Intestinal microbiota diversity of the flat fish *Solea senegalensis* (Kaup, 1858) following probiotic administration. Microb. Ecol., 60: 310-319.

Turnbaugh, P.J., F. Bäckhed, L. Fulton & J.I. Gordon. 2008. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbiol, 3: 213-223.

Vázquez, J.A., M.P. González & M.A. Murado. 2005. Effects of lactic acid bacteria cultures on pathogenic microbiota from fish. Aquaculture, 245: 149-161.

Ventura, M., C. Canchaya, A. Tauch, G. Chandra, G.F. Fitzgerald, K.F. Chater & D. Van Sinderen. 2007. Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum. Microbiol. Mol. Biol. R., 71: 495-548.

Received: 7 December 2016; Accepted: 10 January 2017

Verner-Jeffreys, D.W., R.J. Shields, I.R. Bricknell & T.H. Birkbeck. 2003. Changes in the gut-associated microflora during the development of Atlantic halibut (*Hippoglossus hippoglossus L.*) larvae in three British hatcheries. Aquaculture, 219: 21-42.

Villamil, L., C. Tafalla, A. Figueras & B. Novoa. 2002. Evaluation of immunomodulatory effects of lactic acid bacteria in turbot (*Scophthalmus maximus*). Clin. Diagn. Lab. Immunol., 9: 1318-1323.

Villamil, L., A. Figueras, A.E. Toranzo, M. Planas & B. Novoa. 2003. Isolation of a highly pathogenic *Vibrio pelagius* strain associated with mass mortalities of turbot, *Scophthalmus maximus* (L.), larvae. J. Fish Dis., 26: 293-303.

Wong, S., T. Waldrop, S. Summerfelt, J. Davidson, F. Barrows, P.B. Kenney, T. Welch, G.D. Wiens, K. Snekvi, J. Rawls & C. Good. 2013. Aquacultured rainbow trout (*Oncorhynchus mykiss*) possess a large core intestinal microbiota that is resistant to variation in diet and rearing density. Appl. Environ. Microbiol., 79: 4974-4984.

Xing, M., Z. Hou, J. Yuan, Y. Liu, Y. Qu & B. Liu. 2013. Taxonomic and functional metagenomic profiling of gastrointestinal tract microbiome of the farmed adult turbot (*Scophthalmus maximus*). FEMS Microbiol. Ecol., 86: 432-443.

Ying, C., W. Lei, L. Jiazhong, S. Zhantao & A. Liguo. 2007. Identification and purification of a novel adhesion-associated protein in a new strain of *Lactobacillus*, L15, from flounder (*Paralichthys olivaceus*). Vet. Microbiol., 122: 116-122.