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Short communication

Antibacterial activity evaluation of the Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) skin mucus, against *Vibrio* bacteria affecting the white shrimp *Penaeus vannamei*

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ABSTRACT. Shrimp production has been affected by disease outbreaks caused by *Vibrio* sp. bacteria. Using a polyculture system has been proposed as preventive management, but until now, the mode of action by which the organisms in polyculture obtain some benefits was unknown. Some studies indicate that these benefits are related to the immune system in the skin mucus. The present study aimed to determine the antibacterial potential of skin mucus in two tilapia varieties, *Oreochromis* sp. (marine adapted) and *O. niloticus* (freshwater), against *Vibrio* bacteria that affect the shrimp *Penaeus vannamei*. Skin mucus extracts were obtained from both varieties of tilapia during spring and winter. The extracts were: acidic, aqueous and two organics. In total, 16 extracts were obtained. During the winter season, no antibacterial activity was detected; however, in the spring, the acidic extract from the marine-adapted tilapia inhibited *V. parahaemolyticus*. Additionally, *V. harveyi* was inhibited by the acidic and organic extracts from both varieties. The aim of this study was confirmed: tilapia skin mucus has antibacterial activity against *Vibrio* bacteria, which depends on the tilapia variety, bacterial strain, season and the concentration of colony forming units. SDS-PAGE revealed a protease of 96 kD present in all extracts, even in those without antibacterial activity.

Keywords: *Oreochromis niloticus*; tilapia; bacteria; mucus; tegument; immune system

Shrimp farming is an activity focused on food production, and it has shown exponential growth worldwide. Production has been affected by outbreaks of diseases (Lightner, 1999) caused by bacteria (Nunan *et al.*, 2013), mainly those belonging to the genus *Vibrio* (Soto-Rodriguez *et al.*, 2010a). As a preventive measure against disease outbreaks and economic losses in shrimp farming, polyculture systems, or co-culture, have been implemented (Cruz *et al.*, 2008). In shrimp-tilapia polyculture, the crustacean has benefited, showing high resistance to pathogens (NPPMCI, 2000). However, the mode of action by which the tilapia provide those benefits to shrimp has not yet been defined (Cruz *et al.*, 2008). A pilot study by Austin & McIntosh (1988) revealed the importance of molecules in Fish Skin Mucus (FSM) found in rainbow trout (*Oncorhynchus mykiss*), and later, in a wide range of fish (Subramanian *et al.*, 2007; Guardiola *et al.*, 2017). In acidic, organic and aqueous solvent extraction of FSM has been described the presence of active molecules (Hellio *et al.*, 2002; Subramanian *et al.*, 2008) against pathogens such as bacteria, viruses, fungi, protozoa and some yeasts with human and aquaculture health importance (Fuochi *et al.*, 2017; Guardiola *et al.*, 2017). The antimicrobial capability of FSM depends on the origin of the fish, according to Subramanian *et al.* (2008), marine fish develop higher antimicrobial activity than freshwater fishes. The present study aimed to evaluate the antibacterial activity of extracts obtained from the epidermal mucus of two varieties of tilapia, *Oreochromis* sp. (adapted to seawater), and *Oreochromis niloticus* (freshwater), against pathogenic bacteria *Vibrio parahaemolyticus* E9-2, and *Vibrio harveyi* CAIM 1792 that affect the white shrimp *Penaeus vannamei*.

The study evaluated the antimicrobial activity of 16 extracts from two species of fish during two different seasons on two pathogenic *Vibrio* bacteria. The study was conducted at the Instituto Politécnico Nacional-CIIDIR Sinaloa. The bacteria strain *Vibrio parahaemo-*

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lyticus E9-2 was donated by the biochemistry and cell biology laboratories in CIIDIR-Sinaloa [http://www.ciidirsinaloa.ipn.mx/-investigacion/2qygyubnPaginas/Acuacultura.aspx]. The bacteria strain *Vibrio harveyi* CAIM 1792 was donated by CIAD Mazatlán [https://www.ciad.mx/caim/CAIM] and verified to be pathogenic to shrimp (Soto-Rodríguez *et al.*, 2010b). The freshwater tilapia *O. niloticus* stock was produced in IPN-CIIDIR Sinaloa. The seawater adapted *Oreochromis* sp. stock was captured in Navachiste Bay, Sinaloa, Mexico. Both fish varieties had an average weight of 250 ± 10 g were apparently healthy, and were maintained, following the guidelines of Chervinski (1982) in four concrete (4,000 L) tanks, two tanks for each tilapia variety, and 40 fish per tank, kept at 28 ± 3ºC during the spring and 18 ± 3ºC during the winter; meanwhile, the salinity was kept at 26 and 3 for adapted and freshwater tilapia respectively. Both varieties were fed commercial feed (Nutripec, Purina®) twice per day, corresponding to 3% of their biomass.

The mucus collection was conducted as described by Subramanian *et al.* (2008). Thirty-five fish of each variety were individually sampled by introducing them into a polyethylene bag (Ziploc®) containing 8 mL of 50 mM NaCl for freshwater variety, and 100 mM NaCl for the adapted fish variety, 280 mL total volume. The fish was gently moved back and forth inside the bag for 1-2 min to slough off the mucus. The mucus samples obtained from each fish were mixed and homogenized by variety to test them independently. The homogenized mucus from each fish variety was then divided into three parts, which were subjected separately to acidic, organic, and aqueous solvent extractions (Table 1).

The acidic and aqueous extract of mucus was prepared using the method of Subramanian *et al.* (2008). The elutions were freeze-dried and resuspended in the water when assayed for antimicrobial activity. The organic extracts: aqueous and dichloromethane phase (DCM) of the mucus were prepared as described by Hellio *et al.* (2002).

Both bacteria strains were activated by culturing them at 32ºC in Tryptic Soy Agar plates overnight (TSA, BD®), added with NaCl (2% w/v). A growing kinetic curve was elaborated for each bacterium considering nine points during 8 h in TSA Broth. The optical density was determined per hour analyzing 100 µL of each well, and other 100 µL were utilized to quantify the colony forming units per mL (CFU mL^{-1}) of *Vibrio* sp. bacteria in TCBS plaques. The CFU count and the absorbance per hour were analyzed by linear regression and correlation.

Minimal Inhibitory Concentration (MIC) was calculated following the guidelines of the Clinical & Laboratory Standards Institute (CLSI, 2015), which classifies the intensity of the MIC as susceptible, intermediate or resistant. The antibiotic enrofloxacin (Laboratorios Tornel, Mexico®) was utilized as the control. Nine antibiotic concentrations in triplicate were prepared, and tested to detect the breakpoint: 36, 18, 9, 4.5, 2.25, 1.125, 0.56, 0.28, 0.14 and 0.07 µg mL^{-1}. Each concentration was added to a sterile 96-well plate, with 10 µL of the bacterial solution (1×10^5 CFU mL^{-1}), and 180 µL of TSB (TSB + NaCl 2% w/v). The plate was incubated at 32ºC, and the Optical Density at 610 nm ($OD_{610\ nm}$) was determined every hour for 24 h. Both *Vibrio* strains were analyzed.

The antibacterial activity of the extracts was evaluated according to Fernández (2008) in sterile 96-well plates considering 1×10^7, 5×10^7, and 1×10^8 CFU mL^{-1}. As a positive control, 10 µL of enrofloxacin (4.5 µg mL^{-1}) was added instead of extract, and no antibiotic or extract was used for the negative inhibition control. The percentage of bacterial growth inhibition was calculated as:

$$\text{Percentage inhibition}=100\times\frac{(OD_{\text{negative control}}-OD_{\text{test compound}})}{(OD_{\text{negative control}}-OD_{\text{positive control}})}$$

The protein concentration of the extracts of both varieties was determined following the method of Bradford (1976), with bovine serum albumin as the standard. Acids extracts from both fishes containing protein, and antibacterial activity including the extract FA without antibacterial activity, were analyzed by vertical sodium dodecyl sulfate-PolyAcrylamyde Gel Electrophoresis (SDS-PAGE) 12%, for 2 h at a 20 mA constant current at 4°C. Molecular weights were identified utilizing Low-Range Molecular Weights Marker (SIGMA M3913). Proteases were revealed by the technique of protease activity (Zymogram) in the gel, according to García-Carreño & Haard (1993). The results were expressed in average and standard deviation. For comparing the inhibition percentage values among extracts and proteins concentrations, the Kruskal-Wallis and Dunn tests ($\alpha = 0.05$) were applied to utilize Statistica v.10.

Approximately 300 mL of FSM were collected from each tilapia variety (freshwater or seawater), and season (spring or winter), 280 mL of solution and 20 mL of mucus. From that 300 mL, four extracts were obtained, for 16 extracts total (For extract abbreviations, see table 1).

Results showed that in 0.34 $DO_{610\ nm}$ of a *V. parahaemolyticus* or *V. harveyi* solution the CFU was 1×10^8 CFU mL^{-1}. The antibiogram revealed that the MIC of enrofloxacin was 4.5 µg mL^{-1} for both bacteria strains. According to the Clinical & Laboratory Standards Institute, these strains are classified as susceptible.

Table 1. The protein concentration of skin mucus extracts of both varieties of tilapia (adapted to the marine environment and freshwater) of spring and winter season. ND: Non-Detected.

Keys	Extracts	Spring extract (mg mL^{-1})	Winter extract (mg mL^{-1})
	Adapted tilapia		
AA	Acid	2.9 ± 0.009	0.478 ± 0.007
AAQ	Aqueous	1.4 ± 0.05	0.037 ± 0.002
AO	Aqueous phase organic	ND	ND
ADO	Dichloromethane phase organic	ND	ND
	Freshwater tilapia		
FA	Acid	1.6 ± 0.06	0.495 ± 0.010
FAQ	Aqueous	1.2 ± 0.02	0.281 ± 0.008
FO	Aqueous phase organic	ND	ND
FDO	Dichloromethane phase organic	ND	ND

From the eight extracts obtained during the spring, the AA extract was the only one that showed antibacterial activity against *V. parahaemolyticus*. The highest inhibition was detected for 1×10^7 CFU mL^{-1} and significantly ($P \leq 0.05$) declined with the increment of the bacteria concentration (Table 2). Four extracts showed antibacterial activity against *V. harveyi*. Both acidic extracts AA and AF showed almost 100% inhibition for 1×10^7 CFU mL^{-1} and 5×10^7 CFU mL^{-1}. Meanwhile, the extracts ADO and FDO significantly decreased the Minimal Inhibitory Concentration for 5×10^7 CFU mL^{-1} (Table 2). The AA extract from the spring was the only one that registered antibacterial activity against both strains (*V. parahaemolyticus* and *V. harveyi*). The antibacterial activity was similar for both strains when tested against 1×10^7 CFU mL^{-1}, but inhibition was almost null for *V. parahaemolyticus,* and up to 90% for *V. harveyi* when tested with 5×10^7 CFU mL^{-1} (Table 2). The antibacterial capacity of both extracts, FDO and ADO, was over 90% against 1×10^7 CFU mL^{-1} of *V. harveyi*, and significantly declined at 5×10^7 CFU mL^{-1}. However, the extracts obtained during the winter season did not show antibacterial activity against any bacterial.

The Bradford method, which is used to quantify soluble protein, detected protein in eight extracts: four extracts from the spring, and four from the winter. Acidic extracts from both tilapia varieties contained higher protein concentrations than aqueous extracts from both seasons (Table 1). The four acids extracts analyzed by SDS-PAGE revealed two groups of proteins, the first one is composed of 4 bands of proteins between 6 and 20 kDa, the second one is four bands of proteins between 66 and 97 kDa. Also, extracts from FSM of freshwater tilapia during winter with no antibacterial activity revealed three extra bands between 45 and 60 kDa. Zymogram showed the presence of one protease of 97 kDa in extracts with antimicrobial activity, and in that without antimicrobial activity (Fig. 1).

Diseases caused by bacteria in farmed shrimp have been believed to be latent. According to Noriega-Orozco *et al.* (2007), bacteria populations are always present in aquaculture ponds, including pathogenic and non-pathogenic bacteria of the genus *Vibrio.* In the search for novel alternative practices to control pathogens in aquaculture, the present study shows the potentiality of skin mucus in *O. niloticus* (freshwater) and *Oreochromis* sp. (marine adapted) fish, as a biological control against the bacteria *V. harveyi* CAIM 1792 and *V. parahaemolyticus* E9-2 that have drastically affected shrimp farming (Soto-Rodriguez *et al.*, 2010b). This biological control could act as observed in a variety of pathogenic microorganisms that are vulnerable to FSM including bacteria, viruses, fungi, protozoa and yeasts (Fuochi *et al.*, 2017; Guardiola *et al.*, 2017). In the present study, the FSM of adapted tilapia showed significantly higher protein concentrations than freshwater tilapia, and spring samples from both fish varieties presented significantly higher protein concentrations than those from the winter. These results are similar to those found in other marine fish (Palaksha *et al.*, 2008). A wide range of molecules present in the skin mucus was obtained from different fish according to the extraction type. Acidic extracts rich with basic peptides/proteins were obtained by Diamond *et al.* (1991). Secondary metabolites were extracted with organic solvents (ethanol), and polar metabolites were grouped in the aqueous phase. Subramanian *et al.* (2008) found that the antibacterial activity of the acidic extract is caused by low molecular weight peptides, which act with disruptive (lytic) function or formation of pores in the bacterial cellular

Table 2. Antibacterial activity of skin mucus extracts from tilapia during spring season against *Vibrio parahaemolyticus* E9-2 and *Vibrio harveyi* CAIM 1792 at different CFU concentration. Ctrl-: NaCl 2.5% (w/v) by AA, and FA, dichloromethane solution by ADO and FDO. Ctrl+: Antibiotic enrofloxacin 4.5 µg mL^{-1}. The different superscript letter means a significant difference among concentration per extract.

Strains	Keys	1×10^7 CFU mL^{-1}	5×10^7 CFU mL^{-1}	1×10^8 CFU mL^{-1}
V. parahaemolyticus E9-2	Ctrl -	-	-	-
	Ctrl +	100.0 ± 0.00	100 ± 0.00	100 ± 0.00
	AA	99.2 ± 0.40^a	2.6 ± 1.00^b	-
V. harveyi CAIM 1792	Ctrl -	-	-	-
	Ctrl +	100.0 ± 0.00	100 ± 0.00	100 ± 0.00
	AA	96.8 ± 1.00^a	97.5 ± 1.00^a	8.8 ± 0.00^b
	FA	98.1 ± 1.00^a	97.6 ± 1.00^a	4.3 ± 3^b
	ADO	89.9 ± 0.00^a	3.9 ± 1.00^b	-
	FDO	99.5 ± 1.00^a	1.3 ± 1.00^b	-

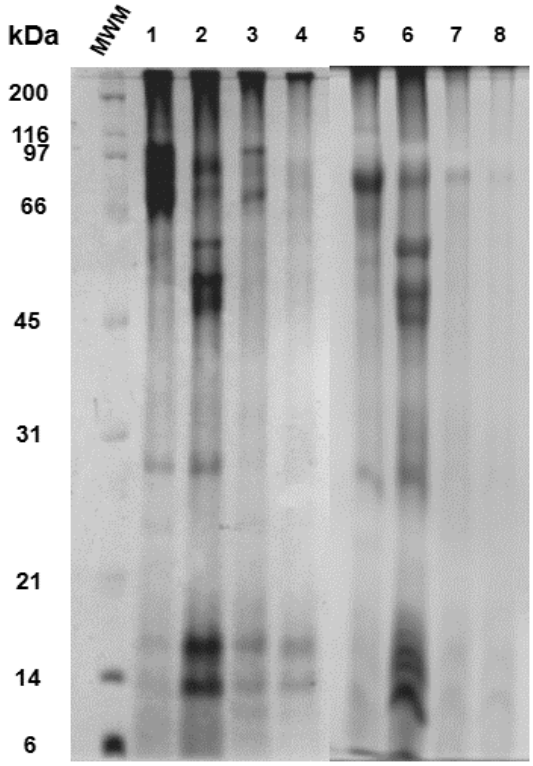

Figure 1. Skin mucus acid extracts electrophoretic analysis of freshwater and adapted tilapia. Lines 1-4: SDS-PAGE analysis and lines 5-8: Zymogram. MWM: molecular weight marker. Lines 1 and 5: winter adapted tilapia, 2 and 6: winter freshwater tilapia, 3 and 7: spring adapted tilapia, 4 and 8: spring freshwater tilapia.

membrane (Shabir *et al.*, 2018). Subramanian *et al.* (2008) described differences according to the fish origin, and the bacteria strains tested, as occurred in our findings where the AA extract inhibited *V. parahaemolyticus* growth; while the AD extract did not. Nonpolar secondary metabolites are free and abundant within the organic extract (DCM). Hellio *et al.* (2002) suggest that antimicrobial activity was correlated with the polarity of extracts, the more polar the extracts, the less active they are. In our results, the DCM extracts of both fish varieties exert antibacterial activity against *V. harveyi* suggesting the action of these molecules at the experimental conditions. Molecules such as hydrolytic enzymes (proteases and lysozymes) and glycoproteins were obtained with aqueous extraction (Nagashima *et al.*, 2001; Subramanian *et al.*, 2007). Our results showed a protein of approximately 97 kD that shows protease activity and is present in extracts from both fish and seasons.

This finding implies that this protease is not responsible for the antimicrobial activity of the aqueous extract because all the extracts contain it. In other studies, Hisar *et al.* (2014) found that aqueous extracts of the FSM do not exert antimicrobial activity against different bacteria. Similar results were recorded in this study. Interestingly, Subramanian *et al.* (2007) found hydrolytic enzymes, such as lysozymes, B cathepsin and protease trypsin, with activity in aqueous extracts of the FSM from some marine and freshwater fish. However, they confirmed that these molecules do not contribute to the antimicrobial activity. We can conclude that the skin mucus of the *O. niloticus* (freshwater) and *Oreochromis* sp. (adapted marine environment), presents antibacterial activity against *V. harveyi* CAIM 1792 and *V. parahaemolyticus* E9-2, that have drastically affected shrimp farming. Now, it is essential to evaluate the functionality of a polyculture system as a biological control method. Shrimp farmers have used *Oreochromis* sp. adapted to a marine environment in polyculture without knowing how to utilize the full benefits the method has for shrimp. The FSM was effective against *Vibrio harveyi* CAIM 1792 and *V. parahaemolyticus* E9-2, in higher concentrations

than 3 to 460 CFU mL^{-1} reported by Noriega-Orozco *et al.* (2007). Meanwhile, in semi-intensive shrimp farming ponds, under normal conditions, Suárez *et al.* (2015) found 21 CFU mL^{-1} in water and 740 CFU g^{-1} of *Vibrio* sp. in the sediment of ponds. In the same form, Lara (2011) found 3×10^5 CFU mL^{-1} of *Vibrio* sp. in the same culture system, while Soto-Rodriguez *et al.* (2010a) found 1×10^3 CFU mL^{-1} and 1×10^5 CFU g^{-1} of *Vibrio* sp.

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REFERENCES

Austin, B. & McIntosh, D. 1988. Natural antibacterial compounds on the surface of rainbow trout, *Salmo gairdneri* Richardson. Journal of Fish Diseases, 11: 275-277.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-254.

Chervinski, J. 1982. Environmental physiology of tilapias. In: Pullin, R.S.V. & Lowe-McConnell, R.H. (Eds.). The biology and culture of tilapias. International Center for Living Aquatic Resources Management, Manila, pp. 119-128.

Clinical and Laboratory Standards Institute (CLSI). 2015. Performance standards for antimicrobial susceptibility testing. Informational supplement (M100-S25). Antimicrobial susceptibility testing standards M02-A12, M07-A10, and M11-A8. CLSI, Pennsylvania.

Cruz, P.S., Andalecio, M.N., Bolivar, R.B. & Fitzsimmons, K. 2008. Tilapia-shrimp polyculture in Negros Island, Philippines: a review. Journal of the World Aquaculture Society, 39: 713-725.

Diamond, G., Zasloff, M., Eck, H., Brasseur, M., Maloy, W.L. & Bevins, C.L. 1991. Tracheal antimicrobial peptide, a cysteine-rich peptide from mammalian tracheal mucosa: peptide isolation and cloning of a cDNA. Proceedings of the National Academy of Sciences, 88: 3952-3956.

Fernández, S. 2008. Estudio exploratorio de la presencia de actividad antibacteriana en la sangre de la concha prieta *Anadara similis*. Tesis de Grado, Facultad de Ingeniería Marítima, Ciencias Biológicas, Oceánicas y Recursos Naturales, Guayaquil, 79 pp.

Fuochi, V., Li-Volti, G., Camiolo, G., Tiralongo, F., Giallongo, C., Distefano, A., Petronio, G., Barbagallo, I., Viola, M. & Furneri, P.M. 2017. Antimicrobial and anti-proliferative effects of skin mucus derived from *Dasyatis pastinaca* (Linnaeus, 1758). Marine Drugs, 15(11): E342.

García-Carreño, F.L. & Haard, N.F. 1993. Characterization of proteinase classes in langostilla (*Pleuroncodes planipes*) and crayfish (*Pacifastacus astacus*) extracts. Journal of Food Biochemistry, 17: 97-113.

Guardiola, F.A., Cuartero, M., Collado-González, M., Baños, F.G.D., Cuesta, A., Moriñigo, M.Á. & Esteban, M.Á. 2017. Terminal carbohydrates abundance, immune related enzymes, bactericidal activity and physico-chemical parameters of the Senegalese sole (*Solea senegalensis*, Kaup) skin mucus. Fish and Shellfish Immunology, 60: 483-491.

Hellio, C., Pons, A.M., Beaupoil, C., Bourgougnon, N. & Le Gal, Y. 2002. Antibacterial, antifungal and cytotoxic activities of extracts from fish epidermis and epidermal mucus. International Journal of Antimicrobial Agents, 20: 214-219.

Hisar, Ş.A., Hisar, O., Yılmaz, S., Çakır, F., Şahin, T. & Uyanık, M.H. 2014. *In vitro* antimicrobial and antifungal activities of aqueous skin mucus from rainbow trout (*Oncorhynchus mykiss*) on human pathogens. Marine Science and Technology Bulletin, 3: 19-22.

Lara, A.G.F. 2011. Manejo de la microbiota para mantener la calidad de agua en cultivos experimentales de camarón *Litopenaeus vannamei* sin recambio de agua. Tésis de Maestría, Instituto Politécnico Nacional - Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Unidad Sinaloa, 56 pp.

Lightner, D.V. 1999. The penaeid shrimp viruses TSV, IHHNV, WSSV, and YHV: current status in the Americas, available diagnostic methods, and management strategies. Journal of Applied Aquaculture, 9: 27-52.

Nagashima, Y., Sendo, A., Shimakura, K., Shiomi, K., Kobayashi, T., Kimura, B. & Fujii, T. 2001. Antibacterial factors in skin mucus of rabbitfishes. Journal of Fish Biology, 58: 1761-1765.

Noriega-Orozco, L., Acedo-Félix, E., Higuera-Ciapara, I., Jiménez-Flores, R. & Cano, R. 2007. Pathogenic and non pathogenic *Vibrio* species in aquaculture shrimp ponds. Revista Latinoamericana de Microbiología, 49: 60-67.

Negros Producers Marketing Coop. Inc. (NPPMCI). 2000. Successful prawn culture management techniques in Bacolod City. Bureau of Fisheries and Aquatic Resources. NPPMCI, Negros Occidental.

Nunan, L. M., Pantoja, C.R., Gomez-Jimenez, S. & Lightner, D.V. 2013. "*Candidatus Hepatobacter penaei*," an intracellular pathogenic enteric bacterium in the hepatopancreas of the marine shrimp *Penaeus vannamei* (Crustacea: Decapoda). Applied and Environmental Microbiology, 79: 1407-1409.

Palaksha, K., Shin, G.W., Kim, Y.R. & Jung, T.S. 2008. Evaluation of non-specific immune components from the skin mucus of olive flounder (*Paralichthys olivaceus*). Fish and Shellfish Immunology, 24: 479-488.

Shabir, U., Ali, S., Magray, A.R., Ganai, B.A., Firdous, P., Hassan, T. & Nazir, R. 2018. Fish antimicrobial peptides (AMP's) as essential and promising molecular therapeutic agents: a review. Microbial Pathogenesis, 114: 50-56.

Soto-Rodriguez, S.A., Gomez-Gil, B. & Lozano, R. 2010b. 'Bright-red' syndrome in Pacific white shrimp *Litopenaeus vannamei* is caused by *Vibrio harveyi*. Diseases of Aquatic Organisms, 92: 11-19.

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Soto-Rodriguez, S.A., Gomez-Gil, B., Lozano, R. & Roque, A. 2010a. Density of vibrios in hemolymph and hepatopancreas of diseased Pacific white shrimp, *Litopenaeus vannamei*, from northwestern Mexico. Journal of the World Aquaculture Society, 41: 76-83.

Suárez, M.G., Medina, Z., Montiel, M., Ibarra, J. & Salcedo, A. 2015. Distribución de *Vibrio* spp. en agua y sedimento de estanques productores de camarón *Litopenaeus vannamei* cultivados con agua del Lago de Maracaibo (Venezuela). Revista Científica, 25: 293-299.

Subramanian, S., MacKinnon, S.L. & Ross, N.W. 2007. A comparative study on innate immune parameters in the epidermal mucus of various fish species. Comparative Biochemistry and Physiology B, 148: 256-263.

Subramanian, S., Ross, N.W. & MacKinnon, S.L. 2008. Comparison of antimicrobial activity in the epidermal mucus extracts of fish. Comparative Biochemistry and Physiology B, 150: 85-92.