

---

## Title

### ***Evaluation of the Biological Activities of Peptides from Epidermal Mucus of Marine Fish Species from Chilean Aquaculture***

## Abstract

The skin of fish is a physicochemical barrier that is characterized by being formed by cells that secrete molecules responsible for the first defense against pathogenic organisms. In this study, the biological activity of peptides from mucus of *Seriola lalandi* and *Seriola violacea* were identified and characterized. To this purpose, peptide extraction was carried out from epidermal mucus samples of juveniles of both species, using chromatographic strategies for purification. Then, the peptide extracts were characterized to obtain the amino acid sequence by mass spectrometry. Using bioinformatics tools for predicting antimicrobial and antioxidant activity, 12 peptides were selected that were chemically produced by simultaneous synthesis using the Fmoc-Tbu strategy. The results revealed that the synthetic peptides presented a random coil or extended secondary structure. The analysis of antimicrobial activity allowed it to be discriminated that four peptides, named by their synthesis code 5065, 5069, 5070, and 5076, had the ability to inhibit the growth of *Vibrio anguillarum* and affected the copepodite stage of *C. rogercresseyi*. On the other hand, peptides 5066, 5067, 5070, and 5077 had the highest antioxidant capacity. Finally, peptides 5067, 5069, 5070, and 5076 were the most effective for inducing respiratory burst in fish leukocytes. The analysis of association between composition and biological function revealed that the antimicrobial activity depended on the presence of basic and aromatic amino acids, while the presence of cysteine residues increased the antioxidant activity of the peptides. Additionally, it was observed that those peptides that presented the highest antimicrobial capacity were those that also stimulated respiratory burst in leukocytes. This is the first work

---

that demonstrates the presence of functional peptides in the epidermal mucus of Chilean marine fish, which provide different biological properties when the fish face opportunistic pathogens. © 2024 by the authors.

## Authors

Álvarez C.A.; Toro-Araneda T.; Cumillaf J.P.; Vega B.; Tapia M.J.; Roman T.; Cárdenas C.; Córdova-Alarcón V.; Jara-Gutiérrez C.; Santana P.A.; Guzmán F.

## Author full names

Álvarez, Claudio A. (56519164500); Toro-Araneda, Teresa (58888514600); Cumillaf, Juan Pablo (34871772700); Vega, Belinda (56407037300); Tapia, María José (58888090600); Roman, Tanya (57210283464); Cárdenas, Constanza (7003841627); Córdova-Alarcón, Valentina (57212675626); Jara-Gutiérrez, Carlos (56997397900); Santana, Paula A. (55364467400); Guzmán, Fanny (7004463815)

## Author(s) ID

56519164500; 58888514600; 34871772700; 56407037300; 58888090600;  
57210283464; 7003841627; 57212675626; 56997397900; 55364467400;  
7004463815

## Year

2024

---

## Source title

Marine Drugs

## Volume

22

## Issue

6.0

## Art. No.

248.0

## DOI

10.3390/md22060248

## Link

<https://www.scopus.com/inward/record.uri?eid=2-s2.0-85197044385&doi=10.3390%2fmd22060248&partnerID=40&md5=c80ef4482fb1064fda15878af8cbeb3c>

## Affiliations

Laboratorio de Cultivo de Peces Marinos, Facultad de Ciencias del Mar, Universidad

---

*Evaluation of the Biological Activities of Peptides from Epidermal Mucus of Marine Fish Species from Chilean Aquaculture*

---

Católica del Norte, Coquimbo, 1781421, Chile; Laboratorio de Fisiología y Genética Marina (FIGEMA), Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Coquimbo, 1781421, Chile; CRC Innovación, Puerto Montt, 5507642, Chile; Núcleo Biotecnología Curauma, Pontificia Universidad Católica de Valparaíso, Valparaíso, 2373223, Chile; Genomics on the Wave SpA, Viña del Mar, 2520056, Chile; Centro Interdisciplinario de Investigación Biomédica e Ingeniería para la Salud, MEDING, Universidad de Valparaíso, Valparaíso, 2362905, Chile; Facultad de Medicina, Escuela de Kinesiología, Universidad de Valparaíso, Valparaíso, 2362905, Chile; Instituto de Ciencias Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, Santiago, 8910060, Chile

## **Authors with affiliations**

Álvarez C.A., Laboratorio de Cultivo de Peces Marinos, Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, 1781421, Chile, Laboratorio de Fisiología y Genética Marina (FIGEMA), Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Coquimbo, 1781421, Chile; Toro-Araneda T., Laboratorio de Fisiología y Genética Marina (FIGEMA), Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Coquimbo, 1781421, Chile; Cumillaf J.P., CRC Innovación, Puerto Montt, 5507642, Chile; Vega B., Laboratorio de Fisiología y Genética Marina (FIGEMA), Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Coquimbo, 1781421, Chile; Tapia M.J., Laboratorio de Fisiología y Genética Marina (FIGEMA), Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Coquimbo, 1781421, Chile; Roman T., Núcleo Biotecnología Curauma, Pontificia Universidad Católica de Valparaíso, Valparaíso, 2373223, Chile; Cárdenas C., Núcleo Biotecnología Curauma, Pontificia Universidad Católica de Valparaíso, Valparaíso, 2373223, Chile; Córdova-Alarcón V., Laboratorio de Fisiología y Genética Marina (FIGEMA), Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Coquimbo, 1781421, Chile, Genomics on the Wave SpA, Viña del

---

Mar, 2520056, Chile; Jara-Gutiérrez C., Centro Interdisciplinario de Investigación Biomédica e Ingeniería para la Salud, MEDING, Universidad de Valparaíso, Valparaíso, 2362905, Chile, Facultad de Medicina, Escuela de Kinesiología, Universidad de Valparaíso, Valparaíso, 2362905, Chile; Santana P.A., Instituto de Ciencias Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, Santiago, 8910060, Chile; Guzmán F., Núcleo Biotecnología Curauma, Pontificia Universidad Católica de Valparaíso, Valparaíso, 2373223, Chile

### **Author Keywords**

antimicrobial; antioxidant; mucus; peptide; respiratory burst; *Seriola lalandi*; *Seriola violacea*

### **Index Keywords**

Animals; Anti-Bacterial Agents; Antioxidants; Aquaculture; Chile; Epidermis; Fishes; Mucus; Peptides; *Vibrio*; *Vibrio anguillarum*; antiinfective agent; antioxidant; peptide; animal; aquaculture; chemistry; Chile; drug effect; epidermis; fish; isolation and purification; mucus; *Vibrio*; *Vibrio anguillarum*

### **Chemicals/CAS**

Anti-Bacterial Agents, ; Antioxidants, ; Peptides,

### **Funding Details**

ANID-Chile; FONDECYT, (1230712, FOVI230160, FOVI230188)

---

## Funding Texts

This work was supported by grants from ANID-Chile, FONDECYT 1210056 to F.G., FONDECYT 1230712 and FOVI230160 to C.A.A., and FOVI230188 to P.A.S.

## References

Nerici C., Merino G., Silva A., Effects of Two Temperatures on the Oxygen Consumption Rates of *Seriolella violacea* (Palm Fish) Juveniles under Rearing Conditions, *Aquac. Eng.*, 48, pp. 40-46, (2012); Alveal K., Silva A., Lohrmann K.B., Viana M.T., Morphofunctional Characterization of the Digestive System in the Palm Ruff Larvae, *Seriolella violacea* under Culture Conditions, *Aquaculture*, 501, pp. 51-61, (2019); Allen P.J., Brokordt K., Oliva M., Alveal K., Flores H., Alvarez C.A., Physiological Insights for Aquaculture Diversification: Swimming Capacity and Efficiency, and Metabolic Scope for Activity in Cojinoba *Seriolella violacea*, *Aquaculture*, 531, (2021); Alvarez C.A., Alvarado J.F., Farias M., Carcamo C.B., Flores H., Guzman F., Martin S.S., Varas J., Messina S., Acosta F., Et al., First Insights about Orexigenic Activity and Gastrointestinal Tissue Localization of Ghrelin from Corvina Drum (*Cilus gilberti*), *Aquaculture*, 571, (2023); Miccoli A., Saraceni P.R., Scapigliati G., Vaccines and Immune Protection of Principal Mediterranean Marine Fish Species, *Fish Shellfish Immunol*, 94, pp. 800-809, (2019); Sicuro B., Luzzana U., The State of *Seriola* Spp. Other Than Yellowtail (*S. quinqueradiata*) Farming in the World, *Rev. Fish. Sci. Aquac.*, 24, pp. 314-325, (2016); Ji Q., Wang S., Ma J., Liu Q., A Review: Progress in the Development of Fish *Vibrio* Spp. Vaccines, *Immunol. Lett.*, 226, pp. 46-54, (2020); Miranda C.D., Rojas R., Vibriosis in the Flounder *Paralichthys adspersus* (Steindachner, 1867) in Captivity, *Rev. Biol. Mar.*, 12, (1996); Rubio-Godoy M., Inmunología de Los Peces Óseos: Revisión Teleost Fish Immunology: Review, *Rev. Mex. Cienc. Pecu.*, 1, pp. 47-57, (2010); Concha K., Olivares P.,

---

Fonseca-Salamanca F., Sanchez R., Serrano F., Parodi J., Mucogenic Additives for the Control of *Caligus Rogerresseyi* in Atlantic Salmon (*Salmo salar*), *Rev. Investig. Vet. Peru*, 28, pp. 477-489, (2017); Benhamed S., Guardiola F.A., Mars M., Esteban M.A., Pathogen Bacteria Adhesion to Skin Mucus of Fishes, *Vet. Microbiol*, 171, pp. 1-12, (2014); Aranishi F., Nakane M., Epidermal proteases of the Japanese eel, *Fish Physiol. Biochem*, 16, pp. 471-478, (1997); Sanahuja I., Fernandez-Alacid L., Ordonez-Grande B., Sanchez-Nuno S., Ramos A., Araujo R.M., Ibarz A., Comparison of Several Non-Specific Skin Mucus Immune Defences in Three Piscine Species of Aquaculture Interest, *Fish Shellfish Immunol*, 89, pp. 428-436, (2019); Zhang L., Falla T.J., Cosmeceuticals and Peptides, *Clin. Dermatol*, 27, pp. 485-494, (2009); Shabir U., Ali S., Magray A.R., Ganai B.A., Firdous P., Hassan T., Nazir R., Fish Antimicrobial Peptides (AMP's) as Essential and Promising Molecular Therapeutic Agents: A Review, *Microb. Pathog*, 114, pp. 50-56, (2018); Valero Y., Saraiva-Fraga M., Costas B., Guardiola F.A., Antimicrobial Peptides from Fish: Beyond the Fight against Pathogens, *Rev. Aquac*, 12, pp. 224-253, (2020); Reddy K.V.R., Yedery R.D., Aranha C., Antimicrobial Peptides: Premises and Promises, *Int. J. Antimicrob. Agents*, 24, pp. 536-547, (2004); Medina M., Prado-Barragan B., Martinez-Hernandez A., Ruiz H.A.A., Rodriguez R.M., Contreras-Esquivel A., Aguilar C.N., Péptidos Bio-Funcionales: Bioactividad, Producción y Aplicaciones. Bio-Functional Peptides: Bioactivity, Production and Applications, *Rev. Científica Univ. Autónoma Coahuila*, 11, pp. 1-7, (2019); Zhou X., Wang C., Jiang A., Antioxidant Peptides Isolated from Sea Cucumber *Stichopus japonicus*, *Eur. Food Res. Technol*, 234, pp. 441-447, (2012); Li Z., Zhang S., Gao J., Guang H., Tian Y., Zhao Z., Wang Y., Yu H., Structural and Functional Characterization of CATH\_BRALE, the Defense Molecule in the Ancient Salmonoid, *Brachymystax lenok*, *Fish Shellfish Immunol*, 34, pp. 1-7, (2013); Guo Z., Qiao X., Cheng R., Shi N., Wang A., Feng T., Chen Y., Zhang F., Yu H., Wang Y., As-CATH4 and 5, Two Vertebrate-Derived Natural Host Defense Peptides, Enhance the Immuno-Resistance Efficiency against Bacterial Infections in Chinese

---

Mitten Crab, *Eriocheir sinensis*, *Fish Shellfish Immunol*, 71, pp. 202-209, (2017); Carvajal-Rondanelli P., Arostica M., Marshall S.H., Albericio F., Alvarez C.A., Ojeda C., Aguilar L.F., Guzman F., Inhibitory Effect of Short Cationic Homopeptides against Gram-Negative Bacteria, *Amino Acids*, 48, pp. 1445-1456, (2016); Santana P.A., Salinas N., Alvarez C.A., Mercado L.A., Guzman F., Alpha-Helical Domain from IL-8 of Salmonids: Mechanism of Action and Identification of a Novel Antimicrobial Function, *Biochem. Biophys. Res. Commun*, 498, pp. 803-809, (2018); Alvarez C.A., Guzman F., Cardenas C., Marshall S.H., Mercado L., Antimicrobial Activity of Trout Hecpudin, *Fish Shellfish Immunol*, 41, pp. 93-101, (2014); Zhang J., Yu L., Li M., Sun L., Turbot (*Scophthalmus maximus*) Hecpudin-1 and Hecpudin-2 Possess Antimicrobial Activity and Promote Resistance against Bacterial and Viral Infection, *Fish Shellfish Immunol*, 38, pp. 127-134, (2014); Lorenzon E.N., Piccoli J.P., Santos-Filho N.A., Cilli E.M., Dimerization of Antimicrobial Peptides: A Promising Strategy to Enhance Antimicrobial Peptide Activity, *Protein Pept. Lett*, 26, pp. 98-107, (2019); Ohno M.K., Kirikae T., Yoshihara E., Kirikae F., Ishida I., Addition of L-Cysteine to the N- or C-Terminus of the All-D-Enantiomer [D(KLAKLAK)<sub>2</sub>] Increases Antimicrobial Activities against Multidrug-Resistant *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Escherichia coli*, *PeerJ*, 8, (2020); Fernandes J.M.O., Ruangsri J., Kiron V., Atlantic Cod Piscidin and Its Diversification through Positive Selection, *PLoS ONE*, 5, (2010); Colorni A., Ullal A., Heinisch G., Noga E.J., Activity of the Antimicrobial Polypeptide Piscidin 2 against Fish Ectoparasites, *J. Fish. Dis*, 31, pp. 423-432, (2008); Zughaier S.M., Shafer W.M., Stephens D.S., Antimicrobial Peptides and Endotoxin Inhibit Cytokine and Nitric Oxide Release but Amplify Respiratory Burst Response in Human and Murine Macrophages, *Cell Microbiol*, 7, pp. 1251-1262, (2005); Chen J., Lin Y.F., Chen J.H., Chen X., Lin Z.H., Molecular Characterization of Cathelicidin in Tiger Frog (*Hoplobatrachus rugulosus*): Antimicrobial Activity and Immunomodulatory Activity, *Comp. Biochem. Physiol. Part—C Toxicol. Pharmacol*, 247, (2021); Wang C., Yan X., Wang G., Liu W., Wang Y., Hao D., Liu H., Zhang M., NKHs27, a Sevenband Grouper



---

NK-Lysin Peptide That Possesses Immunoregulatory and Antimicrobial Activity, *Fish Shellfish Immunol*, 136, (2023); Chen J., Lv Y.P., Dai Q.M., Hu Z.H., Liu Z.M., Li J.H., Host Defense Peptide LEAP-2 Contributes to Monocyte/Macrophage Polarization in Barbel Steed (*Hemibarbus labeo*), *Fish Shellfish Immunol*, 87, pp. 184-192, (2019); Jiang B., Zhang X., Yuan Y., Qu Y., Feng Z., Separation of Antioxidant Peptides from Pepsin Hydrolysate of Whey Protein Isolate by ATPS of EOPO Co-Polymer (UCON)/Phosphate, *Sci. Rep.*, 7, (2017); Kalyanaraman B., Teaching the Basics of Redox Biology to Medical and Graduate Students: Oxidants, Antioxidants and Disease Mechanisms, *Redox Biol*, 1, pp. 244-257, (2013); Sampath Kumar N.S., Nazeer R.A., Jaiganesh R., Purification and Identification of Antioxidant Peptides from the Skin Protein Hydrolysate of Two Marine Fishes, Horse Mackerel (*Magalaspis cordyla*) and Croaker (*Otolithes ruber*), *Amino Acids*, 42, pp. 1641-1649, (2012); Ngo D.H., Ryu B.M., Vo T.S., Himaya S.W.A., Wijesekara I., Kim S.K., Free Radical Scavenging and Angiotensin-I Converting Enzyme Inhibitory Peptides from Pacific Cod (*Gadus macrocephalus*) Skin Gelatin, *Int. J. Biol. Macromol*, 49, pp. 1110-1116, (2011); Jiang H., Tong T., Sun J., Xu Y., Zhao Z., Liao D., Purification and Characterization of Antioxidative Peptides from Round Scad (*Decapterus maruadsi*) Muscle Protein Hydrolysate, *Food Chem*, 154, pp. 158-163, (2014); Guzman F., Gauna A., Roman T., Luna O., Alvarez C., Pareja-Barrueto C., Mercado L., Albericio F., Cardenas C., Tea Bags for Fmoc Solid-Phase Peptide Synthesis: An Example of Circular Economy, *Molecules*, 26, (2021); Lamiable A., Thevenet P., Rey J., Vavrusa M., Derreumaux P., Tuffery P., PEP-FOLD3: Faster de Novo Structure Prediction for Linear Peptides in Solution and in Complex, *Nucleic Acids Res*, 44, pp. W449-W454, (2016); Florez-Castillo J.M., Rondon-Villareal P., Roperov-Vega J.L., Mendoza-Espinel S.Y., Moreno-Amezquita J.A., Mendez-Jaimes K.D., Farfan-Garcia A.E., Gomez-Rangel S.Y., Gomez-Duarte O.G., Ib-M6 Antimicrobial Peptide: Antibacterial Activity against Clinical Isolates of *Escherichia Coli* and Molecular Docking, *Antibiotics*, 9, (2020); Montory J.A., Chaparro O.R., Averbuj A., Salas-Yanquin L.P., Buchner-Miranda J.A.,

---

Gebauer P., Cumillaf J.P., Cruces E., The Filter-Feeding Bivalve *Mytilus chilensis* Capture Pelagic Stages of *Caligus rogercresseyi*: A Potential Controller of the Sea Lice Fish Parasites, *J. Fish. Dis.*, 43, pp. 475-484, (2020); Madrid A.M., Espinoza L.J., Mellado M.A., Osorio M.E., Montenegro I.J., Jara C.E., Evaluation of the Antioxidant Capacity of *Psoralea glandulosa* L. (Fabaceae) EXTRACTS, *J. Chil. Chem. Soc.*, 57, pp. 1328-1332, (2012); Leyton M., Mellado M., Jara C., Montenegro I., Gonzalez S., Madrid A., Free Radical-Scavenging Activity of Sequential Leaf Extracts of *Embothrium coccineum*, *Open Life Sci.*, 10, pp. 260-268, (2015); Stolen J.S., Fletcher T.C., Anderson D.P., Roberson B.S., van Muiswinkel W.B., Techniques in Fish Immunology, pp. 1-220, (1990); Boesen H.T., Larsen M.H., Larsen J.L., Ellis A.E., In Vitro Interactions between Rainbow Trout (*Oncorhynchus mykiss*) Macrophages and *Vibrio Anguillarum* serogroup O2a, *Fish Shellfish Immunol.*, 11, pp. 415-431, (2001)

## Correspondence Address

F. Guzmán; Núcleo Biotecnología Curauma, Pontificia Universidad Católica de Valparaíso, Valparaíso, 2373223, Chile; email: fanny.guzman@pucv.cl; P.A. Santana; Instituto de Ciencias Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, Santiago, 8910060, Chile; email: paula.santana@uautonoma.cl

## Publisher

Multidisciplinary Digital Publishing Institute (MDPI)

## ISSN

16603397

---

## **CODEN**

MDARE

## **PubMed ID**

38921559.0

## **Language of Original Document**

English

## **Abbreviated Source Title**

Mar. Drugs

## **Document Type**

Article

## **Publication Stage**

Final

## **Source**

Scopus

---

## EID

2-s2.0-85197044385