

MARINE SCIENCE AND TECHNOLOGY BULLETIN

In vitro antimicrobial activities of extracts from ballan wrasse (*Labrus bergylta*) skin mucus.

Neslican Katra¹, Olcay Hisar^{1*}, Sevdan Yilmaz², Emre Turgay³, Can Sarvan³, Suheyra Karatas³

¹Department of Basic Science, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

²Department of Aquaculture, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

³Department of Aquaculture, Faculty of Fisheries, Istanbul University, Istanbul, Turkey

ARTICLE INFO

Article history:

Received: 17.05.2016

Received in revised form: 09.06.2016

Accepted : 17.06.2016

Available online : 22.07.2016

Keywords:

Antimicrobial
Skin mucus
Labrus bergylta

ABSTRACT

The increasing bacterial resistance against antimicrobial agents has induced the search for alternative antimicrobial resources. In this regard, antimicrobial peptides in the fish mucus seem to be usable as therapeutic agents. Antimicrobial effects of the mucus extracts obtained from the skin of various fish species. In this study, the antimicrobial effects of organic and aqueous mucus extracts obtained from the skin mucus of *L. bergylta* on fish isolates *Yersinia ruckeri*, *Edwardsiella tarda*, *Listonella anguillarum*, *Aeromonas sobria*, *Shewanella baltica*, *Enterobacter sp.*, *Citrobacter sp.* and *Escherichia coli*, *Bacillus subtilis* and *Lactobacillus plantarum* as control strains were analysed. As a result of this study, aqueous mucus extract, aqueous phase and DCM phase that were obtained from the skin mucus of *L. bergylta* were found not to have any bactericidal effect on the microorganisms tested in this study.

Introduction

The mucus found in the gut, gills, especially on the skin surface of the fish is a highly multifunctional material with its proposed roles in respiration, ionic and osmotic regulation, reproduction, excretion, disease resistance, communication, feeding, nest building and protection (Shephard 1994). Lectins, pentraxins, lysozyme, complement, C - reactive protein, hemolysin, antibacterial peptides and immunoglobulins form protective and antipathogenic characteristics of the mucus (Yano 1996; Magnadóttir 2006).

The increasing bacterial resistance against antimicrobial agents has induced the search for alternative antimicrobial resources. In this regard, antimicrobial peptides in the fish mucus seem to be usable as therapeutic agents (Subramanian et al. 2007; Anbuezhian et al. 2011).

Antimicrobial effects of the mucus extracts obtained from the skin of various fish species including *Scophthalmus maximus*, *Sparus aurata* and *Dicentrarchus labrax* (Magariños et al. 1995), *Conger conger*, *Trisopterus luscus*, *Pollachius pollachius*, *Pollachius virens*, *Gadus morhua*, *Trachurus trachurus*, *Scomber scombrus*, *Labrus bergylta*, *Lophius piscatorius*, *Scophthalmus rhombus*, *Platichthys flesus*, *Pleuronectes platessa* and *Solea solea* (Hellio et al. 2002), *Salmo salar* and *Gadus morhua* (Mozumder 2005), *Salvelinus alpinus*, *S. fontinalis*, *Cyprinus carpio*, *Morone saxatilis*, *Melanogrammus aeglefinus* and *Myxine glutinosa* (Subramanian et al. 2008), *Gadus morhua* (Ruangsri 2010), *Channa striatus* (Wei et al. 2010), *Myxus gulio* and *Arius maculatus* (Anbuezhian et al. 2011), *Oncorhynchus mykiss* (Hisar et al. 2014) were analysed in the scientific literature. However, it was found that antimicrobial effects of the fish mucus differed greatly depending on the fish species, the extraction method and the bacterial strain. In a previous study, the mucus extract of the skin of *Labrus bergylta* (Ballan wrasse) was shown to have antimicrobial effects on *Escherichia coli*, *Klebsiella pneumoniae*, *Serratia*

*Corresponding author

E-mail address: o_hisar@hotmail.com (O. Hisar)

Tel: +90 286 218 00 18 fax: +90 286 218 05 43

marcescens and *Proteus vulgaris* (Hellio et al. 2002). However, there has been no study investigating the antimicrobial effects of the skin mucus of *L. bergylta* against fish isolates in the literature. In this study, the antimicrobial effects of organic and aqueous mucus extracts obtained from the skin mucus of *L. bergylta* on fish isolates *Yersinia ruckeri*, *Edwardsiella tarda*, *Listonella anguillarum*, *Aeromonas sobria*, *Shewanella baltica*, *Enterobacter* sp., *Citrobacter* sp. and *Escherichia coli*, *Bacillus subtilis* and *Lactobacillus plantarum* as control group were analysed.

Material and methods

Collection of the Fish and Mucus

Mucus samples were obtained from ten *Labrus bergylta* (Ballan wrasse) individuals caught by fishing (Dardanelles, Turkey). The mucus were collected into 50 ml centrifuge tubes by peeling of the skin surface of the fish. The ventral surface of the fish was not used for the mucus sampling to prevent the contamination of sperm and faeces (Hellio et al. 2002). The mucus were then frozen at -80°C , lyophilised and kept at -80°C until used.

Preparation of the Extracts

Aqueous supernatant was obtained by the method previously described by Hellio et al. (2000) and Hellio et al. (2002). A little amount of lyophilised fish mucus (1 mg lyophilised mucus/mL pure water) was dissolved at 4°C for 2 hours by means of ultra-turrax. Then the samples were centrifuged for 30 minutes at 30 000 g at 4°C and aqueous supernatant (Extract A) was lyophilised after filtration (Whatman 1822-047).

For organic extract (Hellio et al. 2002; Subramanian et al. 2008), 1 mg/mL of lyophilised mucus sample was dissolved in 95% ethanol and the same procedure was applied for five times. The ethanol extracts were combined together and dried in a nitrogen evaporator at 40°C . 100 mL pure water and dichloromethane (DCM) (4 x 100 mL) were added, and then were taken into the separating funnel. The obtained aqueous phases were lyophilised and then dissolved in pure water (Extract B). The organic phases gathered were combined and dried in nitrogen evaporator and the sample was dissolved in 5% of dimethyl sulfoxide (DMSO) (Extract C).

Organisms and media

In vitro antimicrobial activities of the skin mucus extracts of the *L. bergylta* were tested on previously identified strains of *Escherichia coli*, *Yersinia ruckeri*, *Edwardsiella tarda*, *Listonella anguillarum*, *Aeromonas sobria*, *Shewanella baltica*, *Enterobacter* sp., *Citrobacter* sp., *Bacillus subtilis* and *Lactobacillus plantarum* (Table 1). *E. coli* (ATCC 25922), *B. subtilis* (ATCC 6633) and *L. plantarum* (BC 7321, obtained from Dr. Bülent ÇETİN) were used as control strains. As fish isolates, *Y. ruckeri* (obtained from Dr. Ertan Emek ONUK) was isolated from diseased rainbow trout (*Oncorhynchus mykiss*), *L. anguillarum* was isolated from diseased sea bass (*Dicentrarchus labrax*), *E. tarda* and *Citrobacter* sp. were isolated from diseased tilapia (*Oreochromis niloticus*), *A. sobria*, *S. baltica* and *Enterobacter* sp. were isolated from healthy trout intestines.

Disc diffusion test

The disc diffusion assay was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (2005). Briefly, bacterial strains were inoculated on Mueller-Hinton agar and MRS agar (for *L. plantarum*), at least 3 colonies were taken and their densities were set as 0.5 McFarland and they were placed on the media by using sterilized cotton swab. Then 10 μl of mucus extract (A, B and C) was impregnated to standard sterilized blank disks (OXOID) and the disks were transferred onto the media. Pure water and DMSO (5%) were used for the control disks.

Results and Discussion

As a result of this study, aqueous mucus extract (Extract A), aqueous phase (Extract B) and DCM phase (Extract C), that were obtained from the skin mucus of *Labrus bergylta* (Ballan wrasse), were found not to have any antimicrobial effect on *Escherichia coli*, *Yersinia ruckeri*, *Edwardsiella tarda*, *Listonella anguillarum*, *Aeromonas sobria*, *Shewanella baltica*, *Enterobacter* sp., *Citrobacter* sp., *Bacillus subtilis* and *Lactobacillus plantarum*.

In another study, which the antimicrobial effects of the skin mucus of the fish were analysed, 78 extracts were obtained from 13 different fish species and only 15 extracts from 6 fish species were found to have antimicrobial and/or antifungal effects (Hellio et al. 2002). In the same study, various extracts obtained from the skin mucus of the *L. bergylta* fish were analysed and it was found that extracts had no effect on *Bacillus subtilis*, *B. cereus*, *B. megaterium*, *Streptococcus* sp., *Staphylococcus aureus*, *Candida brusei*, *C. albicans*, *C. tropicalis*, *Saccharomyces cerevisiae* and *Issatchenkia orientalis* strains. However, aqueous phase was reported to have antimicrobial effect on *E. coli*, *K. pneumoniae*, *S. marcescens* and *P. vulgaris* and DCM phase was found to have that effect on *Pseudomonas aeruginosa* strains (Hellio et al. 2002). In this study, we found that the extracts obtained from the *L. bergylta* had no antimicrobial effect on *B. subtilis*; however, the extracts were found not to have any effect on *E. coli* although the extracts were obtained from the same fish species using the same method. This change might be occurred due to the several reasons such as development of antimicrobial resistance in the strains we used, or the season which the fish were caught or changes of the environmental and the nutritional patterns of the fish.

In another study, aqueous mucus extracts obtained from *S. alpinus*, *S. fontinalis*, *C. carpio sub sp. koi*, *Menticirrhus saxatilis*, *Melanogrammus aeglefinus* and *Myxine glutinosa* species were found not to have any antimicrobial effect on *E. coli*, *Salmonella enterica* serovar Typhimurium, *Staphylococcus epidermis*, *P. aeruginosa* Z61, *P. aeruginosa* K799 and *C. albicans* as human pathogens and *Aeromonas salmonicida*, *L. anguillarum* and *Y. ruckeri* as fish pathogens (Subramanian et al. 2008). However, in another study carried out on the same fish species, aqueous mucus extracts were found to have innate antimicrobial agents (Subramanian et al. 2007). In a similar way, aqueous mucus extracts obtained from various fish species were found to have no effects on certain bacterial strains (Wei et al. 2010; Anbuhezian et al. 2011).

Table 1. Characteristics, culture conditions and accession numbers of the bacterial strains used in the study.

Organism	Description	Medium	Temp. (°C)	Acc. no.
<i>Aeromonas sobria</i> , SY-A100	Non-pathogenic, isolated from rainbow trout intestines	MH	22	KX388235
<i>Citrobacter</i> sp., SY-C10	Fish pathogen, isolated from spleen of Nile tilapia	MH	28	KX388233
<i>Edwardsiella tarda</i> , SY-ED14	Fish pathogen, isolated from spleen of Nile tilapia	MH	28	KX388234
<i>Enterobacter</i> sp., SY-EN560	Non-pathogenic, isolated from rainbow trout intestines	MH	22	KX388232
<i>Listonella anguillarum</i> , SY-L24	Fish pathogen, isolated from European sea bass	MH*	24	KX388236
<i>Shewanella baltica</i> , SY-S145	Non-pathogenic, isolated from rainbow trout intestines	MH	22	KX388237
<i>Yersinia ruckeri</i> , E42	Fish pathogen, isolated from spleen of rainbow trout	MH	22	KX388238
<i>Escherichia coli</i> , ATCC 25922	Control Strain	MH	37	-
<i>Bacillus subtilis</i> , ATCC 6633	Control Strain	MH	37	-
<i>Lactobacillus plantarum</i> , BC 7321	Control Strain	MRS	30	-

MH: Mueller-Hinton agar, *1.5% NaCl added to the medium.

The reasons why the aqueous extracts did not show any antimicrobial effect in the studies can be explained with the possibilities of that the enzyme contents (lysozyme, proteases, etc.) of the extracts could not become active as the temperature and pH values were not appropriate *in vitro* environment or that the effect could not be determined during the analysis since the enzyme levels in the media were in low rates (Subramanian et al. 2007). In addition, it is obvious that the antimicrobial effect may vary depending on the fish species, extraction methods and bacterial strains.

As with our study, organic extracts obtained from the mucus of certain fish species (DCM and/or aqueous phase) were reported not having bactericidal effects (Hellio et al. 2002; Subramanian et al. 2008), while the organic extracts in some fish species were noted having bactericidal effects (Hellio et al. 2002). This can be explained with the antimicrobial agents not being in sufficient concentration to be active in the extracts and/or small molecules being able to be extracted rather than organic solvents and active antimicrobial constituents (Subramanian et al. 2008). Consequently, aqueous and organic mucus extracts obtained from the *Labrus bergylta* were found not having any bactericidal effect on the microorganisms tested in this study.

References

- Anbuechezian, R., Gobinath, C. and S. Ravichandran. 2011. Antimicrobial peptide from the epidermal mucus of some estuarine cat fishes. *World Applied Sciences Journal*, **12**(3):256-260.
- Clinical and Laboratory Standards Institute (CLSI). *Methods for antimicrobial disk susceptibility testing of bacteria isolated from aquatic animals; Proposed Guideline*. CLSI document M42-P (ISBN 1-56238-576-3). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2005.
- Hellio, C., Bourgougnon, N. and Y.L. Gal. 2000. Phenoloxidase (EC 1.14. 18.1) from the byssus gland of *Mytilus edulis*: Purification, partial characterization and application for screening products with potential antifouling activities. *Biofouling*, **16**(2-4):235-244.
- Hellio, C., Pons, A. M., Beupoil, C., Bourgougnon, N., and Y. Le Gal. 2002. Antibacterial, antifungal and cytotoxic activities of extracts from fish epidermis and epidermal mucus. *International Journal of Antimicrobial Agents*, **20**(3):214-219.
- Hisar, O., Hisar, S.A., Uyanik, M.H., Sahin, T., Cakir, F. and S. Yilmaz. 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**(1):19-22.
- Magariños, B., Pazos, F., Santos, Y., Romalde, J.L. and A.E. Toranzo. 1995. Response of *Pasteurella piscicida* and *Flexibacter maritimus* to skin mucus of marine fish. *Diseases of Aquatic Organisms*, **21**:103-103.
- Magnadóttir, B. 2006. Innate immunity of fish (overview). *Fish & Shellfish Immunology*, **20**(2):137-151.
- Mozumder, M.M.H. 2005. Antibacterial activity in fish mucus from farmed fish. Master Thesis, University of Tromsø, Norway.
- Ruangsi, J., Fernandes, J.M., Brinchmann, M. and V. Kiron. 2010. Antimicrobial activity in the tissues of Atlantic cod (*Gadus morhua* L.). *Fish & Shellfish Immunology*. **28**(5): 879-886.
- Shephard, K.L. 1994. Functions for fish mucus. *Reviews in Fish Biology and Fisheries*, **4**(4): 401-429.
- Subramanian, S., Ross, N.W. and S.L. MacKinnon. 2008. Comparison of antimicrobial activity in the epidermal mucus extracts of fish. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, **150**(1):85-92.
- Wei, O.Y., Xavier, R. and K. Marimuthu. 2010. Screening of antibacterial activity of mucus extract of snakehead fish, *Channa striatus* (Bloch). *European Review for Medical and Pharmacological Sciences*. **14**(8):675-681.