aquaculture europe 22

JOIN US IN ITALY FOR AQUACULTURE EUROPE 2022

INNOVATIVE SOLUTIONS IN A CHANGING WORLD

27-30 SEPTEMBER 2022
RIMINI, ITALY

#AE22RIM

www.aquaeas.or g

SILVER SPONSOR

GOLD SPONSOR

european aquaculture society

SUPPORTED BY

SESSION SPONSOR

BIOFILM INHIBITION OF PATHOGENIC STRAINS BY EXTRACELLULAR PRODUCTS (ECPS) OF *Shewanella .sp* PROBIOTIC

O.Pérez-Gómez *[1], M. Domínguez-Maqueda[1], C. Esteve [2], ST. Tapia Paniagua [1], M.C. Balebona [1], M.A. Moriñigo[1]

[1]Departament of Microbiology. Universidad de Málaga, Málaga, Spain
[2]Department of Microbiology and Ecology, Universidad de Valencia, Valencia, Spain
Correspondence: olipergom@uma.es

Introduction

Shewanella putrefaciens Pdp11 and *V.proteolyticus* DCF 12.2 are strains isolated by our research group. *S. putrefaciens* Pdp11 has been described as a probiotic for farmed fish species such as *Solea senegalensis* and *Sparus aurata* [1].Although the probiotic potential is well described some studies reference to application of postbiotic as functional bioactive compounds produces by probiotics. Recent works have been focused in the identification of substances as alternative of anti-biofilm methods and their implication in surface attachment inhibition [2]. In this research, *S. putrefaciens* Pdp11 and *V.proteolyticus* DCF 12.2 have been cultured under different growth conditions (temperature, culture media and during 24 and 48 hours of incubation) and their extracellular products (ECPs) have been extracted and tested as potential postbiotics that affect the biofilm formation of several fish pathogenic strains.

Materials and Methods

The probiotic strains were grown on tryptic soja agar plates supplemented with NaCl (1,5%) for 24h at 23ºC. Then, one to two colonies were picked up in 10ml of tryptic soy broth supplemented with NaCl (1.5%) (TSBs) and incubated at 23ºC and 15 ºC for 36h (stationary phase). Then, extracellular products (ECPs) from solid medium3, 1 ml of the cultures were spread on TSAs plates as control condition. Another 1 mL was spread on plates containing: Pdp11 was culture in a medium with 25% of algae mix (diet 1) and supplement with a commercial diet (diet 2) were incubated to 23ºC for 24h. While *V. proteolyticus* DCF 12.2 was incubated in a commercial and algae mix diet (diet 3) and with 25% of algae mix (diet 4) to 15 and 23 º C during 48 and 24h respectively. After incubation, the ECPs were recovered with 5 ml of sterile phosphate buffer saline (PBS), and centrifuged (10000xg, 4ºC, 10 min) and the supernatant was filtered (0.22µm, pore diameter). ECPs were conserved at -80ºC until use. The biofilm inhibition was determined for the pathogenic strains: *S. putrefaciens* SH4, SH12, SH6 and SH9 4damage of the mouth, extensive skin discoloration, exophthalmia, ascites and bad odour. The S. putrefaciens group was recovered from freshwater samples taken at the L′Albufera system, along autumn–winter 2015. Its counts significantly increased in freshwater parallel to hypoxia and temperature rising. Shewanellae strains were identified as S. putrefaciens and S. xiamenensis by 16S rRNA gene sequencing. These isolates recovered from sick eels or freshwater were virulent for European eel by IP challenge (LD50 106 CFU g−1 body weight using 96 well plates by adding 90µl ECPs + 90 µl TSBs + 20 µl of pathogenic bacterial suspension adjusted to OD600nm ~ 0.5, per well. The plates were incubated at 23ºC during 24h. The biofilm formation assay was performed by crystal violet (CV) staining5”ISSN”:”0044 8486”,”abstract”:”The use of effective biocides as disinfectants is essential in aquaculture facilities. However, while most biocides act effectively on free-living planktonic pathogens, they are seldom useful against biofilms. In this study, we evaluate the biocidal efficacy and antimicrobial specific contact time of three disinfectants, Virkon™Aquatic (VirA and quantify at OD595nm in a plates reader. The results were analysis by two-way Anova method.

Results and Discussion

Two different ECPs concentration were assayed to check the potential inhibition of the biofilm formation, 0.45 and 0.25 µg protein/ml (Figure 1). The SH4 and SH12 pathogenic strains did not present biofilm formation in contrast to SH6 and SH9 strains. The biofilm of SH6 presented the highest inhibition at a concentration of 0,25 µg/ml of respect to control in each condition. On the contrary, the biofilm of SH9 presented inhibition by at 0,014 µg/ml of ECPs. Some authors describe that the biofilm inhibition can be mediated by soluble antimicrobial peptides (AMPs) secreted on ECP of probiotics strains [6]. This assay results evidence that Pdp11_Pmix_2324 ECPs have showed the most impact in the biofilm formation of pathogenic strains. Therefore, ECPs secreted by Pdp11 and *V.proteolyticus* DCF 12.2 are implicated in the inhibition to adhesion of pathogens on surfaces.

(Continued on next page)

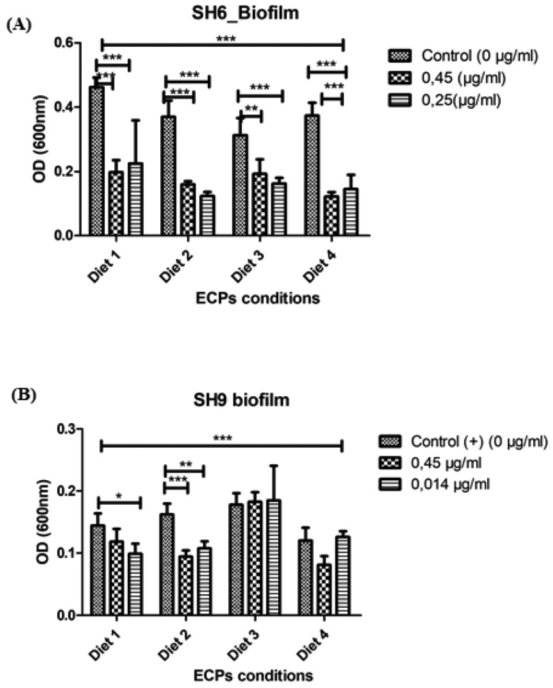

Figure 1. Biofilm inhibition in SH6 and SH9 pathogenic strains by *S.putrefaciens* Pdp11 and *V.proteolyticus* DCF 12.2 ECPs. The results were analysis by two-way Anova with P-value < 0.05 (*), P-value <0.01 (**), P-value < 0.001 (***).

Reference

1. Tapia-Paniagua, S. T. *et al.* (2012). *Aquac. Int.* doi:10.1007/s10499-012-9509-5.
2. Mishra, R. *et al.* (2020). *Frontiers in Microbiology* doi:10.3389/fmicb.2020.566325.
3. LIU, P. V. (1957). *J. Bacteriol.* doi:10.1128/jb.74.6.718-727.1957.
4. Esteve, C. *et al*.(2017). *J. Fish Dis.* doi:10.1111/jfd.12574.
5. Acosta, F. *et al*. (2021). *Aquaculture* doi:10.1016/j.aquaculture.2020.736004.
6. Barroso, C. *et al.* (2021). *Front. Immunol.* doi:10.3389/fimmu.2021.754437.