Investigation of biofilm control strategies for *Flavobacterium johnsoniae*-like isolates

Hafizah Y. Chenia

Discipline: Microbiology, School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Private Bag x54001, Durban 4001, South Africa

Correspondence: cheniah@ukzn.ac.za

Abstract

Flavobacterium johnsoniae-like isolates play a major role in disease of farmed fish in South Africa [1]. *F. johnsoniae*-like isolates are known to form biofilms [2], which allows for a saprophytic mode of existence [3] and persistence in aquaculture systems. The persistance of the pathogenic *Flavobacterium* spp. in aquaculture tanks results in recurrent disease outbreaks causing high mortality and large economic losses. The pathogenicity and persistence of *F. johnsoniae*-like isolates associated with outbreaks of fish disease in South Africa is linked to their biofilm-forming ability, which facilitates their survival in aquaculture systems [2]. By understanding the stages of biofilm formation, mechanisms involved in biofilm formation and biofilm physiology, different strategies may be applied to eliminate biofilms. These strategies include the use of matrix-degrading enzymes, quorum-sensing inhibitors, phage inducers, bacteriophages, sugars, etc [4].

In order to effectively remove these biofilms, the effect of a matrix-degrading enzyme, efflux pump inhibitors (EPIs), quorum-sensing inhibitors (QSIs) and phage inducers on initial attachment and detachment from mature biofilms of 15 *F. johnsoniae*-like isolates and four *Flavobacterium* spp. type strains, was assessed using modified microtiter plate assays [2]. Treatments included: Matrix-degrading enzyme (DNase I); EPIs (phenylalanine arginine β-naphthylamide, PaβN and 1-(1-naphthylmethyl)-piperazine, NMP); QSIs [(Z-)-4-bromo-5-(bromomethylene)-2(5H)-furanone, S-adenosylhomocysteine, sulfathiazole and dodecanamide]; phytochemicals (*trans*-cinnamaldehyde, vanillin); sugar (N-acetyl neuraminic acid); and phage inducers (mitomycin C, ciprofloxacin and 5-fluorouracil). Treatments were added at the time of inoculation to determine their effect on initial attachment and to mature biofilms to determine their detachment effect. The percentage biofilm reduction was also assessed for each treatment [5]. One-way Repeated Measures ANOVA and Student *t*-tests (SigmaStat) were used to examine the statistical significance of all treatments, i.e., treated *vs* untreated. A p value of 0.05 was considered significant.

Decreased adhesion was observed for *F. johnsoniae*-like isolates, following treatments at the time of inoculation, in the following order: ciprofloxacin > mitomycin C > DNase I = *trans*-cinnamaldehyde > furanone > dodecanamide >S-adenosylhomocysteine > vanillin = 5-fluorouracil > N-acetyl neuraminic acid > PAβN > sulfathiazole > NMP. However, increased detachment from mature biofilm was observed in the following order: *trans*-cinnamaldehyde > furanone > S-adenosylhomocysteine = ciprofloxacin > mitomycin C = 5-fluorouracil > N-acetyl neuramininc acid > dodecanamide = sulfathiazole = vanillin > PAβN > NMP > DNase I.

All treatments did not result in statistically significant alterations in initial attachment, whilst *trans*-cinnamaldehyde, S-adenosylhomocysteine and furanone treatments of pre-formed biofilms resulted in decreased adhesion that was statistically significant ($p < 0.05$). However, individual isolates displayed statistically significant differences following both initial attachment treatments, as well as mature biofilm treatments. Isolate-specific differences and differences between isolates following the different treatments may be explained by the diversity of phenotypes, genotypes, isolation source and biofilm-forming abilities of isolates studied. This can be observed in Figures 1 and 2, where the treatment effects on the *F. johnsoniae* type strain and two isolates YO64 (a strong biofilm-former) and YO12 (a poor biofilm-former) are compared. For isolate YO64, a strong biofilm-former, all six treatments resulted in significant reduction in biofilm formation, while the weak biofilm-forming *F. johnsoniae* type strain and *F. johnsoniae*-like isolate YO12 did not show significant treatment effects. For YO12, an increase in adhesion was observed with NMP (Fig. 1). A similar trend was observed following the treatment of mature biofilms (Fig. 2).

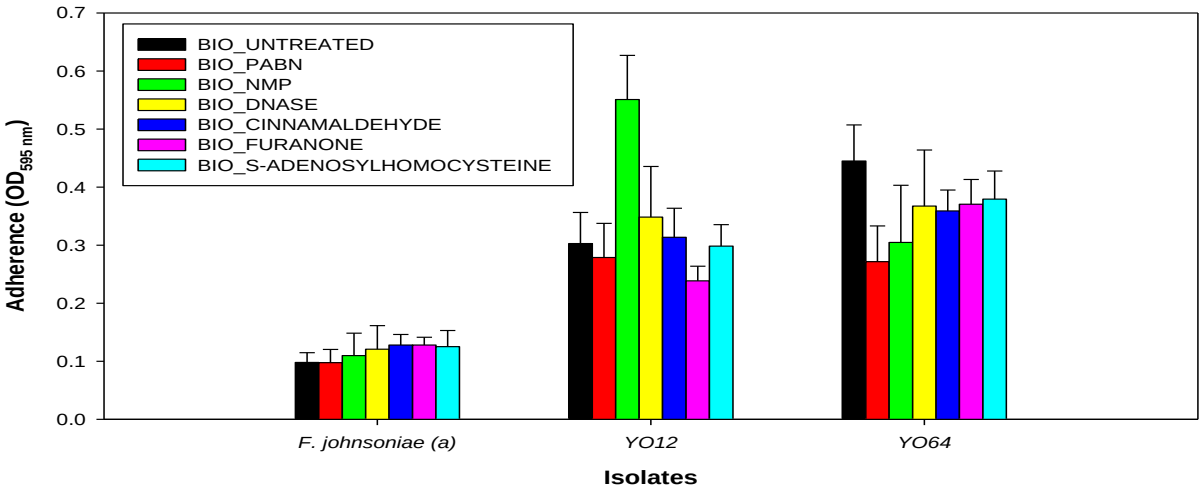

Figure 1. Effect of six treatments [efflux pump inhibitors (PAβN, NMP); matrix-degrading enzyme DNase I; phytochemical (cinnamaldehyde) and quorum sensing inhibitors (furanone and S-adenosylhomocysteine)] on initial attachment of the *F. johnsoniae* type strain and *F. johnsoniae*-like isolates YO12 and YO64.

Biofilms are associated with many problems and their control is crucial for the treatment of diseases and prevention of disease outbreaks. For *F. johnsoniae*-like isolates, cinamaldehyde exposure was the most effective biofilm removal strategy, while ciprofloxacin treatment was most effective to prevent attachment and thus biofilm formation. Cinnamaldehyde is a bio-renewable phytochemical, with potential application on a large-scale, it may be incorporated into feed; unlike DNase I and furanone treatments, which are expensive and often toxic. Anti-pathogenic compounds, like cinnamaldehyde, attenuate virulence and thus reduce the resistance potential. Thus alternative strategies to antimicrobial agent use may be proposed to control biofilm formation by *F. johnsoniae*-like isolates, to potentially limit outbreaks of disease in aquaculture systems.

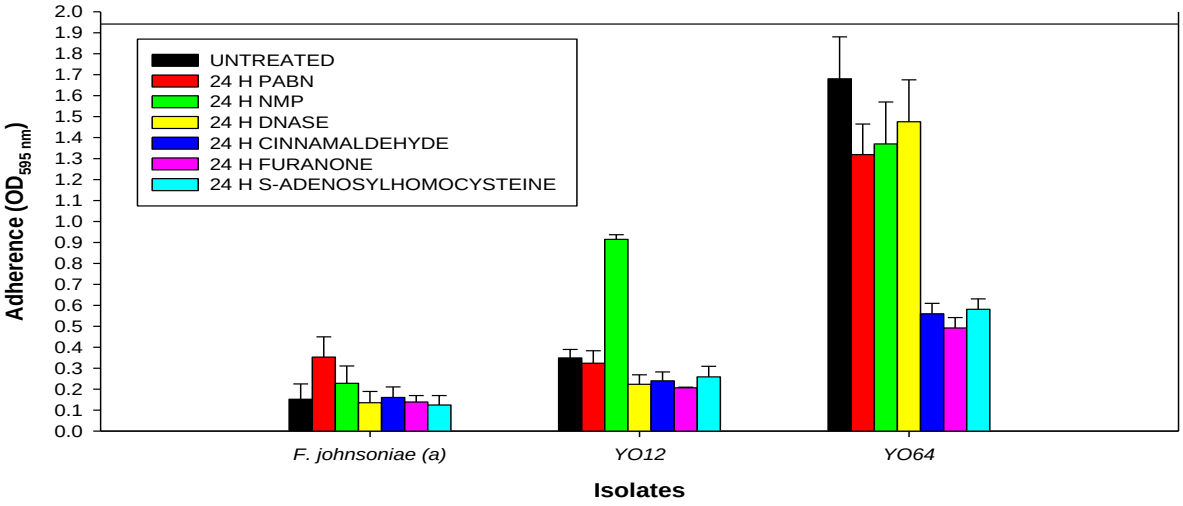

Figure 2. Effect of six treatments [efflux pump inhibitors (PAβN, NMP); matrix-degrading enzyme DNase I; phytochemical (cinnamaldehyde) and quorum sensing inhibitors (furanone and S-adenosylhomocysteine)] on mature biofilms of the *F. johnsoniae* type strain and *F. johnsoniae*-like isolates YO12 and YO64.

References

[1] Flemming L., Rawlings D., Chenia H., 2007. Phenotypic and molecular characterisation of fish-borne *Flavobacterium johnsoniae*-like isolates from aquaculture systems in South Africa. Research in Microbiology, 158:18–30

[2] Basson A., Flemming L.A., Chenia H.Y., 2008. Evaluation of adherence, hydrophobicity, aggregation, and biofilm development of *Flavobacterium johnsoniae*-like isolates. Microbial Ecology, 55:1–14

[3] Kunttu H.M.T., Valtonen E.T., Jokinen I.E., Suomalainen L.-R., 2009. Saprophytism of a fish pathogen as a transmission strategy. Epidemics, 1:96–100

[4] Hentzer M., Givskov M., 2003. Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. Journal of Clinical Investigation, 112:1300–1307

[5] Pitts B., Hamilton M.A., Zelver N., Stewart P.S., 2003. A microtiter-plate screening method for biofilm disinfection and removal. Journal of Microbiological Methods, 54:269–276