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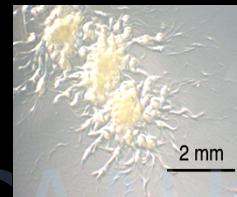
ADHESION DYNAMICS OF *FLAVOBACTERIUM COLUMNARE* TO CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) AND ZEBRAFISH (*DANIO RERIO*) AFTER IMMERSION CHALLENGE

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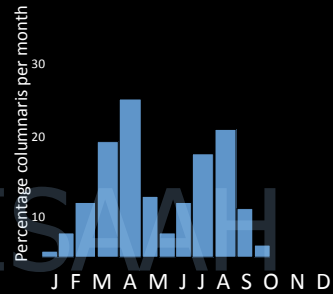
Flavobacterium columnare

- Columnaris disease
 - Described by Davis in 1922
 - *Bacillus columnare*, *Flexibacter columnaris*, *Cytophaga columnaris*, *Flavobacterium columnare*...
 - Columnaris disease affects wild, farm-raised and ornamental fish species
 - Main disease for channel catfish



Columnaris disease in catfish

- Mortality rates
 - 50-60% in food fish
 - Up to 90% in fingerlings
- Epizootiology
 - Early spring & late summer
 - Acute and chronic manifestations
 - Environmental stressors



Genetic heterogeneity

- Three genomovar described within the species
 - Genomovar I
 - Europe
 - USA
 - Genomovar II
 - USA
 - Japan
 - Genomovar III
 - Japan
 - USA



Virulence

- Genomovar II is more virulent than genomovar I in channel catfish

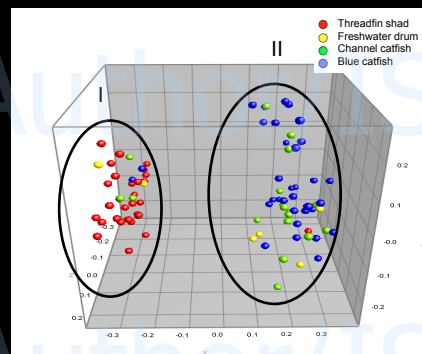
Strain	Genomovar	Dose	Mean Cumulative % mortality
LSU	II	7.0×10^5	100.0 (± 0)
530	II	7.2×10^5	100.0 (± 0)
515	II	7.7×10^5	96.2 (± 0)
02-26	II	6.9×10^5	92.3 (± 0)
ARS-1	I	7.3×10^5	46.2 (± 0)
HS	I	7.5×10^5	3.9 (± 0)
463	I	7.7×10^5	0.0 (± 0)
Control	NA	NA	0.0 (± 0)



Shoemaker, C.A., Olivares-Fuster, O., Arias, C.R. & Klesius, P.H. (2008) Veterinary Microbiology 127 353-359.

Specific host-species association

- In wild fish populations genomovar II has been found almost exclusively associated to catfish



Olivares-Fuster, O., Baker, J.L., Terhune, J.S., Shoemaker, C.A., Klesius, P.H. & Arias, C.R. (2007). Systematic and Applied Microbiology 30, 624-633.

Early stages of host-pathogen interaction

- Chemotaxis towards catfish mucus
 - Genomovar II has a higher chemotaxis response towards catfish mucus than genomovar I
- Adhesion to gill tissue plays main role in colonization and invasion
 - Contradictory studies on correlation between virulence and adhesion in *F. columnare*

Klesius, P.H., Shoemaker, C.A. & Evans, J.J. (2009) FEMS Microbiology Letters 288, 216-220.

Decostere, A., Haesebrouck, F., Van Driessche, E., Charlier, G. & Ducatelle, R. (1999) Journal of Fish Diseases 22, 465-474.

Kunttu, H.M.T., Suomalainen, L.-R., Jokinen, R.I. & Valtonen, E.T. (2009) Microbial Pathogenesis 46, 21-27.



Objective

- To compare adhesion of *Flavobacterium columnare* genomovar I and II to channel catfish and zebrafish



Material and Methods

- Static aquaria (35 L)
 - Each tank is an independent unit
 - 15 or 35 fish/tank (catfish/zebrafish)
- Bath immersion
 - 30 min with $\sim 10^6$ CFU/ml
 - No stressor
- Treatments (4 tanks/treatment)
 - ARS-1 genomovar I
 - BGFS 27 genomovar II
 - Control
 - Terminated at 24 h post-challenge



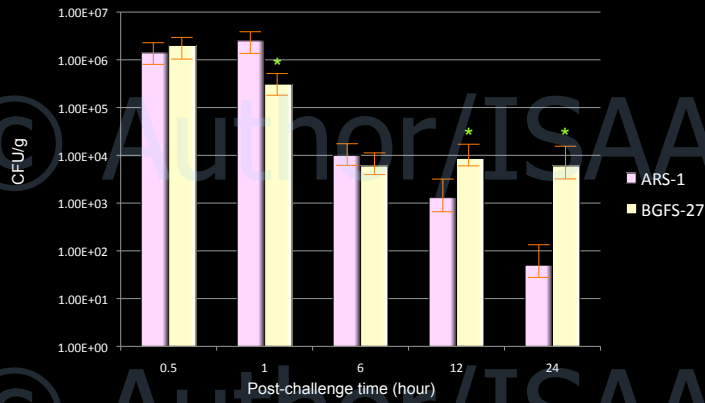
Materials and Methods, cont.

- Fish
 - Catfish: experiment 1 and 3
 - Zebrafish: experiment 2 and 4
- Samples
 - Gill clips at 0.5, 6, 12 and 24 h post-challenge (+ control pre-challenge)
 - Skin ~ 0.5 cm² (Experiment 1 and 2)
 - 3 fish pooled/treatment X 3
 - *F. columnare* CFU/g (selective medium)
 - Scanning electron microscopy (SEM)



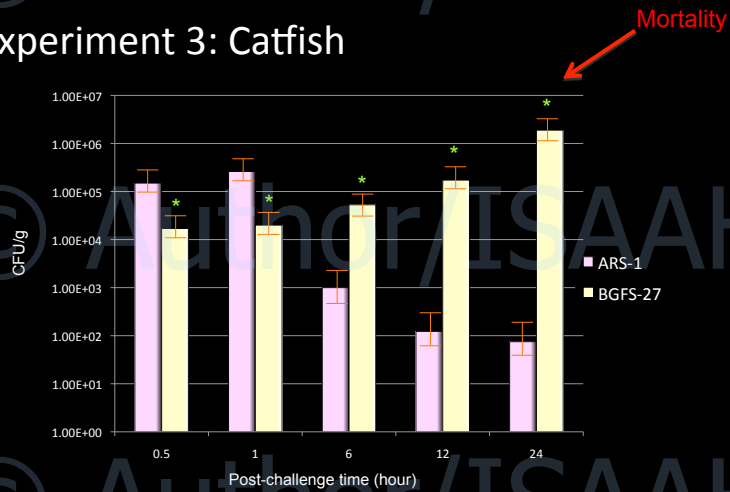
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• Experiment 1: Catfish



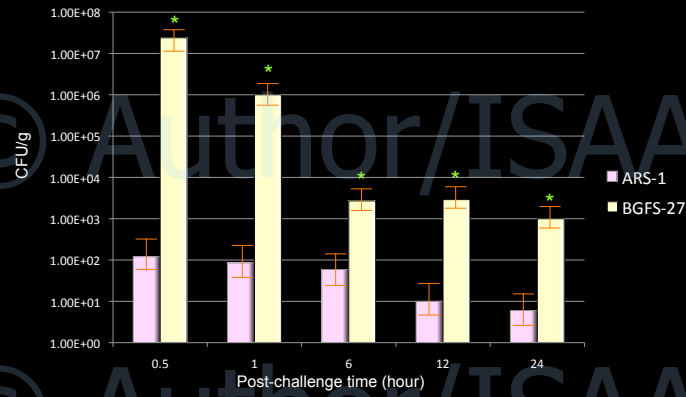
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• Experiment 3: Catfish



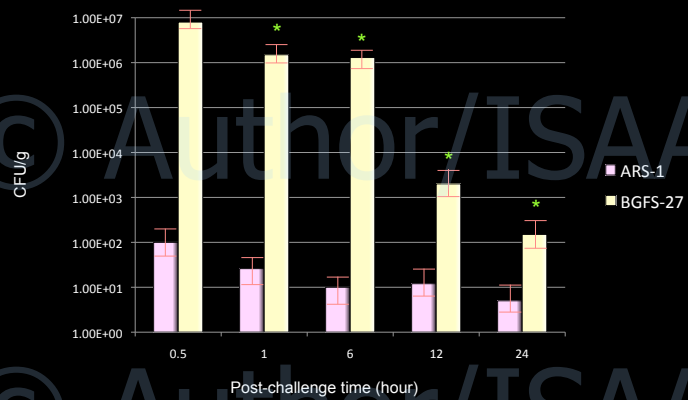
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• Experiment 2: Zebrafish



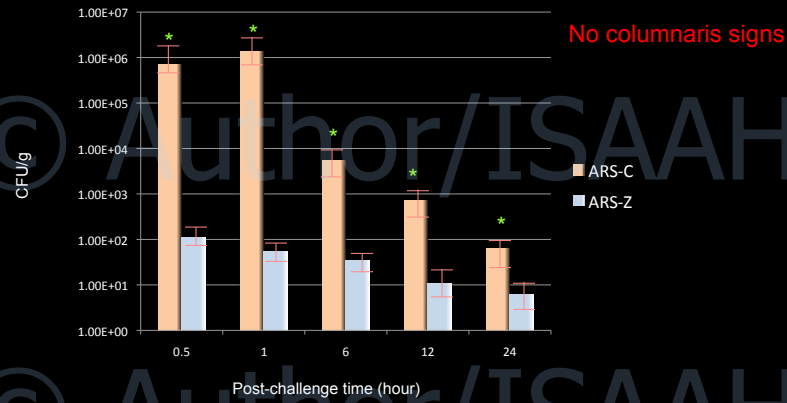
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• Experiment 4: Zebrafish



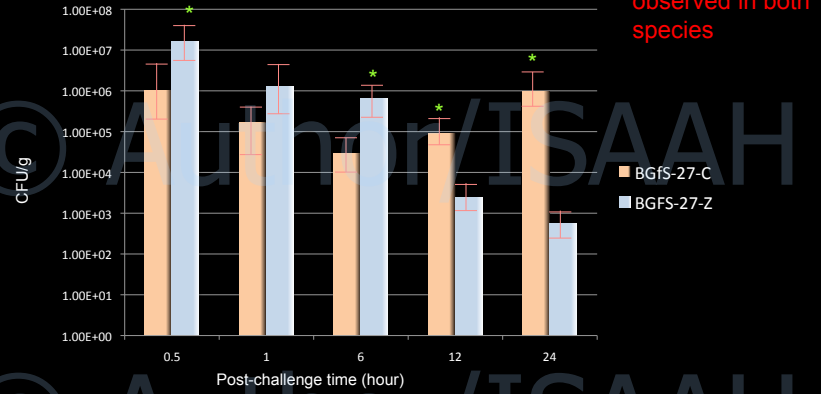
Results

- Genomovar I: catfish vs zebrafish

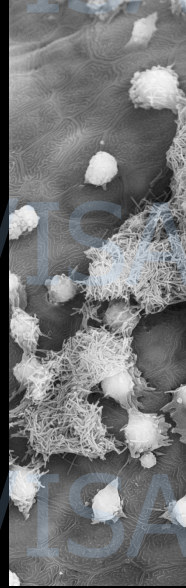
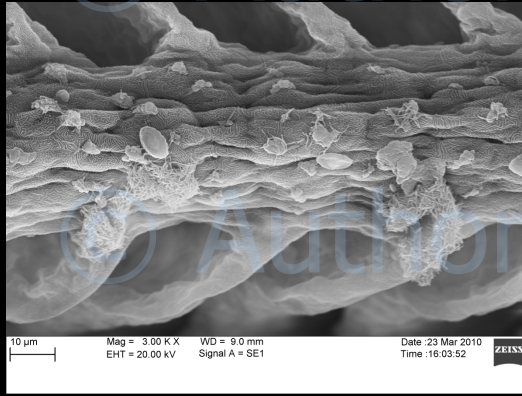


Results

- Genomovar II: catfish vs zebrafish



Results



- No difference between ARS-1 and BGFS-27 observed by SEM (@ 0.5 h post-challenge)



Conclusions

- Marked difference in adhesion dynamics between genomovars I and II
 - Both genomovars can adhere to catfish gills at high levels ($>10^4$ CFU/g) within 1 h post-challenge
 - Genomovar II adheres to zebrafish gill tissue at significantly higher levels than genomovar I
 - Genomovar II persists longer (24 h) at higher numbers ($>10^3$ CFU/g) in both species



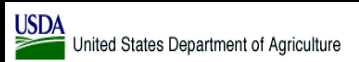
© Author/ISAAH Conclusions cont.

- Adhesion of *F. columnare* to gill tissue is insufficient to cause columnaris disease
- Persistence in gill tissue is better correlated with virulence of *F. columnare* strains than attachment
 - Critical to follow the dynamics of adhesion: analyze more than one time point



© Author/ISAAH Acknowledgments

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