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DEVELOPMENT, CHARACTERIZATION AND EARLY EVALUATION OF NEW MODIFIED LIVE VACCINES AGAINST COLUMNARIS DISEASE

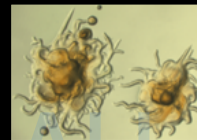
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Columnaris disease

- Caused by *Flavobacterium columnare*
 - Ubiquitous / opportunistic bacteria
- Acute to chronic infection
 - Up to 90% mortality in catfish fingerlings
- Affects most freshwater fish
 - Aquaculture and ornamental species



Columnaris still the 2nd
most important
disease for channel
catfish aquaculture



Strategies against columnaris

- Prevention and treatment
 - Best management practices
 - Reduce stress
 - Therapeutants
 - Oxytetracycline
 - Romet 30 (sulphadimethoxine and ormetoprim)
 - Aquaflor®-CA1 (florfenicol)
 - Potassium permanganate
 - Diquat® (herbicide)



Vaccines

- Columnaris vaccines
 - Fryvacc 1-2 / Novartis
 - Bacterin
 - AQUAVAC-COL™/Intervet-Schering-Plough
 - Developed by Shoemaker, Evans and Klesius (ARS/USDA)
 - Modified live vaccine
 - Showed protection against columnaris in lab experiments
 - No available data from pond studies
 - Its efficacy has been questioned in some forums



Objective

- Develop new modified live vaccines against columnaris disease

Why?

- AQUAVAC-COL™ derives from a low virulence strain
- Adhesion and persistence in fish tissues after vaccination is low



Justification

- *Flavobacterium columnare*
 - Three distinct genetic groups or genomovars
 - Genomovar II has been proved to be more virulent to catfish than genomovar I
 - AQUAVAC-COL™ contains a genomovar I strain
 - Attempts to obtain genomovar II rifampin-resistant mutants have previously failed



Rifampin-resistant mutants

- Passes on media with increasing concentrations of rifampicin
 - From 50 µg to 200 µg/ml
- Avirulent, stable mutants
 - Altered LPS structure
 - *Brucella abortus*, *Edwardsiella ictaluri* and *Flavobacterium columnare* genomovar I mutants
 - Lack of high molecular weight bands in the LPS



F. columnare genomovar II mutants

- Four genomovar II strains selected based on their genetic properties and virulence
- Modification of the process of obtaining the mutants
- 13 new mutants
 - All stable, and avirulent for channel catfish

AL-CC-11	AL-CC-15	AL-CC-16	AL-CC-17
11-131	15-131	16-532	17-13
11-132	15-132	16-534	17-23
11-133	15-134		
11-232	15-231		
	15-232		



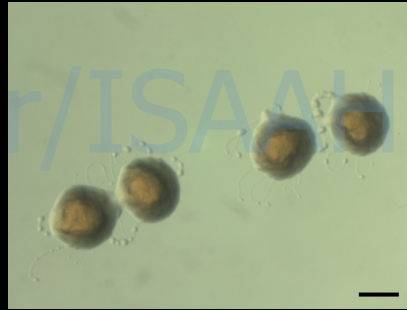
© Author/ISAAH Mutant characterization

- Morphology



Parent 15-132

1 mm



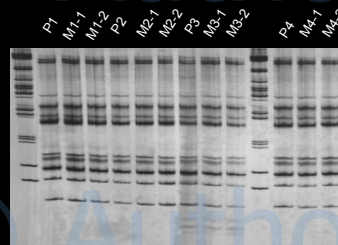
Mutant AL-CC-15

1 mm

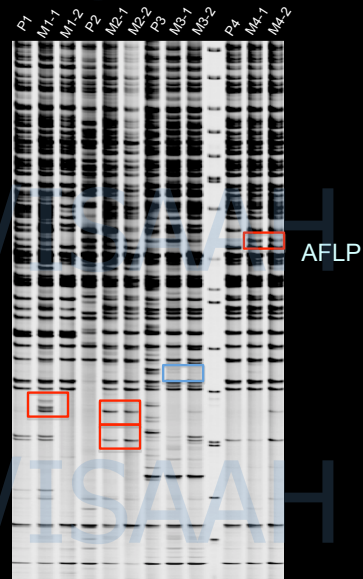


© Author/ISAAH Mutant characterization

- Genomics
 - 16S rDNA
 - ITS
 - Whole-genome (AFLP)



16S-SSCP

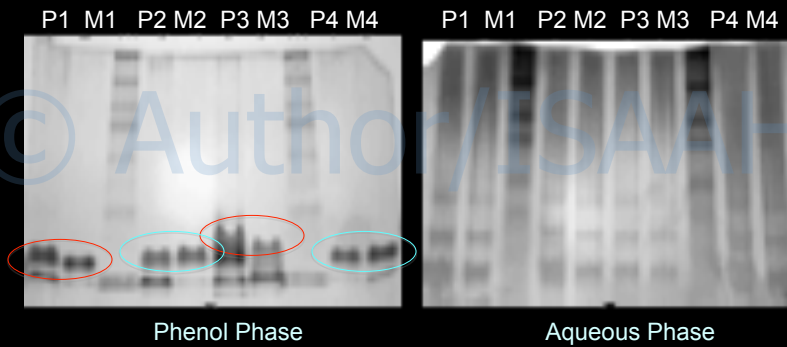


AFLP



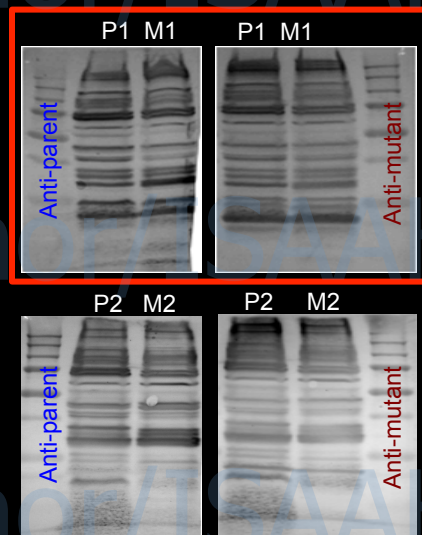
© Author/ISAAH Mutant characterization

- LPS



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- Immunoblots
 - Total proteins
 - Sera from immunized catfish



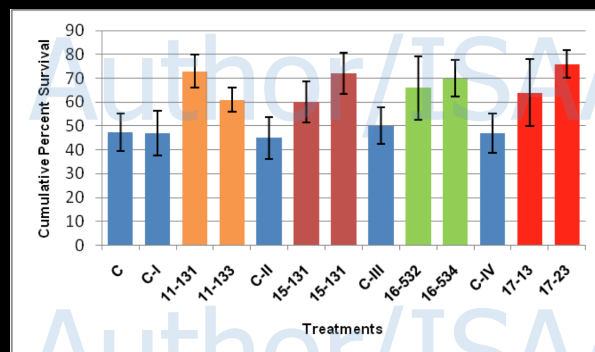
Vaccination experiments

- Catfish; 25 fish/tank (2.2-2.6 g)
 - Small system 4 tanks/treatment (15 fish/tank)
 - Large system 6 tanks/treatment (35 fish/tank)
- Acclimation – Vaccination – Challenge
- Challenged with highly virulent *F. columnare* 28 days post-vaccination
- Acute columnaris disease



Comparison between mutants

- Graphic representation of the cumulative percent survival recorded after four independent vaccination/challenge experiments. C: mean of all four control treatments



New Vaccines

- New rifampin-resistant mutants developed from different strains of *F. columnare*

Treatment	Cumulative Percent Survival (mean \pm S.D.)	ODDS ratio ³	RPS ⁴
Control	69.2 \pm 8.3	1.0000	-
11-131	71.0 \pm 29.8	1.0981	5.8
15-132	71.0 \pm 10.1	1.0851	5.8
16-534*	80.1 \pm 14.4	1.7910	35.4
17-23*	84.5 \pm 13.2	2.4956	49.7
AquaVac-COL ¹	74.7 \pm 20.6	1.2664	17.7

1- FCRR corresponds to Aquavac-Col® (Intervet/Schering-Plough Animal Health)

2- Different superscript letters indicate a significant difference at $P < 0.05$

3- Odds of survival for fish in a given treatment when compared to fish in the control

4- Relative Percent Survival as defined by Amend (1981)



Conclusion

- We have developed new rifampin-resistant mutants of *F. columnare* that provide a better protection than AQUAVAC-COL™
- Provisional patent (international)
- Future work
 - Ornamental species
 - Pond studies with catfish in 2011



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