Aeromonas salmonicida proliferation and quorum sensing in response to mucins isolated from Atlantic salmon skin and intestine



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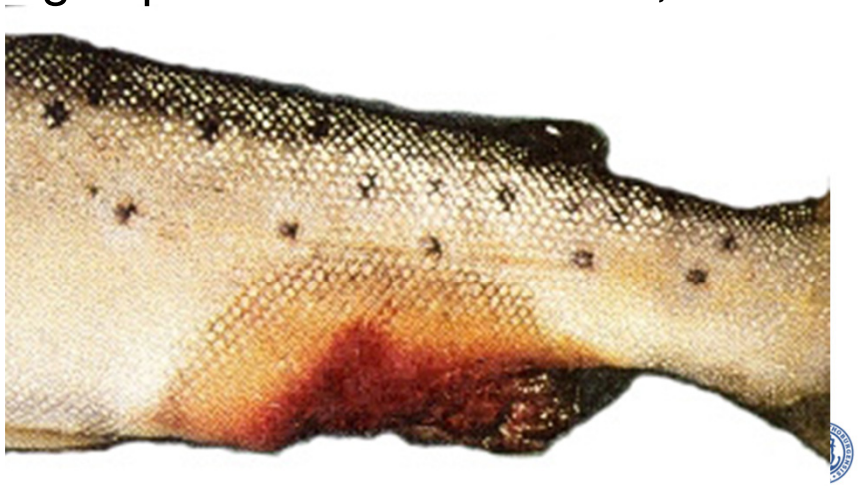
Mucins in Infection and Cancer Team

The Sahlgrenska Academy

UNIVERSITY OF GOTHENBURG

Furunculosis disease

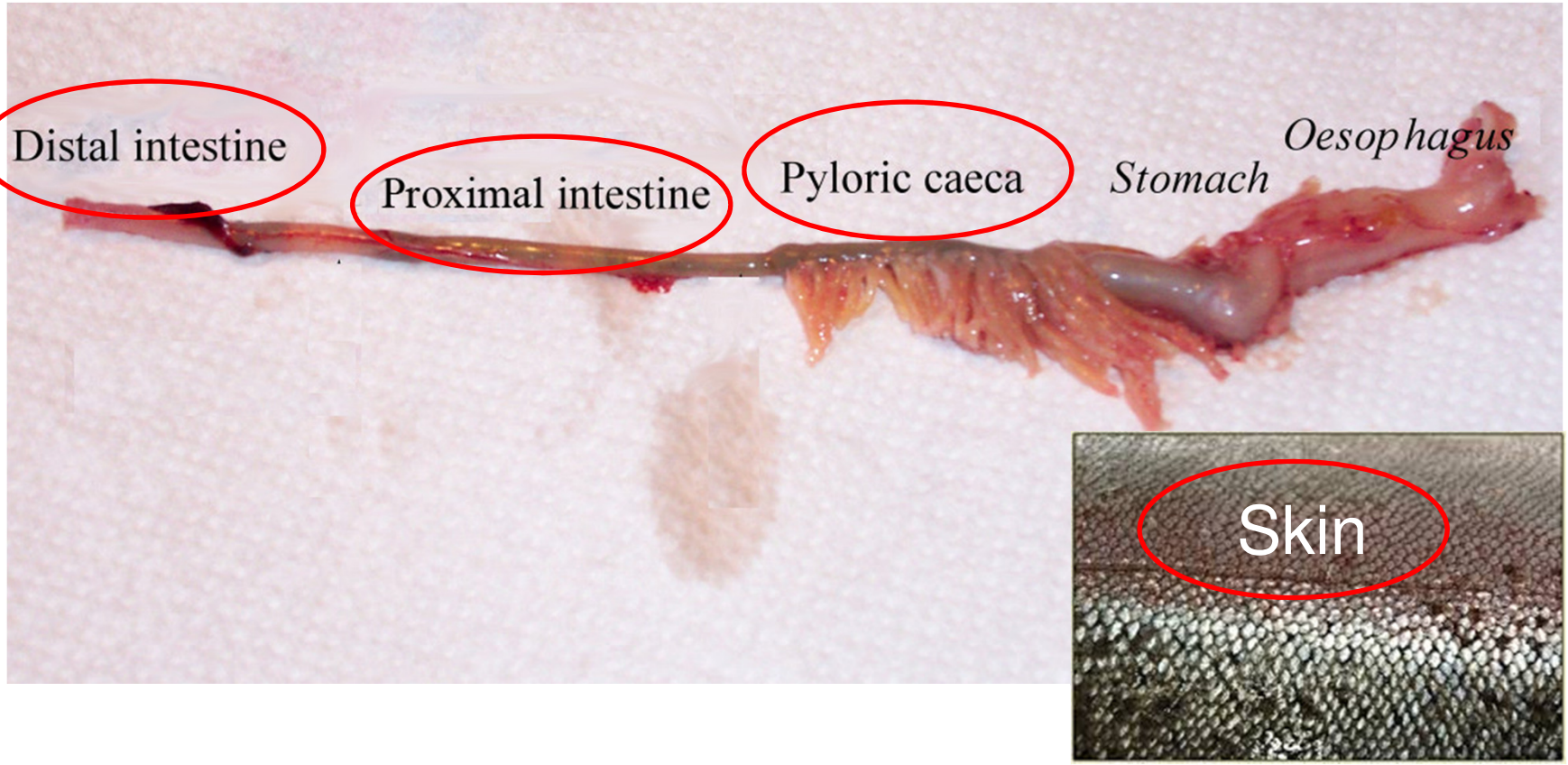
- Causative agent of furunculosis disease: *Aeromonas salmonicida*
- Gram-negative, facultative anaerobic, non-motile rod
- Furuncle-like swelling, ulcerative lesions, bowel inflammation – often fatal
- Infects mainly salmonids but described in other fish species in contact with salmonids
- *A. salmonicida* has a devastating impact on cultivated fish, particularly the *Salmonids*



Role of Mucins

- Form mucus layer with other secretions
- **Barrier function**
- Dense carbohydrate coating can hold water
- Protects against proteolysis
- Lubrication
- Other

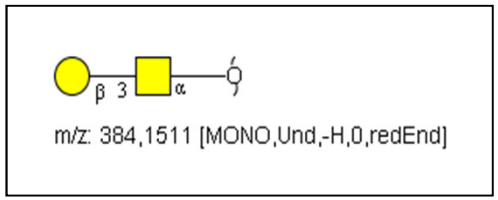
Sites of interest

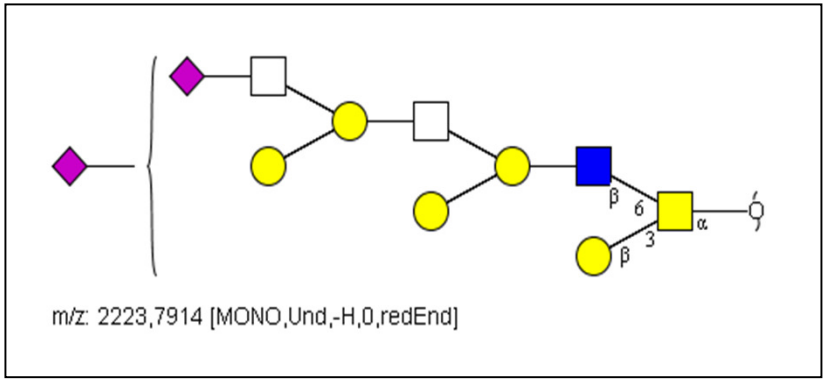


Mucin structure



- 2-11 residues in glycan chains
- Skin *O*-glycans are short (2-5)
- Intestinal mucin *O*-glycans are longer (up till 11)





Previous results

IAI
Journals.ASM.org

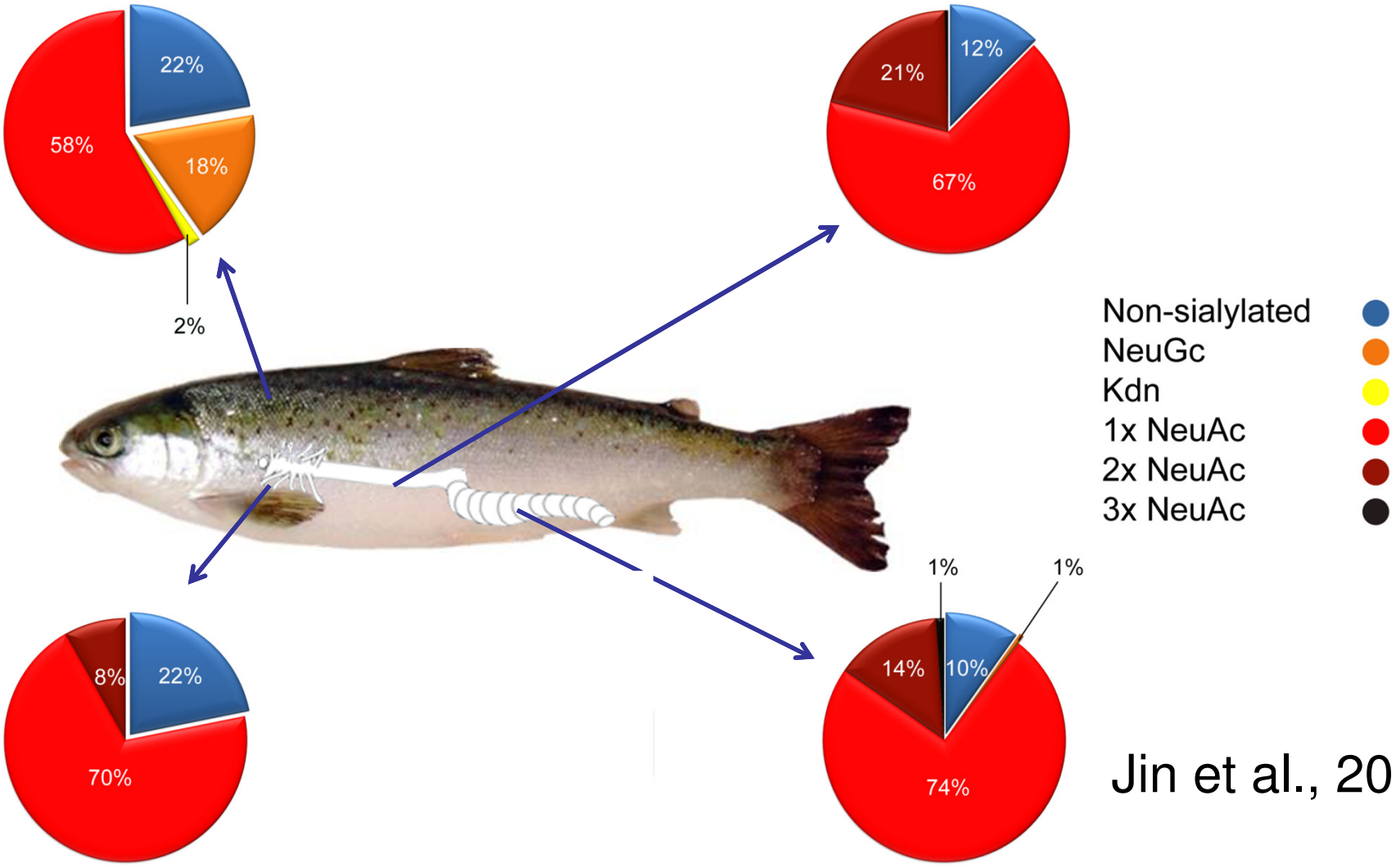
Aeromonas salmonicida Binds Differentially to Mucins Isolated from Skin and Intestinal Regions of Atlantic Salmon in an *N*-Acetylneuraminic Acid-Dependent Manner

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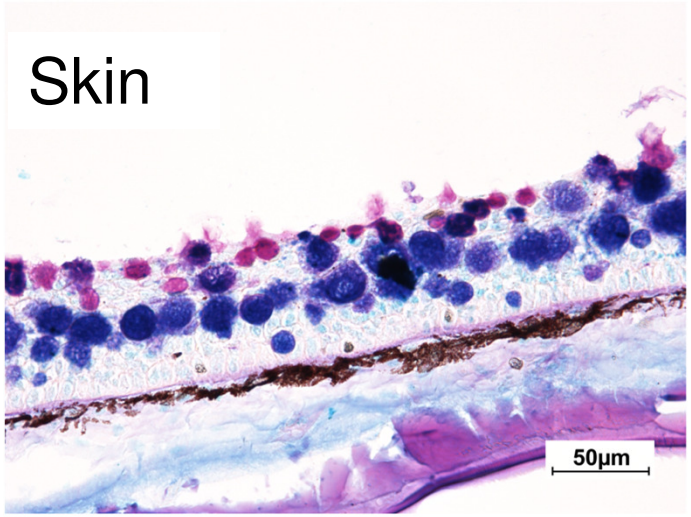
***Aeromonas salmonicida* subsp. *salmonicida* infection, also known as furunculosis disease, is associated with high morbidity and mortality in salmonid aquaculture. The first line of defense the pathogen encounters is the mucus layer, which is predominantly comprised of secreted mucins. Here we isolated and characterized mucins from the skin and intestinal tract of healthy Atlantic salmon and studied how *A. salmonicida* bound to them. The mucins from the skin, pyloric ceca, and proximal and distal intestine mainly consisted of mucins soluble in chaotropic agents. The mucin density and mucin glycan chain length from the skin were lower than were seen with mucin from the intestinal tract. *A. salmonicida* bound to the mucins isolated from the intestinal tract to a greater extent than to the skin mucins. The mucins from the intestinal regions had higher levels of sialylation than the skin mucins. Desialylating intestinal mucins decreased *A. salmonicida* binding, whereas desialylation of skin mucins resulted in complete loss of binding. In line with this, *A. salmonicida* also bound better to mammalian mucins with high levels of sialylation, and *N*-acetylneuraminic acid appeared to be the sialic acid whose presence was imperative for binding. Thus, sialylated structures are important for *A. salmonicida* binding, suggesting a pivotal role for sialylation in mucosal defense. The marked differences in sialylation as well as *A. salmonicida* binding between the skin and intestinal tract suggest interorgan differences in the host-pathogen interaction and in the mucin defense against *A. salmonicida*.**

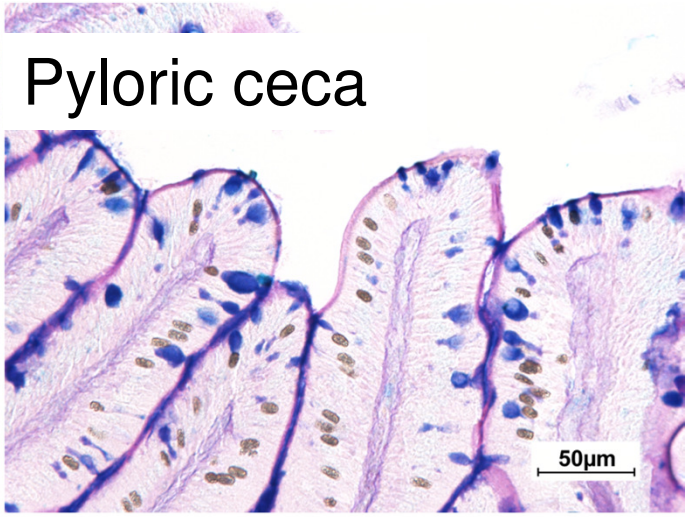
The salmon *O*-glycans are highly sialylated (78-90%)

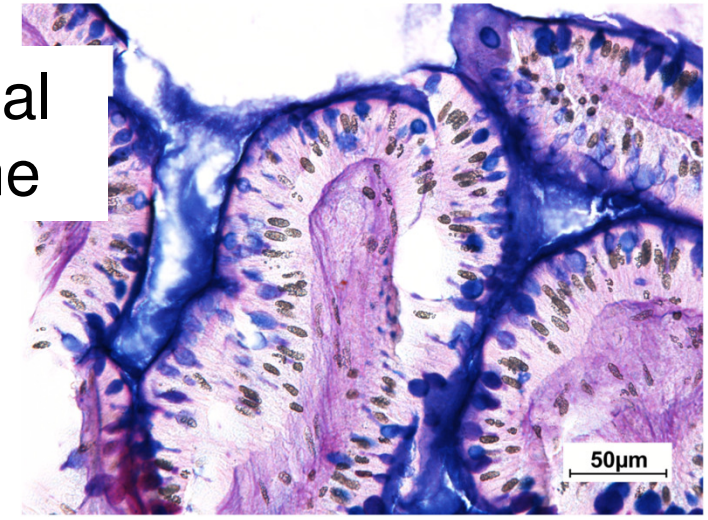


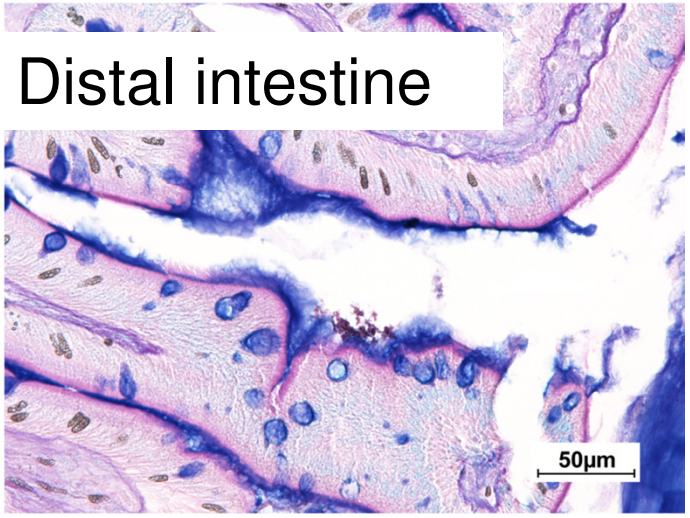
Jin et al., 2015

AB-PAS staining





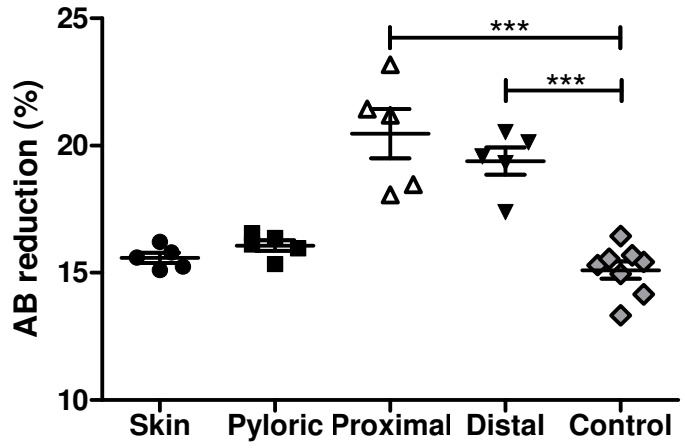




Jin et al., 2015

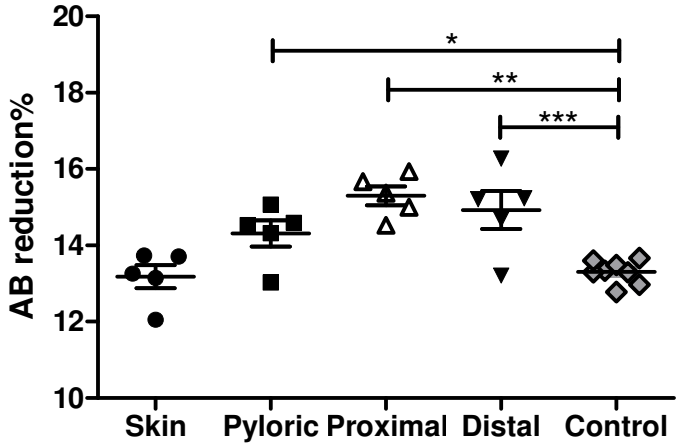
Intestinal mucins promote *A. salmonicida* growth

Defined medium (DM)+Glucose



30 min

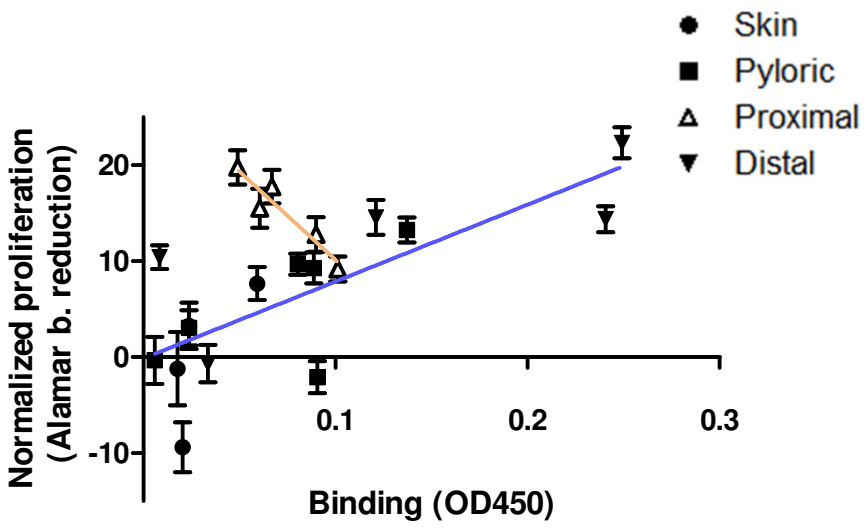
DM only



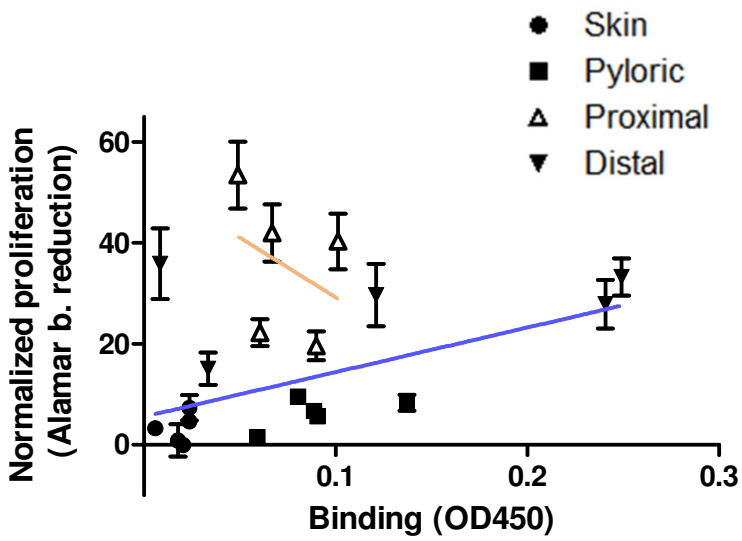
160 min

Adhesion – dual role?

DM+Glucose

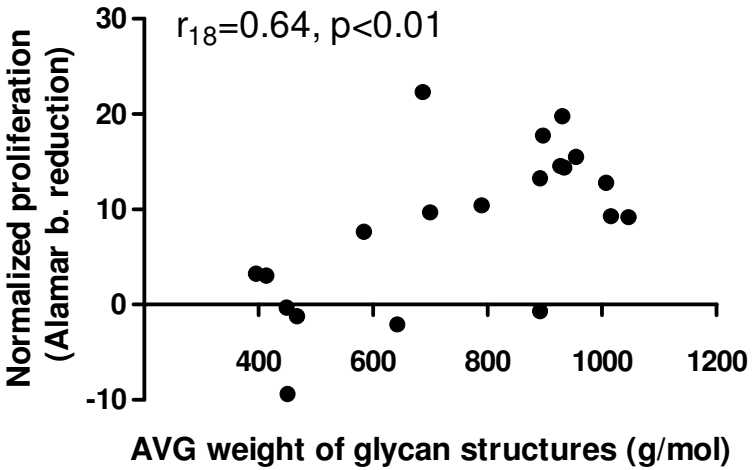


DM

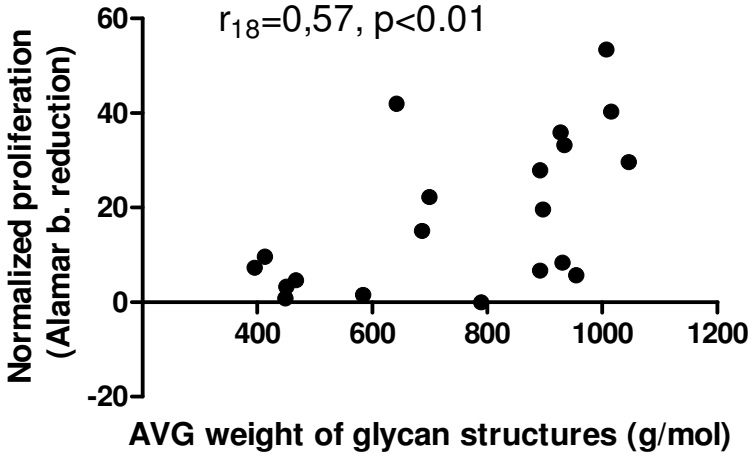


”Large” mucins nurture better

DM+Glucose

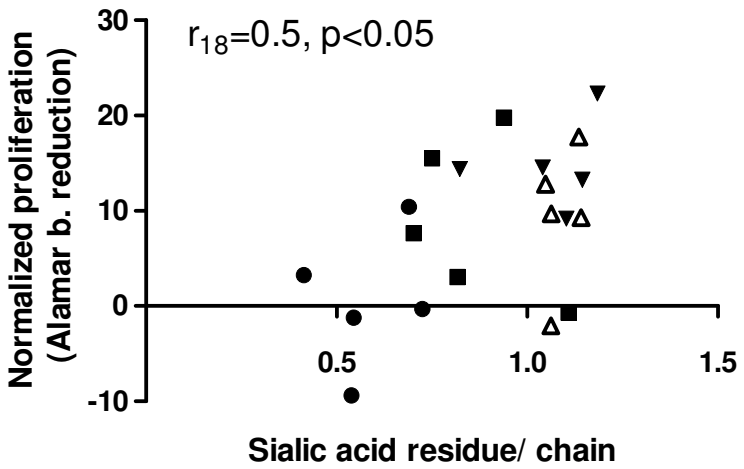


DM

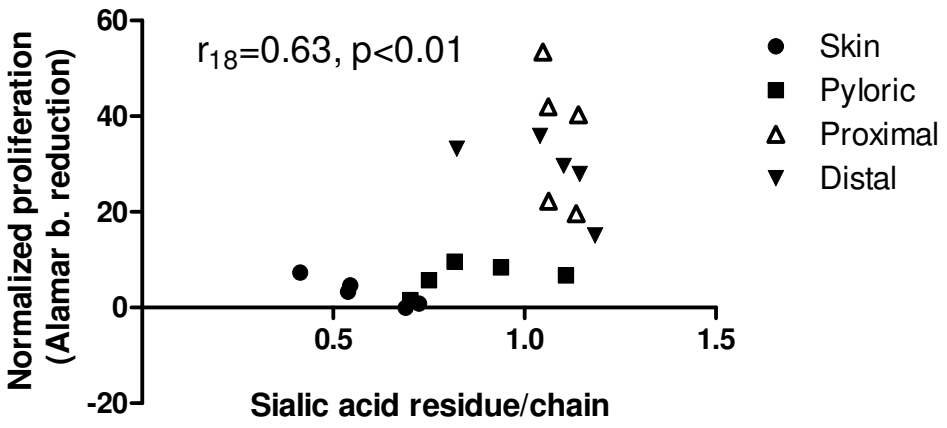


Do sialic acids promote growth?

DM+Glucose



DM

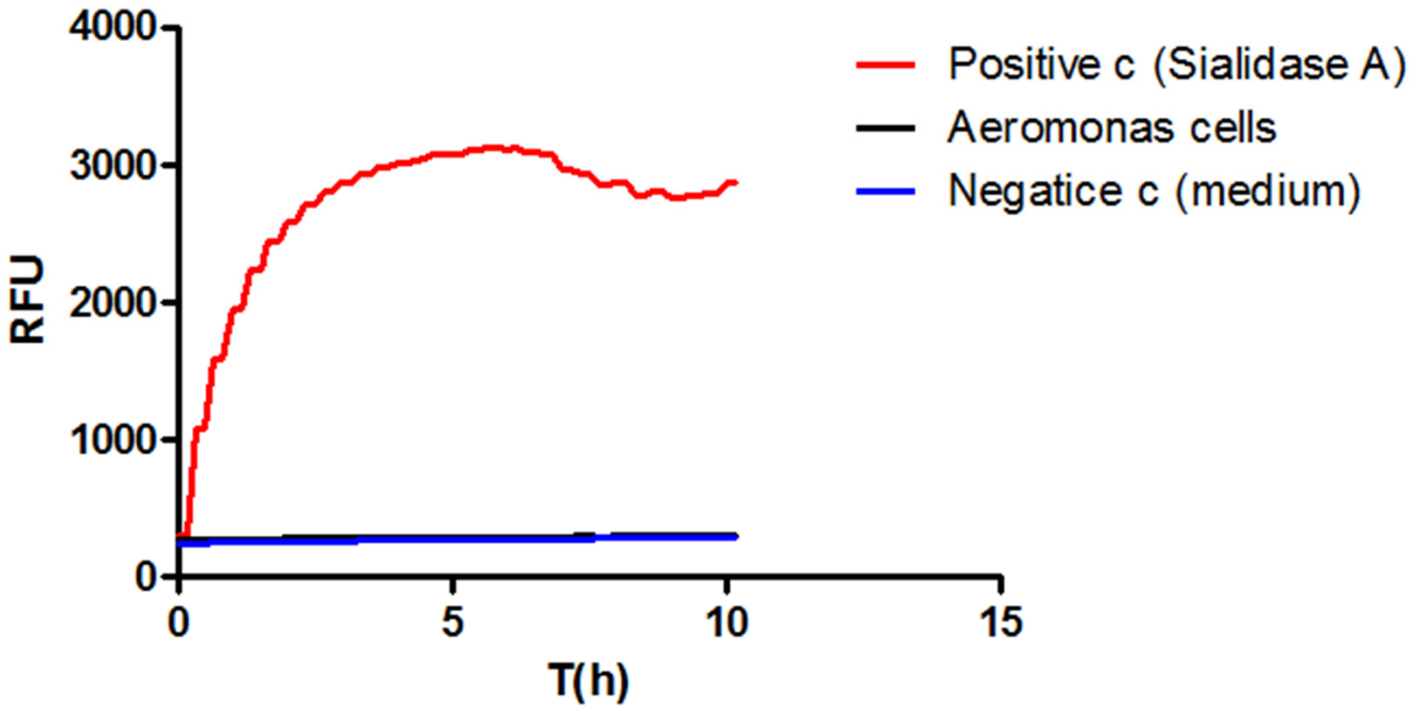


Aeromonas salmonicida – tactics of colonization

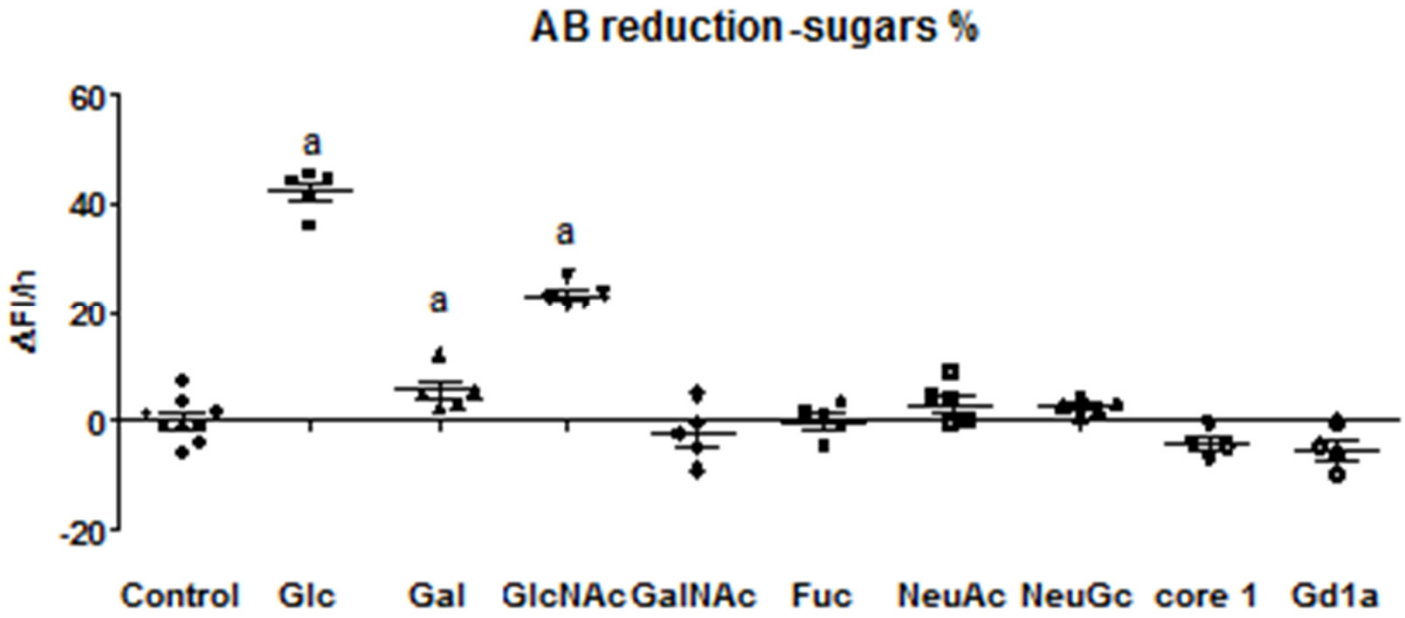
A. salmonicida is provided with tools to degrade mucins and use as C-source

- β-galactosidase
- β-N-acetylhexosaminidase
- GlcNAc-6-phosphate deacetylase
- Glucosamine-6-phosphate deaminase
- **Sialidase?**

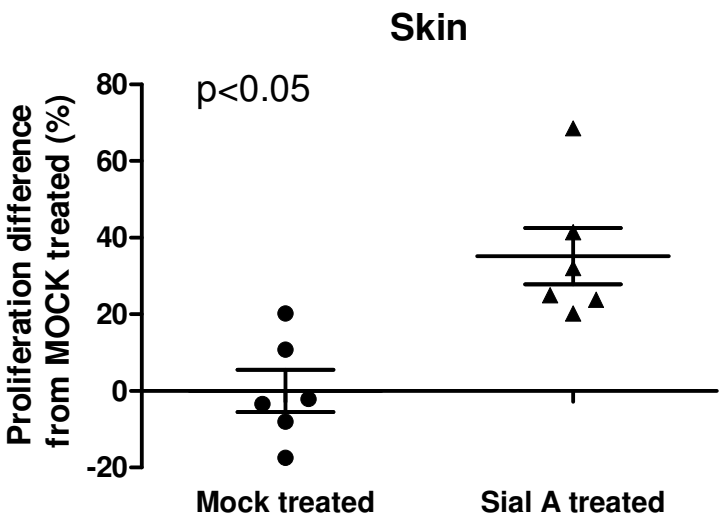
Sialidase present?

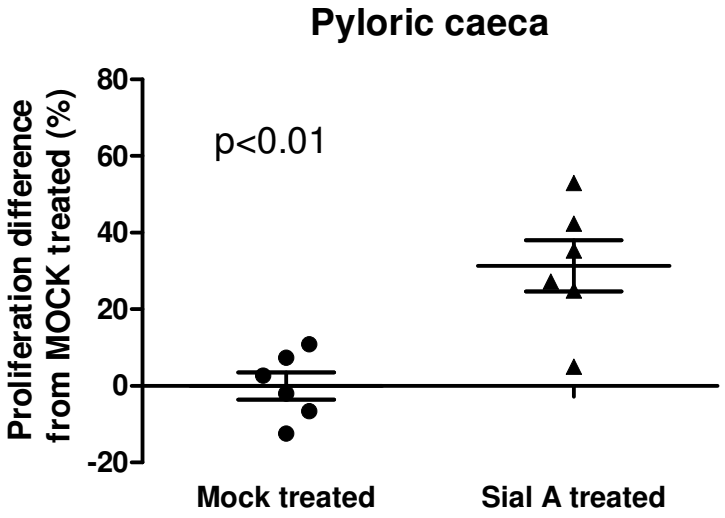


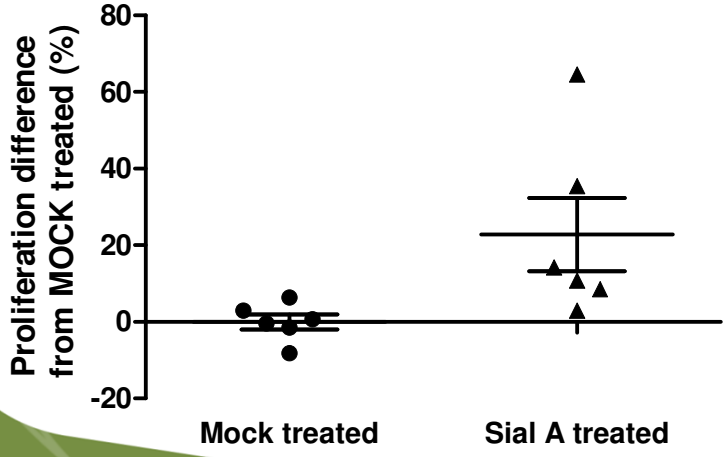
Mucin building blocks on proliferation

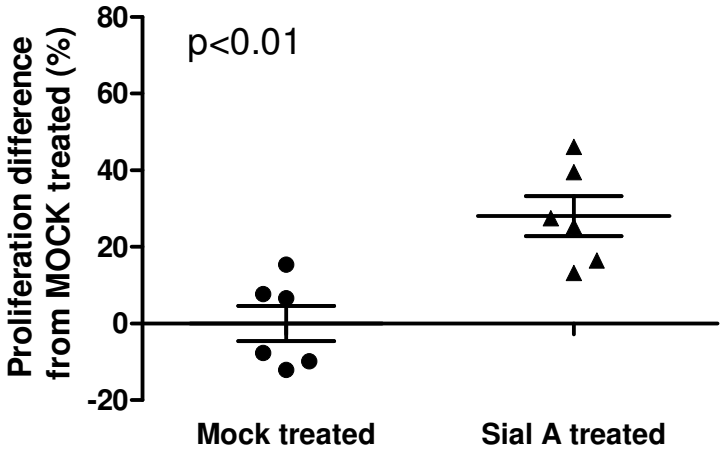


Desialylated mucins further increase growth









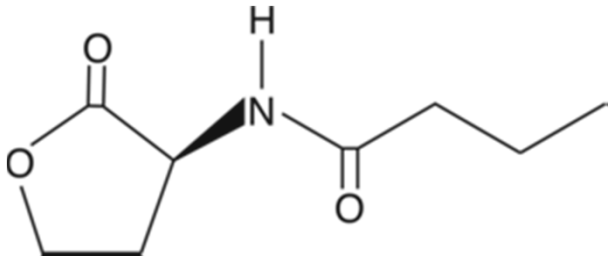
Quorum sensing

- A form of communication with small molecules
- Responsible for the group behaviour of bacteria
- Alters metabolism, luminescence, **virulence** etc.
- Three forms are known at present:
  - Intra-species communication
  - Inter-species communication
  - Vibrio intra genera communication

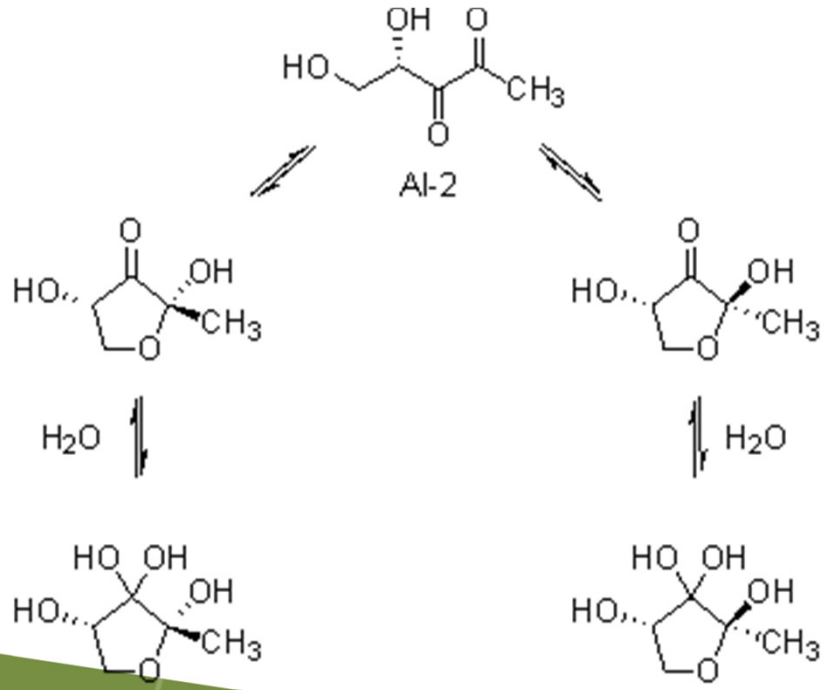
Aeromonas salmonicida ”language”

- **Autoinducer-1** – Acylhomoserine lactons
  - AsaI-autoinducer synthase
  - AsaR-autoinducer receptor
- **Autoinducer-2 system** – ***A. hydrophila*** – *4,5-Dihydroxy-2,3-pentanedione*
  - LuxS-autoinducer synthase
  - LuxP-autoinducer receptor
  - LuxU-autoinducer phosphorelay protein
  - LuxO-autoinducer regulator
- **Other system(s)???**

Effect of autoinduction on growth

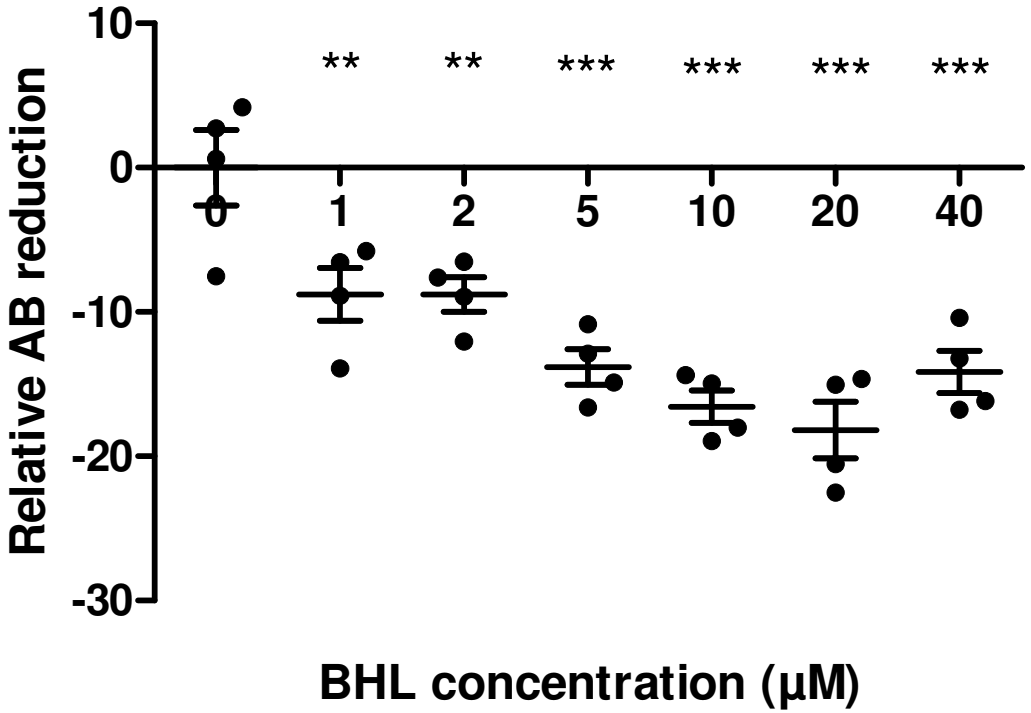


N-Butyryl-homoserine lactone
(BOC Sciences)

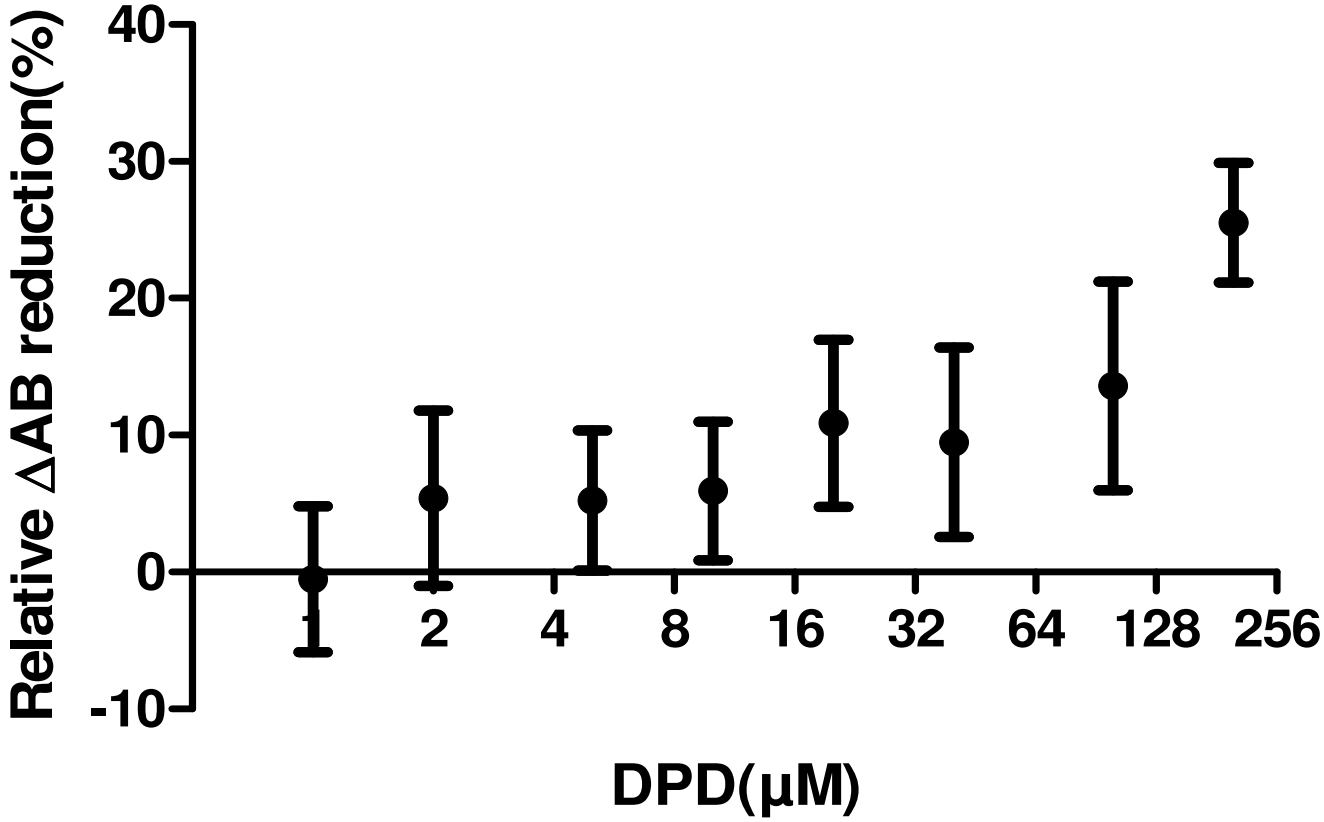


(S)-4,5-Dihydroxy-2,3-
pentandione (DPD, AI-2)
Autoinducer-2

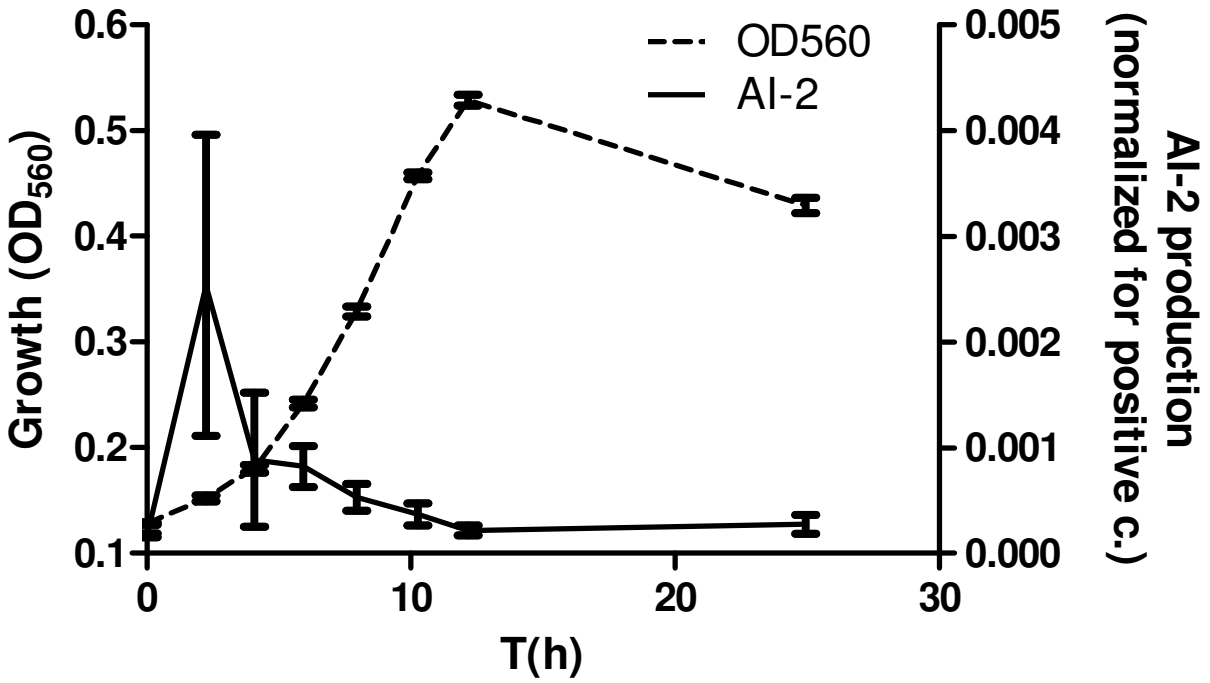
BHL (C4 HSL) growth response



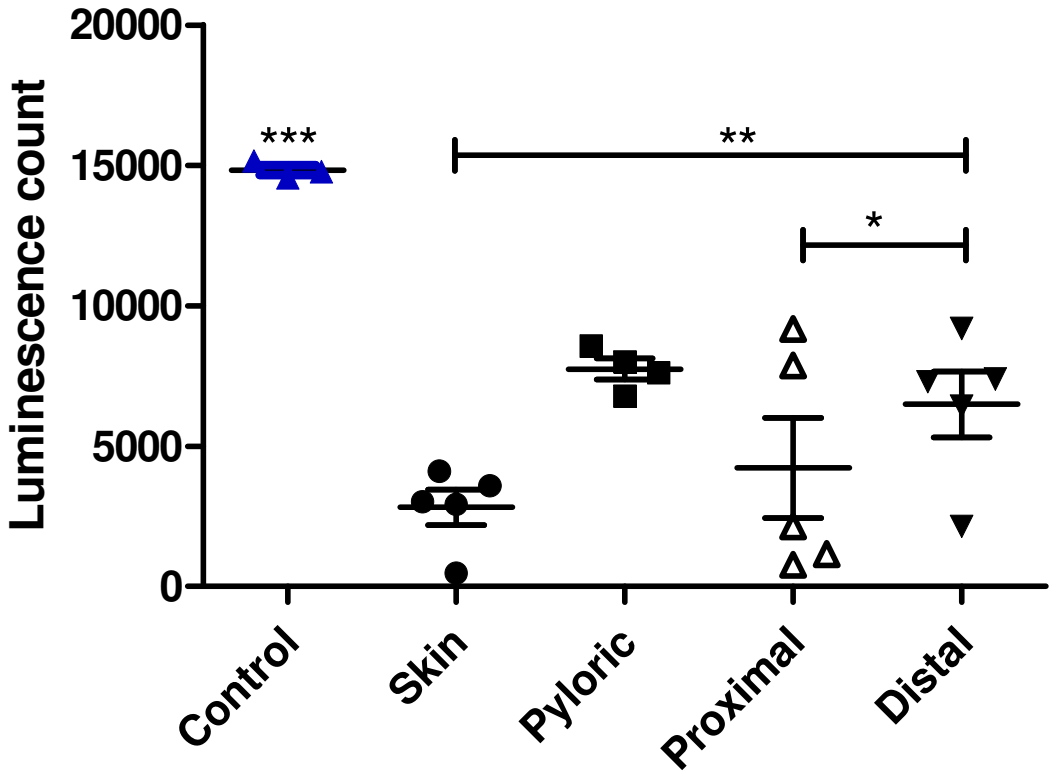
DPD growth response



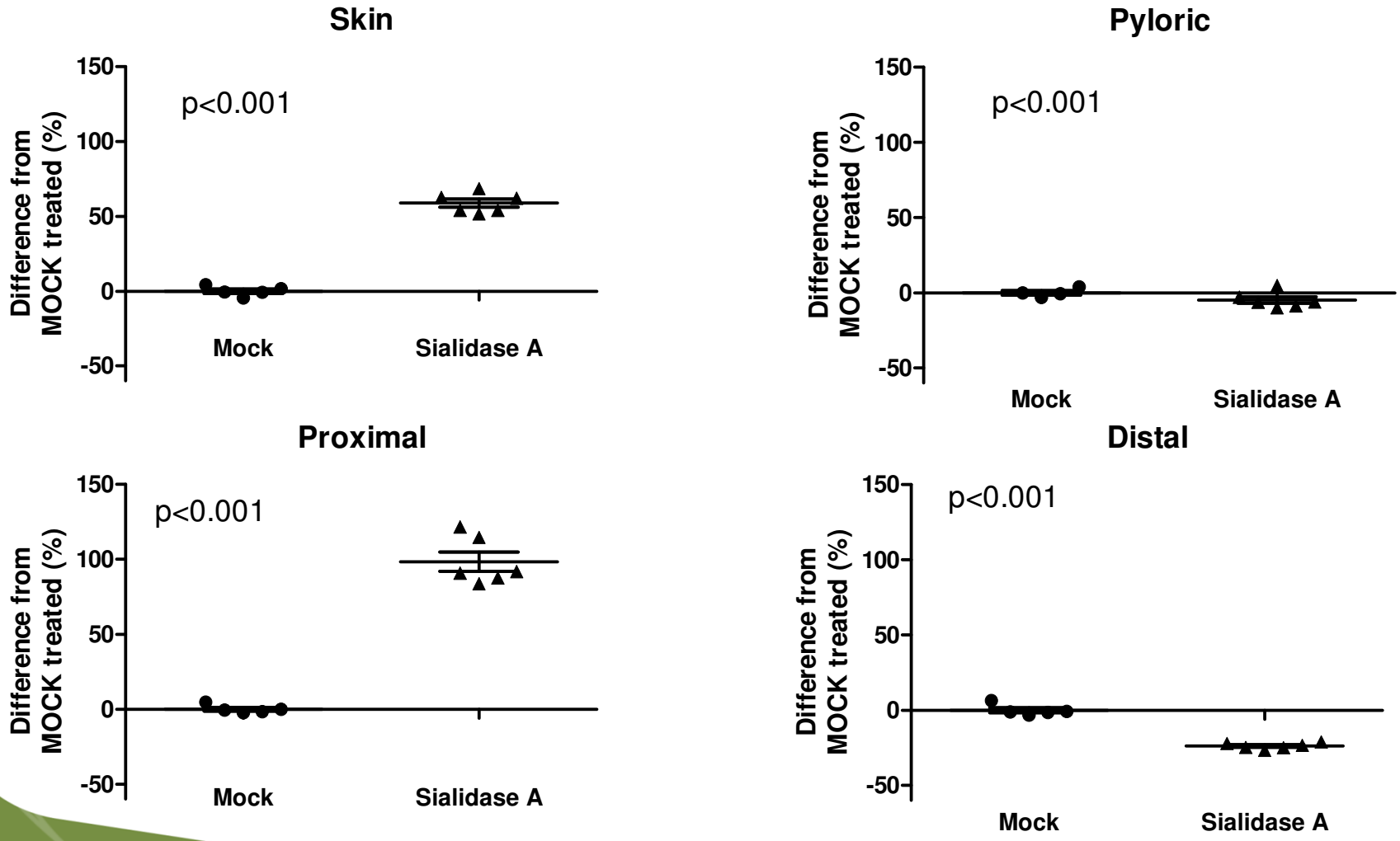
Ai-2 timecourse



Mucins Ai-2 communication



Reduction in sialic acids differentially affects QS in the organ groups



Conclusion

- Intestinal mucins feed the bacteria but reduce the level of communication. Balancing between commensals and pathogens through AI-1 system?
- Skin mucins seem to be inhibitory for *A. salmonicida* colonization and infection.
- Sialic acids in Proximal intestine and Skin has an especially high importance in QS
- AI-1 and AI-2 timecourse with mucin effect

Acknowledgements

Prof. Sara Lindén , Dr. Chunsheng Jin and the rest of the Mucins in Infection and Cancer Team

Prof. Kristina Sundell

Dr. Henrik Sundh