

# Cost of virulence in fish farm isolates of *Flavobacterium columnare* in outside host environment

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## Abstract

Fish farms have been suggested to promote virulence of the harmful fish bacterium *Flavobacterium columnare* [1], causing columnaris disease in farmed salmonids in freshwaters. Skin lesions, fin erosion and gill necrosis associated with the bacterial infection are fatal, especially to fingerling fish if left untreated. This makes the bacterium as one of the most serious threats to fresh water salmon farming in Finland and elsewhere in northern Europe. Only recently, isolates of *F. columnare* were obtained from the environment outside fish farming and the fish hosts [2]. In challenge tests with the zebra fish model, these isolates exhibited lower virulence than isolates originating from fish hosts. This suggests that fish farms and the environment could be differentially selecting for the traits that might be underlying the emergence of disease outbreaks in fish farms.

Earlier, *F. columnare* has been shown to produce different colony morphologies on Shieh agar plate cultures, suggested to possess different biological functions in transmission and survival outside the host and during infection [3]. Rhizoid colony morphotype is isolated in primary cultures from diseased fish and most often associated with virulence, while rough morphotype forms during culture in laboratory and is avirulent. Unlike obligatory bacterial pathogens, which cannot survive outside their hosts, *F. columnare* is capable to grow on and transmit from dead fish and to survive in water for extended periods [4], and can therefore be considered as an opportunistic pathogen. Opportunistic pathogens do not face the trade-off between within-host growth and transmission as obligatory pathogens do, i.e. transmission to a new living host before the death of the previous host is not mandatory for their survival. For this reason, opportunistic pathogens are a challenge for the virulence evolution theory. However, opportunistic pathogens are subject to environmental trade-offs: growth in different environments may incur costs associated with different traits selected in different environments. For example, ability for rapid exploitation of host tissues for growth on the fish (virulence) might be a disadvantage in the environment outside the host, leading to a trade-off between virulence and environmental performance [5].

For *F. columnare*, it is not known if growth between environmental and fish farm isolates differ, or if virulent rhizoid and non-virulent rough colony types have different growth characteristics. To test this, we measured growth parameters of both rhizoid and rough colony types of ten isolates of *F. columnare* (five from the environment and five from fish farms) in culture medium, i.e. outside host environment. The fish farm isolates originated from disease outbreaks at farms in Finland, but included also strain JIP39/87 isolated from France (kindly donated by J-F Bernardet). The environmental isolates all originated from the same location in Central Finland, upstream of a fish farm in 2010 [2]. For each isolate we had two different

morphotypes: rhizoid and rough. Rough morphotype originated from the ancestral rhizoid type spontaneously during laboratory culture. Both resource level and temperature affect growth through their effects on biochemical processes, thus we measured the growth in five different resource concentrations (0.05×, 0.1×, 0.5×, 1× and 2× Shieh medium) and at two temperatures (15 and 25 °C). Bacterial growth was measured with Bioscreen C<sup>®</sup> temperature controlled spectrophotometer (Growth Curves AB Ltd, Finland) as increase in turbidity (OD at wide band 420–580 nm) at 5 min intervals. The maximum growth rates and yield (biomass) were assessed from the OD data (unpublished Matlab script by J. Laakso).

Bacteria grew faster at 25 °C than at 15 °C (Fig. 1) and growth rates were higher at higher resource concentrations (Fig. 2). The growth rates differed between all resource concentrations, except between the two lowest ones (pairwise testing).

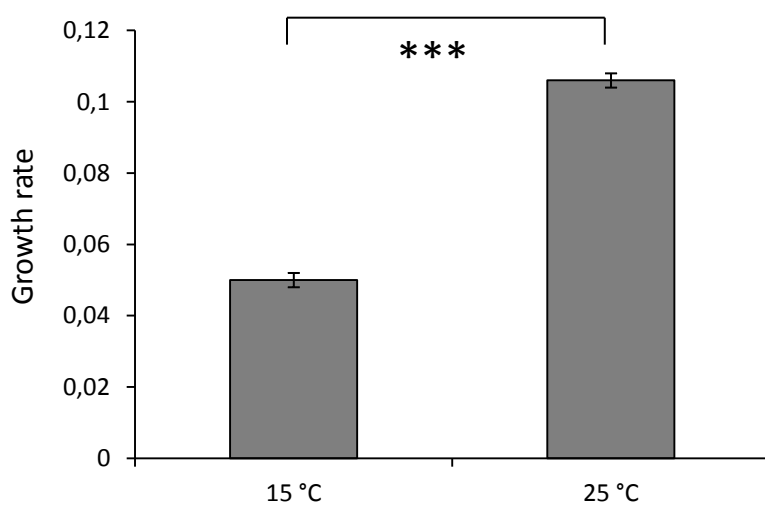


Figure 1. Effect of temperature on maximal growth rate of *Flavobacterium columnare*. Statistical significance between treatments is denoted as \*\*\* =  $p < 0.001$ .

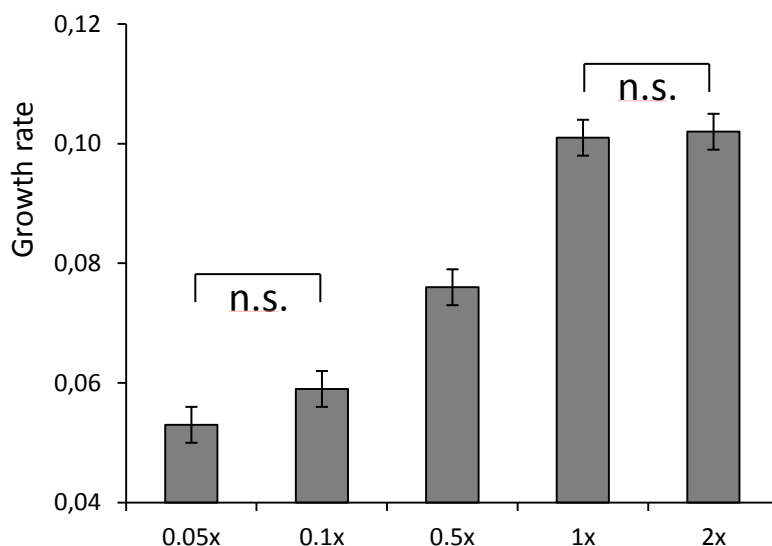


Figure 2. Effect of resource concentration on maximal growth rate of *Flavobacterium columnare*. Resource concentrations used were 0.05×, 0.1×, 0.5×, 1× and 2× Shieh medium. Only non-significant (n.s) differences in pairwise t-tests between treatments are shown, all other pairwise comparisons were significant.

Origin of isolate or colony morphotype did not affect the growth rate individually, but via interactions. Isolates from fish farms outperformed the environmental isolates in growth only if the morphotype was rough, but not if the morphotype was rhizoid. However, at low temperatures there were no differences between morphotypes, or between isolates from

different origins. The results concerning the biomass yield were consistent with the results on growth rate. Biomass yield was higher at 25 °C than at 15 °C and increased with resource concentration. Higher yield was achieved at the higher temperature in all resource concentrations apart from the lowest 0.05× concentration.

Our results confirm the importance of temperature for growth of *F. columnare*, being one explanatory factor why columnaris disease outbreaks generally occur during warm summer months. Our results also indicate that growth of *F. columnare* is strongly affected by resource concentration, which could be one of the factors at fish farms selecting for faster growing strains. Isolates from fish farms outperformed the environmental isolates in growth, which suggests that fish farms might select for faster growth ability of *F. columnare*. However, fish farm isolates achieved faster growth only as rough, avirulent morphotype. This suggests that maintaining the virulent rhizoid morphotype might be costly and traits selected during growth in fish host decrease the ability to grow outside the host environment. Our findings support the hypothesis that different morphotypes in *F. columnare* have different functions in the infection cycle [3]. In addition, selection between morphotypes occurs in fish farms rather than in the natural environment.

## References

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