Paramoeba perurans
interaction with Atlantic salmon gills

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Amoebic gill disease infected Atlantic salmon

Introduction

Amoebic Gill Disease (AGD) caused by the ectoparasite *Paramoeba perurans* affects several cultured marine fish species worldwide. The amoeba has been thoroughly investigated due to the associated economic loss in the salmon industry[1], but the intimate nature of *P. perurans* interaction with the branchial epithelium is still not clear. In this study, the morphologies of cultured *P. perurans* and the surface relationships between *P. perurans* and the Atlantic salmon gill epithelium during development of AGD is described using scanning and transmission electron microscopy (resp. SEM and TEM).

Material & methods

P. perurans was isolated from the gills of AGD-affected Atlantic salmon, *Salmo salar* L., according to Morrison *et al*.[2]. Gill arches were obtained from an ongoing infection trial as part of a larger AGD-study. Cultured *P. perurans* and gill branches were prepared for ultrastructural examination in SEM and TEM according to standard procedures.

Results

P. perurans cultures contained several different morphologies ranging from a distinct rounded cell structure (fig. 3b) to polymorphic cells with pseudopodia of different lengths and shapes (fig. 3c-d). SEM studies of the gills of AGD affected Atlantic salmon revealed the presence of enlarged swellings in affected gill filaments (fig. 2a) and syncytia between adjacent lamellae (fig. 2b). Spherical amoebae appeared to embed within the epithelium (fig. 2d), and subsequently leave hemispherical indentations following their departure (fig. 2e). These fenestrated structures corresponded to the presence of pseudopodia which could be seen by TEM to 'penetrate' into the epithelium (fig. 2f). However, *P. perurans* associated with the pavement epithelium did not appear to directly attach to the epithelial cell membranes but were rather separated by a diffuse amorphous matrix (fig. 2g).

Conclusions

Cultured *P. perurans* revealed several different morphologies. The amoeba seems to form a rounded morphology in response to environmental stressors. This might be a semi-resistant pseudocyst stage which may affect the efficacy of treatment of amoebic gill disease. The formation of extended pseudopodia enables the amoeba to transport over large distances probably aiding in fish-to-fish transmission during disease.

***P. perurans* attachment to the gill surface resulted in considerable fenestrated indentations and holes in the gill pavement epithelium cells.**

The demonstrated diffuse amorphous matrix separating the amoeba - epithelial cell membranes suggest the existence of cellular glycocalyces and a role for extracellular products in mediating pathological changes in amoebic gill disease.

Unaffected gill branch

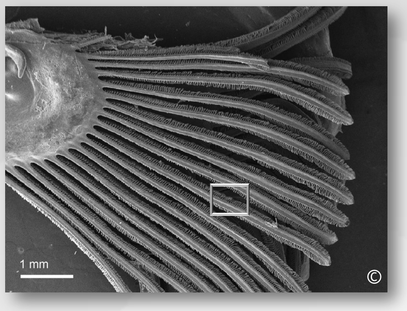


Figure 1a. Filaments with normal structure(SEM)

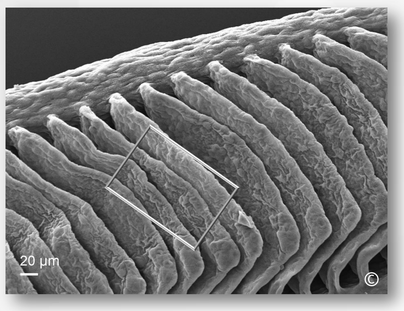


Figure 1b. Gill filament with numerous lamellae (SEM)

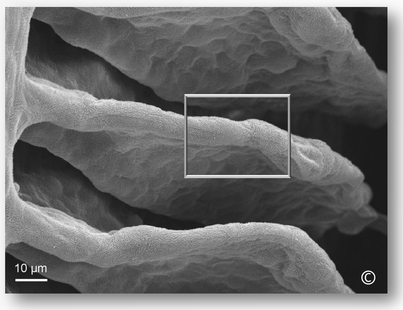


Figure 1c. Gill lamellae (SEM)

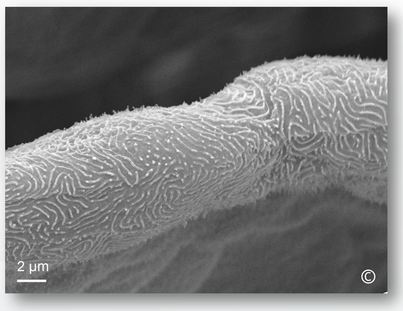


Figure 1d. Gill lamella with intact micro-ridged surface (SEM)

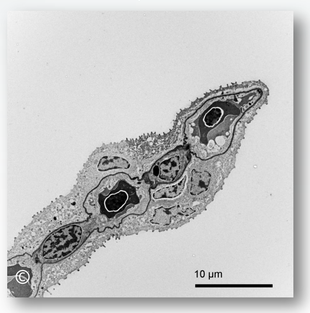


Figure 1e. Ultrathin section of a gill lamella (TEM)

AGD affected gill branch

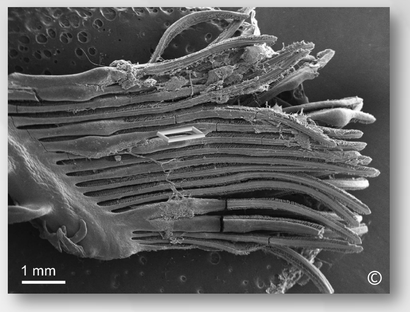


Figure 2a. Several filaments with hyperplastic areas (SEM)

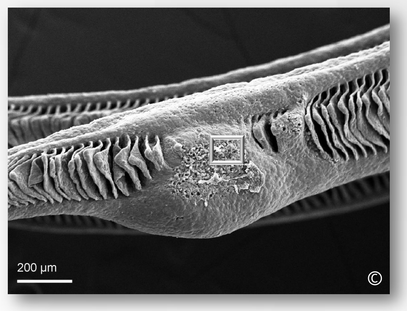


Figure 2b. Swelling in gill filament and syncytia between lamellae (SEM)

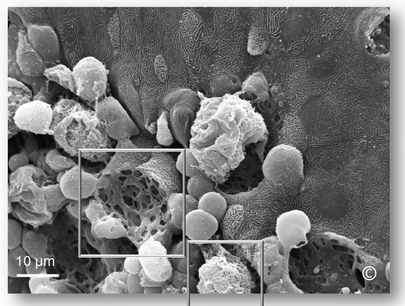


Figure 2c. Paramoeba infected pavement epithelium (SEM)

Figure 2d. *P. perurans* attached to the epithelium

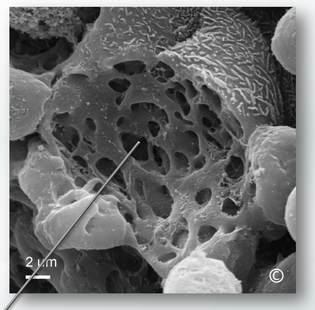
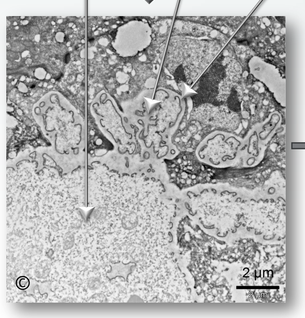


Figure 2e. Holes in the epithelial membrane (SEM)



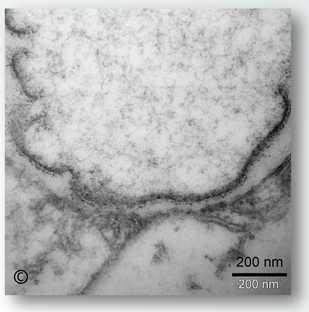
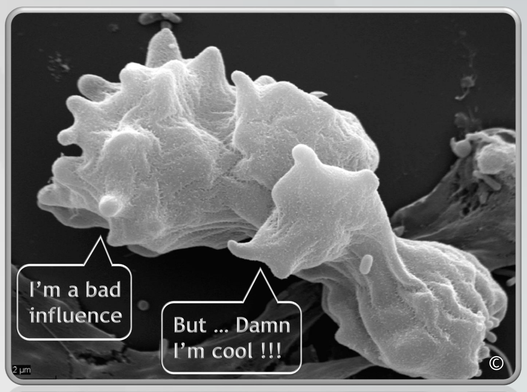


Figure 2 f-g. *P. perurans* pseudopods 'penetrating' an epithelial cell and close-up of the membrane assosiation (TEM)

Cultured *Paramoeba perurans*



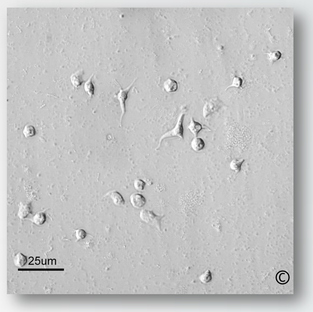


Figure 3a. Several morphologies of monocultured *P. perurans* (phase contr.)

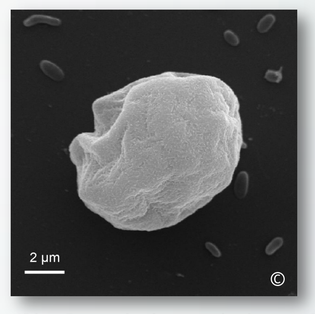


Figure 3b. Rounded morphology (SEM)

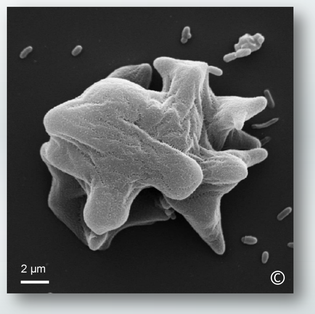


Figure 3c. Intermediate morph. (SEM)

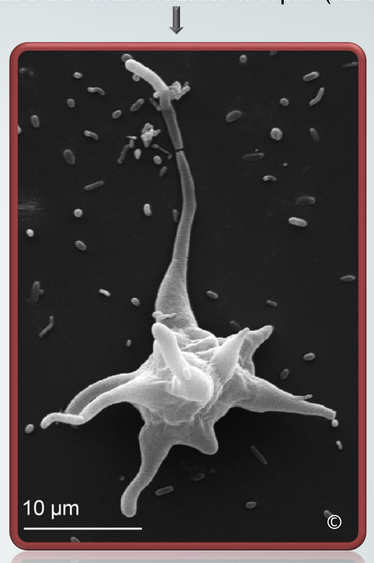


Figure 3d. Long pseudopodia (SEM)

References

1. Nowak B., Valdenegro-Vega V., Crosbie P. & Bridle A. (2014) Review; Immunity to Amoeba. *Dev. Comp. Immunol*. **43**, 257-267

2. Morrison R.N., Crosbie P.B. & Nowak B.F. (2004) The induction of laboratory-based amoebic gill disease revisited. *J. Fish. Dis.* **27,** 445-449.

Acknowledgements

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