



## GROWTH AND MORPHOLOGICAL CHARACTERIZATION OF FLAVOBACTERIUM OREOCHROMIS ISOLATED FROM TAMBAQUI (COLOSSOMA MACROPOMUM)

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### RESUMO

*Flavobacterium oreochromis* is one of the major pathogens of tambaqui. This fastidious bacterium makes diagnostic techniques complex and hinders the implementation of effective protocols for the prevention and control of the disease. In this context, the objective of this study was to characterize the growth curve and investigate the morphology of four genetic groups of *F. oreochromis* isolated from tambaqui. To achieve this objective, 4 strains representative of each genetic group (GG) of *F. oreochromis* (GG<sub>1</sub>-AMFO04, GG<sub>2</sub>-AMFO12, GG<sub>3</sub>-AMFO14 and GG<sub>4</sub>-SPFO12) were previously isolated from farmed tambaqui and selected based on the results of REP-PCR (the molecular tool used for the prospection of GG). For the characterization of the growth curve, each strain was initially incubated on G agar at 28°C for 24h. Then, two colonies of each strain were transferred to the respective culture medium, containing 500mL of sterile G broth. Bacteria were incubated at 28°C/140rpm/192h, and every 12h, an aliquot of each GG (1mL) was sampled to determine the bacterial concentration in optical density (spectrophotometer, OD<sub>625</sub>) and also in colony-forming units (CFU/mL) through plating and counting of serial dilutions (1:9, inoculum: sterile-PBS). The data was estimated according to the period of incubation to determine the growth curve phases as following: lag, exponential, stationary, and death. For morphological analysis, one colony of each strain was selected for Gram staining and also for processing (fixed, dehydrated, coated with a conductive layer) for image at optical microscopy and scanning electron microscopy, respectively. The results showed that all strains showed significant growth after 24h in G medium and remained viable until 120h. The lag-phase lasted from 0-12h, the exponential-phase was reached after 24h of incubation, and the stationary-phase after 96h. The death-phase was not achieved until 120h of incubation. The surface morphology revealed that the 4 strains produced similar colonies with a yellow, opaque, rhizoid and flat appearance, adherent aspect, and irregular shape, but in different degrees. The 4 strains appeared as typically Gram-negative filamentous morphology (long-bacilli) and at SEM, showed the presence of biofilm. The aspects described in this are fundamental to understand the dynamics of proliferation and pathogenicity of GG of *F. oreochromis* that infect tambaqui. These factors facilitate the development of biosecurity measures to protect fish health, and also to reduce risks to human health and maintain the integrity of the aquatic ecosystem, promoting a single integrated health approach. Founding source: Amazônidas/Fapeam and INCT-Peixes, MCTIC/CNPq (proc. 405706/2022-7).

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