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IDENTIFICATION, VIRULENCE AND GENETIC DIVERSITY OF FLAVOBACTERIUM OREOCHROMIS OF TAMBAQUI (COLOSSOMA MACROPOMUM) IN DIFFERENT BRAZILIAN STATES

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RESUMO

Aquaculture in Brazil has increased. However, the efficient control of bacterial diseases is still one of the greatest concerns for development of the activity in the context of One-Health (fish: consumer: environment). Flavobacteriosis outbreaks are one of the greatest constraints for intensification and have raised as a major concern for the farming of tambaqui. Current limitations in epidemiological data obfuscate the risk these pathogens pose to its farming. Therefore, the aim of this work was to accomplish an epidemiological study (CEUA:008/2021) to establish the pathogen-identity, the prevalence, virulence and genetic diversity of *Flavobacterium* sp. isolated from diseased tambaqui in different states in Brazil. Hence, approximately 23.500 juveniles from Amazonas, São Paulo and Rondônia were monitored (2019-2022). A total of 27 mortality outbreaks (27 batches of up to 1000 fish/each) suggestive of Flavobacteriosis were investigated, resulting in 270 examined fish for the target-bacteria. More than 300 strains presumptively ascribed to the target-bacteria were isolated from kidney and muscle on G agar (incubation at 28°C/48h), resulting in 64 isolates prospected to confirm the identity through PCR-Multiplex (Promega-GoTaq), following the amplicon-size according related-species: *F. columnare* (415bp), *F. covae* (320bp), *F. davisii* (8949bp) and *F. oreochromis* (659bp). Then, one isolate from each outbreak was prospected (27 strains) to determine the virulence genes and the genetic groups (GG) diversities. Virulence was determined through PCR (Promega-GoTaq) for the following target-genes: nitric-oxide reductase (254bp), thioredoxin (341bp) and glycosyltransferase (678bp). Genetic diversity was determined by REP-PCR (Promega-GoTaq) using the same primer-mix as PCR-multiplex, besides 16SrRNA. Following cladistic analysis, the genetic diversity was categorized in GG_1, GG_2, GG_3 and GG_4, and considered identical (same GG) with the 90% cut-off point. The results identified all 64 isolates as *F. oreochromis*, and the pathogen prevalence was recorded in 100%. All investigated virulence-genes were present in the 27 selected-strains. A total of 4GG of *F. oreochromis* were identified, resulting in $6GG_1$-strains (Amazonas), $4GG_2$-strains (Amazonas), $15GG_3$-strains (Amazonas and Rondônia) and $2GG_4$-strains (São Paulo). This study proves that *F. oreochromis* poses a significant threat to farmed tambaqui in Brazil, with a high prevalence of infection. Additionally, the study confirms the virulence of *F. oreochromis* and identifies four distinct genetic varieties of the pathogen, with higher prevalence of GG_3. This information is valuable for implementing targeted control measures and understanding the

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epidemiology of the disease and allow an integrated One-Health approach to mitigate risks to human, animal and environmental health. Funding source: Amazônidas/Fapeam and INCT-Peixes,MCTIC/CNPq(proc.405706/2022-7).

PALAVRAS-CHAVE: Flavobacteriosis, Epidemiology, Native species

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