



## MULTILOCUS SEQUENCE TYPING OF LACTOCOCCUS PETAURI STRAINS ISOLATED FROM BRAZILIAN NATIVE FISH SPECIES REVEALS GENETIC DIFFERENCE IN RELATION TO ISOLATES OBTAINED IN OUTBREAKS ON THE AMERICAN TILAPIA AND TROUT FARMS

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### RESUMO

*Lactococcus petauri* is an emergent fish pathogen detected in mortality outbreaks in Nile tilapia and rainbow trout farms on the American continent. Diseased fish exhibits erratic swimming, eye disorders, dark skin pigmentation among other clinical signs. The multilocus sequence typing (MLST) is a well-established methodology for the study of bacterial genetic diversity, which makes it possible to evaluate the ancestry for different isolates. Therefore, this study performed the genetic investigation of *L. petauri* strains isolated from Brazilian native fish species through MLST approach. A total of 24 *L. petauri* strains were used in this study. The isolates were obtained from six species of native fish (*Arapaima gigas*, *Brycon amazonicus*, *Colossoma macropomum*, *Pseudoplatystoma corruscans*, *Pseudoplatystoma fasciatum* and a hybrid of *Pseudoplatystoma*) reared between 2012 and 2023 on commercial farms in four Brazilian states (Amazonas, Bahia, Mato Grosso do Sul and Minas Gerais). These isolates were previously identified at the species level using *gyrB* gene sequencing. MLST was performed by sequencing the internal fragments from seven housekeeping genes (*als*, *atpA*, *tuf*, *gapC*, *gyrB*, *rpoC* and *galP*) according to Ferrario et al. (2013). Sequence types (ST) were defined by analyzing the seven sequences in the *Lactococcus garvieae* MLST database (<https://pubmlst.org/organisms/lactococcus-garvieae>). Phyloviz software was used to identify clonal complexes (CC) among *L. petauri* strains isolated from fish on the American continent and deposited on PubMLST database. The 24 isolates analyzed were grouped in fifteen different STs, three previously reported (ST25, n=1; ST29, n=8; and ST35, n=3) and twelve new STs (nST1, nST2, nST3, nST4, nST5, nST6, nST7, nST8, nST9, nST10, nST11 and nST12). The ST29, ST35, nST2, nST3, nST4, nST5, nST7, nST8 and nST9 were grouped into CC29. nST1 was grouped into CC27 with ST27 and ST61, whereas the nST6 grouped with ST47. The ST25, nST10, nST11 and nST12 were characterized as singletons. Brazilian *L. petauri* isolates obtained from Nile tilapia were previously classified as ST24 (n=29 isolates) and ST47 (n=1), whereas North American strains from rainbow trout were classified as ST14 (n=3) and ST145 (n=11). In conclusion, the Brazilian *L. petauri* strains from native fish species constitute a genetically diverse population, where, the

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majority of the isolates (75%) belong to the same clonal complex. However, the CC29 together with other five STs, except nST6, are unrelated to MLST lineages identified in Nile tilapia and rainbow trout outbreaks, suggesting that piscine American *L. petauri* populations evolved from distinct ancestors. Funding: CAPES, FAPEMIG (APQ-01227-22), FAPEAM

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