IAFP Latino 2024
Simpósio Latinoamericano em Segurança dos Alimentos
Santos - SP - Brasil
11 a 14 Nov, 2024

Multilocus sequence typing of Cronobacter malonaticus, C. dublinensis and C. turincensis isolated from foods in Brazil

Carine da Fonseca Cechin[1], Gabriela Guimarães Carvalho[2], Dirce Yorika Kabuki[1]

[1.] Universidade Estadual de Campinas, Faculdade de Engenharia de Alimentos, Campinas / São Paulo, Brasil
[2.] Universidade Estadual de Campinas, Instituto de Biologia, Campinas / São Paulo, Brasil

Cronobacter spp. are opportunistic pathogens associated with infections in neonates and infants, mostly due to the consumption of contaminated infant formula. Over the years, this pathogen has been isolated from various foods, including those with low water activity. This genus has seven species, of which *C. sakazakii* is the most studied, as it is most associated with cases of infection in infants. However, other species such as *C. malonaticus*, *C. turincensis*, and *C. dublinensis* have been isolated from food and require further study. *C. malonaticus* is also associated with clinical cases in adults. This genus is very diverse and new strains are constantly emerging around the world. Multilocus Sequence Typing (MLST) is widely used for the typing of *Cronobacter* strains. The aim of this study was to characterize *C. malonaticus*, *C. dublinensis,* and *C. turincensis* isolated from low water activity foods (granola, chia, and flaxseed seeds and flours) in Brazil using MLST. Twelve isolates were evaluated, of which six were *C. dublinensis*, three were *C. malonaticus,* and three were *C. turincensis*. Seven housekeeping genes (*atp*D, *fus*A, *gln*S, *glt*B, *gyr*B, *inf*B and *pps*) were sequenced for MLST. PCR for the seven genes was performed according to the protocol available in the PubMLST *Cronobacter* database and sequenced at Life Sciences Core Facility (LaCTAD) of the University of Campinas (UNICAMP), using the primers available in PubMLST. Alternative primers available in the database were also used for PCR and sequencing of some isolates. The data were analyzed using Mega and Bioedit software, and the sequences were consulted in PubMLST *Cronobacter* to assign sequence types (ST) and clonal complexes (CC). Of the 12 isolates, it was possible to sequence all 7 MLST genes in 8 isolates, and 8 different STs were found, including two new ones in *C. turicensis* and *C. dublinensis* (ST1011 and ST1012, respectively) and, in addition, ST350, ST7, ST923, ST258, ST462 and ST251. Among these, ST7 of *C. malonaticus* stands out due to the larger number of clinical isolates compared to the other STs, which have more isolates from food and environmental samples. Clonal complexes have not yet been assigned to most of the STs found, except for ST7, which is already well reported and is assigned to CC7. In 4 *C. dublinensis* isolates, it was not possible to complete the MLST due to difficulties in sequencing the *pps* gene. Other authors have also reported these problems and may be related to the high diversity of this species. In these isolates new alleles were found for the other genes, ranging from 1 to 5 new alleles. Several regulatory agencies around the world have microbiological criteria for the analysis of the *Cronobacter* genus in infant formula, so species other than *C. sakazakii* deserve attention. *Cronobacter* genus is very diverse, and investigation of the molecular characteristics of the strains provides very important information about their evolution. In addition, difficulties in sequencing some genes need to be solved to better characterize the strains, and complete genome sequencing is a tool that can help overcome these challenges.

Agradecimentos: This study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) - finance code 001, and Fundo de Apoio ao Ensino, Pesquisa e Extensão (FAEPEX) from University of Campinas.