

ORT_06 - Misidentification of *Curtobacterium*, *Leifsonia*, *Microbacterium*, *Pseudarthrobacter* and *Paenibacillus* as *Cronobacter* spp. isolated from a pharmaceutical industry

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Introduction: *Cronobacter* spp. are Gram-negative rods belonging to the *Enterobacteriaceae* family and included into the group of thermotolerant or fecal coliforms. This genus has the soil of plants as its primary niche, however, certain species have great pathogenic potential. Certain characteristics of this genus seem to favor the survival of *Cronobacter* spp. in manufacturing environments, which is critical, depending on the production stage that this microorganisms can be isolated.

Objective: The objective of this study was to identify strains isolated from a pharmaceutical industry previously identified as *Cronobacter* spp. by 16S rDNA full gene sequencing.

Methodology: Thirteen strains isolated from 2013 to 2021 were submitted to Gram stain and showed morphological characteristics of Gram-negative rods. Subsequently, they were submitted to Vitek 2[®] and identified as *Cronobacter sakazakii* group. These strains were identified by 16S rDNA gene sequencing using the MicroSEQ[™] Full Gene 16S rDNA kit. Sequences were processed using DNA Star LaserGene SeqMan, and identification results were obtained from <https://www.ezbiocloud.net/>. Only retrieved results with identification $\geq 96\%$ were considered valid and only species whose identification was $\geq 98.7\%$ were considered identified.

Results: From the 13 strains, eight (61.5%) met the criteria for species identification: three (37.5%) were identified as *Leifsonia shinshuensis*, two (25.0%) as *Curtobacterium Oceanosedimentum*, one (12.5 %) as *Microbacterium foliorum*, one (12.5%) as *Paenibacillus cineris*, one (12.5%) as *Paenibacillus lactis*, and one (12.5%) as *Paenibacillus phoenicis*. From the other strains, four (80.0%) were identified as *Paenibacillus* spp. and one (20.0 %) as *Pseudarthrobacter* spp.

Conclusion: In conclusion, Gram staining followed by identification with Vitek 2[®] system resulted in false-positive results for the genus *Cronobacter*. Environmental origin bacteria can be Gram variable due to the stress state, that may be caused by the use of sanitizers in controlled areas of pharmaceutical industries. Thus, other techniques not dependent on Gram stain should be implemented for reliable identification of the genus *Cronobacter*.

Keywords: *Cronobacter* spp.; Microbial identification; Quality control