

**TITLE:** CLONALITY OF *Phytobacter diazotrophicus* ISOLATED FROM BLOOD CULTURES OF PATIENTS FROM A KIDNEY DISEASES CLINIC IN SOUTHERN BRAZIL, USING THE IR BIOTYPER® METHODOLOGY

**AUTHORS:** Geiziane Aparecida Gonçalves<sup>1</sup>; Débora Nicole de Oliveira Kulek<sup>1,2</sup>; Amanda Dal Lin<sup>1,2</sup>; Thais Oliveira Gaio<sup>1</sup>; Gisele Aparecida Bernardi<sup>1</sup>; Lavinia Nery Villa Stangler Arend<sup>1</sup>; Marcelo Pillonetto<sup>1,2</sup>.

**INSTITUTIONS:** <sup>1</sup>LABORATÓRIO CENTRAL DO ESTADO DO PARANÁ (R. Sebastiana Santana Fraga, 1001 – Guatupê, São José dos Pinhais – PR, CEP 83060-500)

<sup>2</sup>PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ (R. Imaculada Conceição 1155 - Prado Velho, Curitiba - PR, 80215-901)

**ABSTRACT:**

*Phytobacter* is an emerging pathogen associated with human infections. It has been responsible for several outbreaks since 1975 although it is misidentified in most episodes. The tracking of outbreaks is of paramount importance. For this, a reliable, fast, and accurate methodology is necessary. The IR Biotyper® analyzes molecular vibrations caused by the absorption of infrared light with a Fourier transform, where bands of carbohydrates, lipids, and proteins offer discriminatory power comparable to molecular genetics methods. In November 2021 we received five supposed *Pantoea* or *Phytobacter* isolates from blood samples from a Kidney Diseases Clinic (KDC) for ID confirmation. These strains were identified by MALDI-TOF, API 20E, *Phytobacter*-specific qPCR, and 16S rRNA sequencing. After identification, a molecular typing of these isolates was performed using the IR Biotyper®, including 11 isolates previously identified (2013-2021) as *P. diazotrophicus* (seven from Curitiba and four from São Paulo). A heavy suspension was prepared in specific microtubes, containing 50µL deionized water and 50µL 70% ethanol. Microtubes were vortexed for 2 min and 15µL of this suspension was placed in triplicate on the IR Biotyper plate, with positive and negative controls. The results showed the presence of four distinct clusters according to temporality. Cluster A contains strains 5020RM, 5110RM, 33098RM, and 37396RM, the first two isolates are part of a 2013 outbreak, associated with Total Parenteral Nutrition (Pillonetto *et al.*, 2018). Cluster B contains strains 32063RM and 32065RM isolated in São Paulo from different hospitals. Cluster C contains the isolate 29310RM and the strains 38397RM and 38453RM, these two are from KDC. Cluster D contains four isolates: strains 38394RM and 38468RM coming from KDC, one isolate from the community (33822RM), and the fourth from another city (32064RM - São Paulo). Among the 16 strains studied, three were singlets. Clusters C and D have at least two strains with strong epidemiological correlation (probable outbreaks). However, some related isolates don't have an epidemiological link, and further investigation is needed. The methodology used proved to be reliable, as cluster A contains two known clonal isolates, confirmed by automated rep-PCR (Diversilab®, BioMerieux) as described previously (2018). The proposed method is fast (four hours) and cheap, as it uses only reagents commonly found in laboratory routine (70% ethanol) for the analysis.

**Keywords:** *Phytobacter diazotrophicus*, outbreaks, infrared analysis, clusters, molecular epidemiology

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