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**DECODING THE ENVIRONMENTAL RESISTOME:  
CHARACTERIZATION OF A CHROMOSOME-ENCODED PER-LIKE  $\beta$ -  
LACTAMASE FROM *Rheinheimera mesophila* IITR-13**

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$\beta$ -Lactamases are the main resistance mechanism to  $\beta$ -lactams in Enterobacterales. The encoding genes may be located on mobile genetic elements. They can also exist as ubiquitous genes in the chromosomes of environmental microorganisms, from where they might be recruited and transferred to pathogens. Class A PER  $\beta$ -lactamases from clinical isolates have distinctive biochemical and structural features compared to other class A enzymes. These include high catalytic efficiency on oxyimino-cephalosporins and an enlarged and inverted omega-loop fold. PER-2 is a plasmid-encoded extended-spectrum  $\beta$ -lactamase (ESBL) present in clinical isolates of Enterobacterales in Argentina. The reservoir and origin of this family of  $\beta$ -lactamases is thought to be the chromosome of environmental species of the recently proposed genus *Pararheinheimera*, which was split from the genus *Rheinheimera*. In this study, we performed the biochemical characterization of the chromosome-encoded PER-like  $\beta$ -lactamase from an environmental *Rheinheimera mesophila* isolate. The chromosome-located blaPER gene from *Rheinheimera mesophila* IITR13 (SAMN10496970) was cloned into a pUC57-kan vector and transformed into *Escherichia coli* Top10. Minimum inhibitory concentrations (MIC) were determined for this clone and *E. coli* expressing PER-2. Then, the blaPER gene was then cloned into a pET28a vector, and the  $\beta$ -lactamase was expressed and purified by affinity chromatography (Ni Sepharose His-Trap). The main steady-state kinetic parameters were determined and compared with PER-2. Additionally, in silico model of the PER-IITR13 variant was obtained. Kinetic data suggest that PER-IITR13 has lower cephalosporinase activity compared to PER-2, especially for ceftazidime. The relative kcat/Km drops to 3% of the PER-2 value (0.02 vs 0.71  $\mu\text{M}^{-1} \text{s}^{-1}$ ). In contrast, for ceftriaxone, the relative kcat/Km of PER-IITR13 is higher compared to PER-2 (5.0 vs 1.8  $\mu\text{M}^{-1} \text{s}^{-1}$ ). A similar behavior is observed for cephalothin (3.9 vs 7.6  $\mu\text{M}^{-1} \text{s}^{-1}$ ). For penicillin G and ampicillin, the kcat/Km values were comparable to those of PER-2. These results are consistent with the MIC values obtained for recombinant isogenic clones expressing each protein in the same vector. The in

silico model reveals that the Thr237Tyr substitution could have a significant impact on the modification of the activity. We previously demonstrated that Thr237 plays an important role as it participates in the hydrogen bond network that stabilizes the active site in PER-2. Under proper expression conditions (e.g., a suitable promoter and/or intensive antibiotic usage), the recruitment of the PER-IIIR13-encoding gene by pathogenic bacteria could result in the dissemination of novel ESBL variants of the PER family.

Palabras clave: Resistome - bacterial resistance - antibiotics -  $\beta$ -lactamases