



# The Open Microbiology Journal Supplementary Material

Content list available at: <https://openmicrobiologyjournal.com>



## Identification of *Klebsiella Variicola* T29A Genes Involved In Tolerance To Desiccation

Osvaldo Rodríguez-Andrade<sup>1</sup>, Andrés Corral-Lugo<sup>2</sup>, Yolanda Elizabeth Morales-García<sup>1,3,4</sup>, Verónica Quintero-Hernández<sup>5</sup>, América Paulina Rivera-Urbalejo<sup>1,4</sup>, Dalia Molina-Romero<sup>1,3</sup>, Rebeca Débora Martínez-Contreras<sup>6</sup>, Patricia Bernal<sup>7</sup> and Jesús Muñoz-Rojas<sup>1,\*</sup>

<sup>1</sup>Ecology and Survival of Microorganisms Research Group (ESMRG), Laboratorio de Ecología Molecular Microbiana (LEMM), Centro de Investigaciones en Ciencias Microbiológicas (CICM), Instituto de Ciencias (IC), Benemérita Universidad Autónoma de Puebla (BUAP), Puebla, Mexico

<sup>2</sup>Department of Virology, Institut de Biologie Intégrative de la Cellule (I2BC), CNRS, Gif-Sur-Yvette, France

<sup>3</sup>Licenciatura en Biotecnología, Facultad de Ciencias Biológicas, BUAP, Puebla, Mexico.

<sup>4</sup>Facultad de Estomatología, BUAP

<sup>5</sup>CONACYT, EMSRG, LEMM, CICM, IC, BUAP, Puebla, México

<sup>6</sup>LEMM, CICM, IC, BUAP

<sup>7</sup>Imperial College London, MRC Centre for Molecular Bacteriology and Infection, Department of Life Sciences, South Kensington Campus, London, United Kingdom

### Article History

Received: May 22, 2019

Revised: September 07, 2019

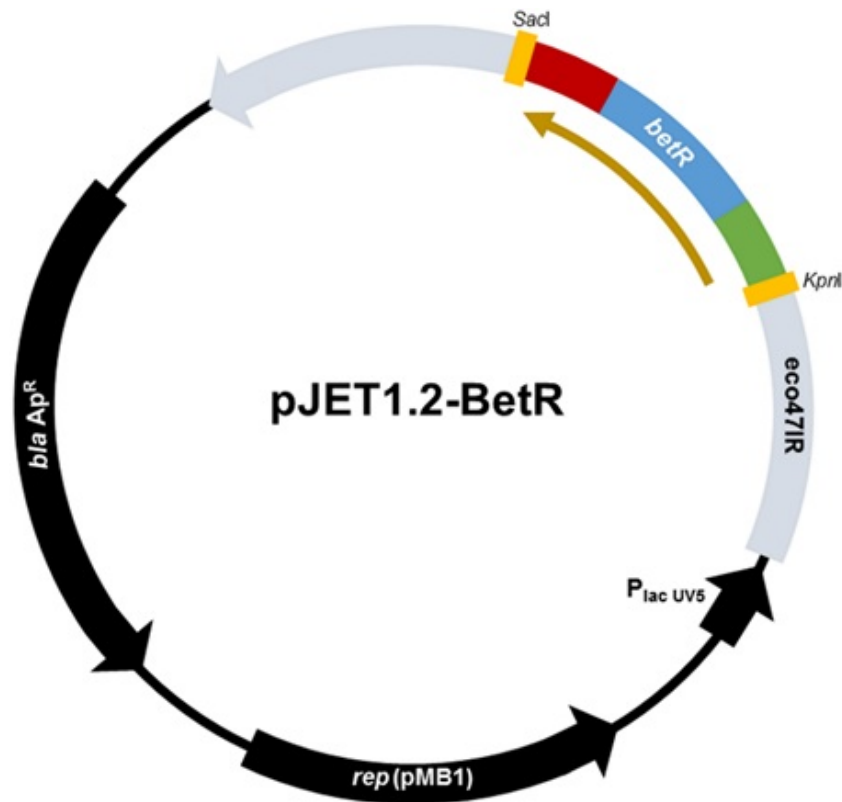
Accepted: September 09, 2019

### SUPPLEMENTARY TABLES AND FIGURES

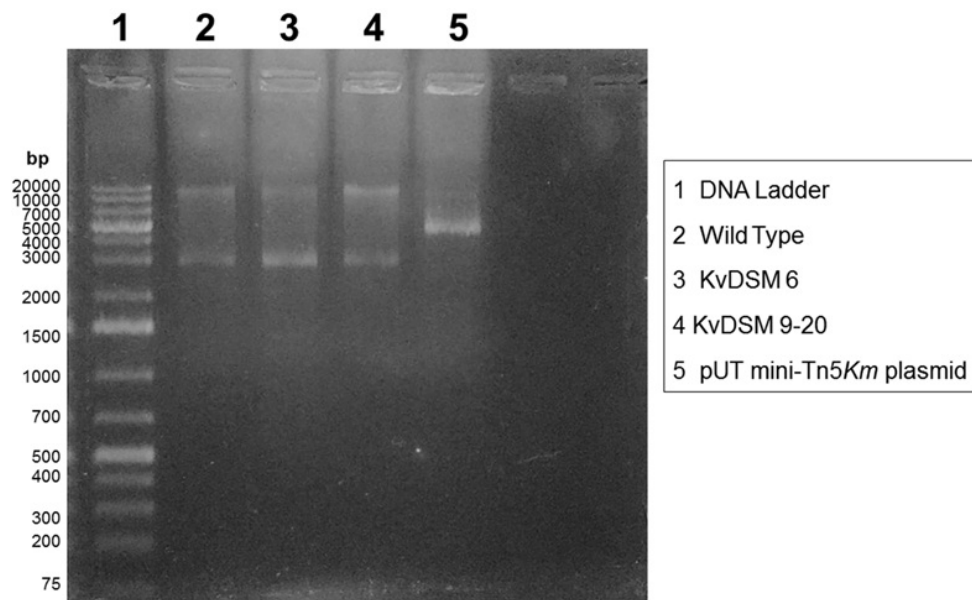
**Supplementary Table 1. Nucleotidic sequences of the *BetR* gene fragments interrupted by the mini-Tn5Km transposon in the KvDSM 6 mutant. The pair of oligonucleotides: Mut 6 start-Rv/Tn5-Fw, allowed to amplify a region of 490 base pairs between the transposon and a region of 223 base pairs upstream of the start of the *BetR* gene, while the pair: Mut 6 stop-Fw/Tn5-Rv, allowed to amplify a region of 695 base pairs between the transposon and the end of the *BetR* gene.**

PCR Product	Sequence
DNA fragment amplified with the oligonucleotides: Mut 6 start-Rv/Tn5-Fw	<p>CTTCAGGAACATTTTATACACATCTACAACAACCTTACAAGACGCCGCAACATAAAAAACGATTTTTATGTTGTTCAAGCAA  TGGAGGAGAACAAAAAGGACGCCAAAAGAACCAGCGCAACATTACATATCGCTTTGTTTTAATTGAAATTAATCTCAGGATACCTACAAT  AACAAACCTAAAATGTCATTTTACCCACCCTACAGAAGGCCGATATGATACATCTAAATCGACACCAATTGAACCTAAGATTAGTGAGAT  GTTAAGCAACATCATGAATGAAAGGGGTATTTCAAGAACAACATACTACTCATATAGCTAATGTACTCGGTATAACCATACCCACGCAAAACCG  TAAAATGAAAGGTTTTGCGTCATGGGAAAATCAGCTGAAAAAGTCGCTAAGTCTTTAGGCTGACTCTTATACACAAGTGCG  GCCGCGGCTAGGCGGCCGAAAGCTTGCAAT</p>
DNA fragment amplified with the oligonucleotides: Mut 6 stop-Fw/Tn5-Rv	<p>GAATTCGGCCTAGGCGGCCAGATCTGATCAAGAGACAGGCTTTAGGTGTAAGCCTCAGTGATTATTTAAAAT  GGTTGAGGACAGCTATGAGGAGAAACACGCCGAAAAATATATTTTCAACAAGGAAGAAATGGAGGGAGAGATAACCTTTTCCCTG  ATACAGAAAGAATATTCAGCTGTTAAAATTAACGATCAATGGCATATTTTAAAACAGATGATATTGAAGGCAATGACATTATGA  AGAATCGCGCAAGGTGTTTTAAGATTTCCGTTGAAAGTTCAGCAAGAGACAAGTCTAAGCCACGTATCGCTCTCTGGATGACGATA  AGGAGATTCTGACAACAACAGCTGAACCTCTGAAGAAAGGCCATATAAAAATGACACCTTCACCAGTTGTAAGATCTGATAGCAAG  AATTCAAATCAAGCCATACGATGGATATATACTGGACTGGGTGGTGAATGATAAATCGCATATGATGTTTGAAGAAAATCCGCGA  ATCAAAAAACCATGCAATGATTATCATCTTAACAGGACAAACAGGAGATTTATAGATCAGGAAAATGCAGAAGCGTTTAA  TGACTTCGACATCTAGGC</p>

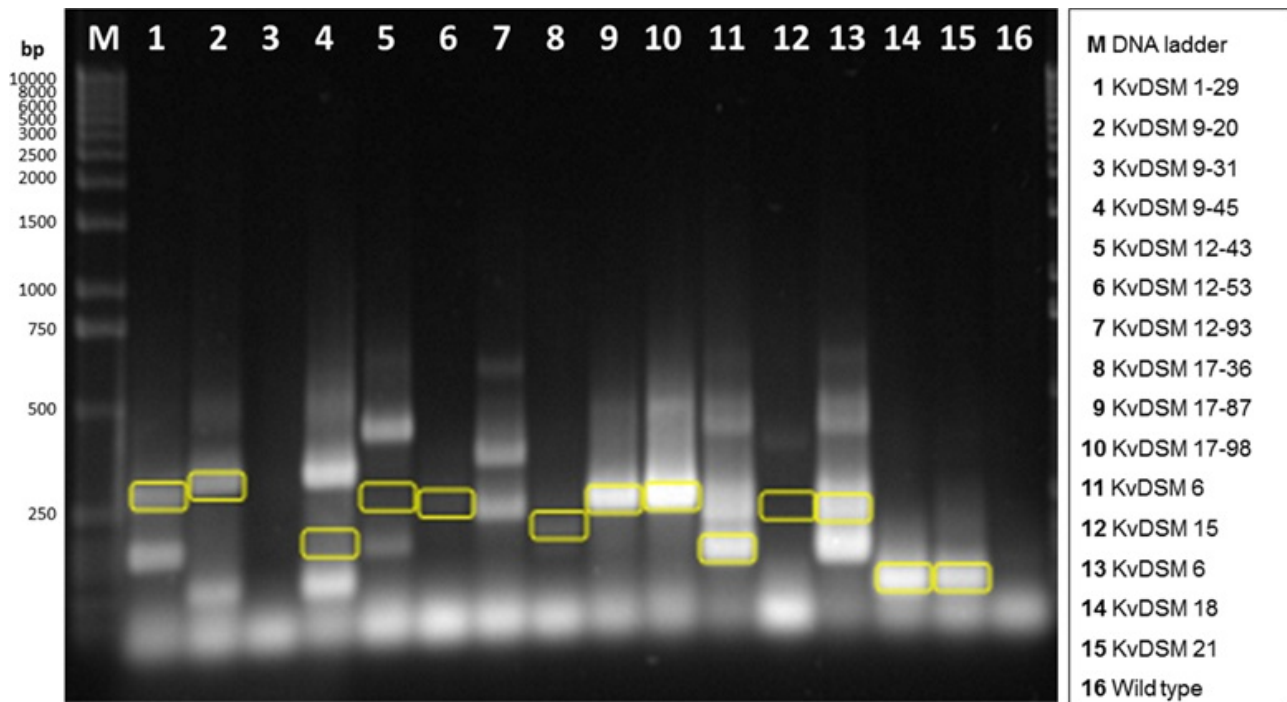
The blue letters correspond to the sequence of the *BetR* gene, the red ones to the mini-Tn5Km transposon and the green ones to a sequence upstream of the start of the gene. The underlined letters correspond to the repeated sequences given by the insertion of the transposon mini-Tn5Km.



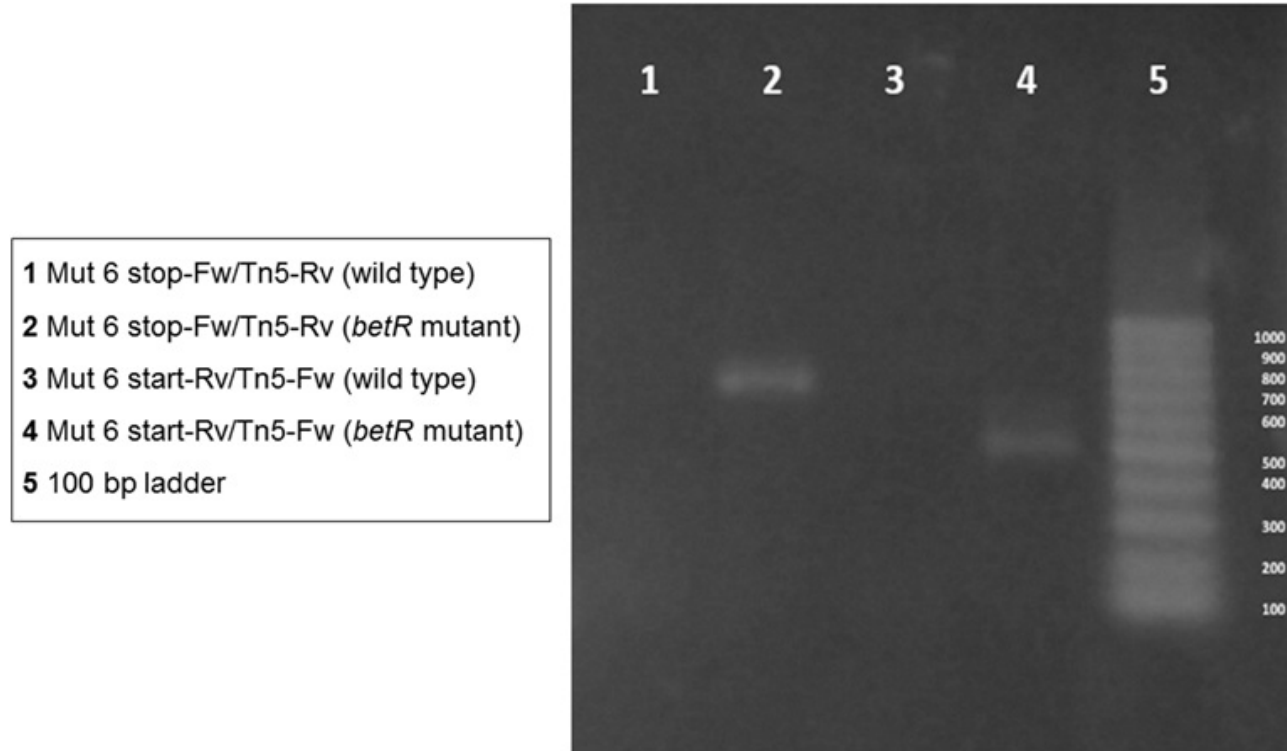
**Supplementary Fig. (1).** Construction of a plasmid vector for *trans* complementation of the *BetR* gene in *K. variicola* T29A::mini-Tn5Km. The *BetR* gene (blue) as well as a 223 bp sequence upstream of the gene start (green) and a 228 bp sequence downstream of the gene end (red) were amplified by PCR using primers with restriction sites *KpnI* and *SacI*. The amplification product was cloned into the vector pJET1.2/blunt. The brown arrow indicates the direction in which the insert was cloned.



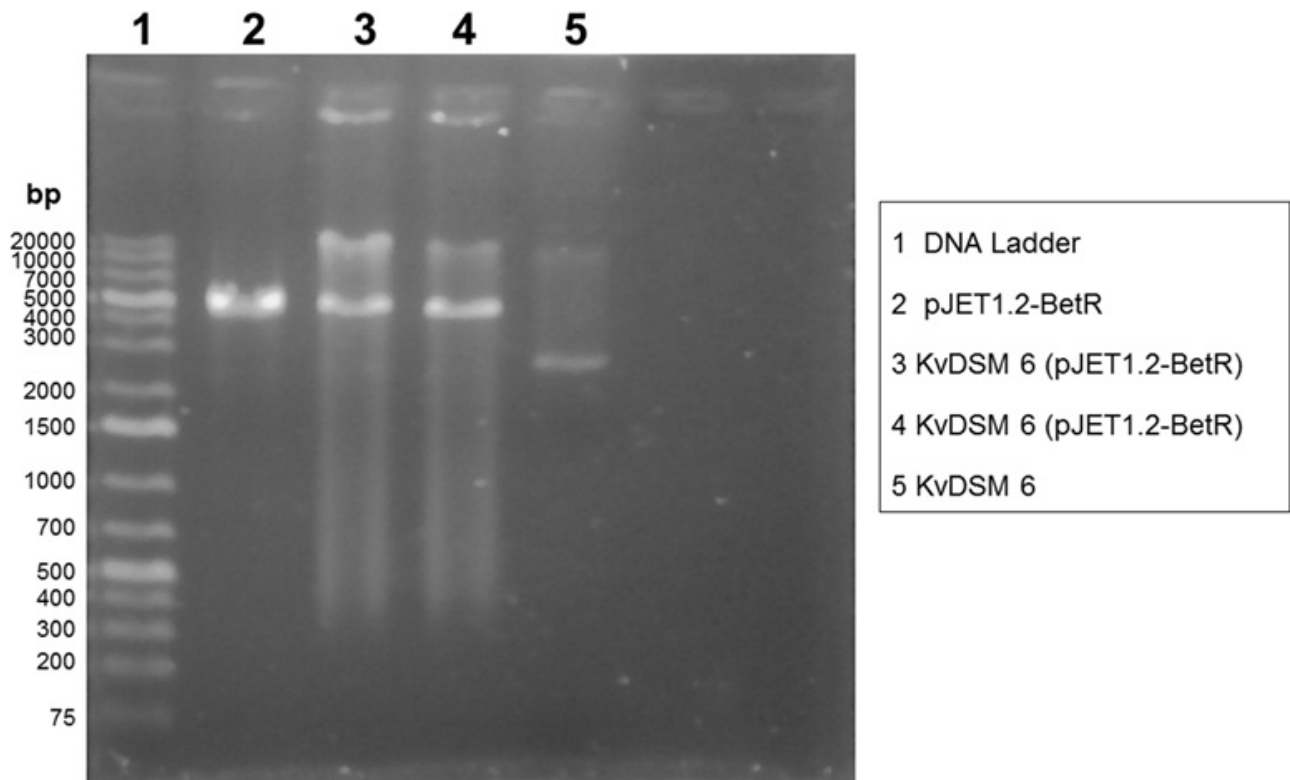
**Supplementary Fig. (2).** Plasmidic profile of some mutants of *K. variicola* T29A library (*K. variicola* T29A::mini-Tn5Km). The natural plasmid of this bacterium is observed, but the pUT mini-Tn5Km plasmid (7.05 Kb) is absent, which suggests that the mini-Tn5 transposon was integrated into the chromosome of the bacterium.



**Supplementary Fig. (3).** Band profile of the 14 mutants sensitive to desiccation-rehydration in a 2% agarose gel after amplification by an arbitrary PCR. For the wild-type strain, there was no amplification. The DNA purified from the selected bands (denoted with a rectangle) was sequenced and compared with the databases to determine the insertion site of the mini-Tn5Km transposon.



**Supplementary Fig. (4).** The electrophoretic shift of the amplification products using oligonucleotides at the start and end of the *BetR* gene as well as oligonucleotides specific to the mini-Tn5 transposon. Mut 6 stop-Fw/Tn5-Rv and Mut 6 start-Rv/Tn5-Fw oligonucleotide pairs were used for the wild-type strain and the *BetR* mutant of *K. variicola* T29A, where the amplification products of 695 and 490 base pairs were observed, respectively.



**Supplementary Fig. (5).** Plasmid profile of the mutant interrupted in the *BetR* gene, with respect to some transformants of the mutant complemented *in trans* with the construction of pJET1.2-*BetR*.

© 2019 Rodríguez-Andrade *et al.*

This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International Public License (CC-BY 4.0), a copy of which is available at: (<https://creativecommons.org/licenses/by/4.0/legalcode>). This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.