EFFECT OF AN ALKALINE-MIMICKING MUTATION IN *Acremonium chrysogenum pacC* GENE ON β-LACTAM ANTIBIOTICS PRODUCTION

Alberto Cristian López, Francisco Fierro, Octavio Loera, Francisco José Fernández; Universidad Autónoma Metropolitana-Iztapalapa, Depto. de Biotecnología, México, D.F., C.P. 09340;
e-mail: fierrof@xanum.uam.mx

Key words: β-Lactam biosynthesis, genetic improvement, Acremonium chrysogenum

Introduction. β-Lactam antibiotics are the most prescribed antimicrobials and the most important drugs (from an economical viewpoint) (1). Among these compounds, cephalosporin C (CPC) is the core of all semisynthetic cephalosporins for clinical use. CPC is exclusively produced by *Acremonium chrysogenum*, and its biosynthesic pathway is regulated by pH signaling through transcription factor PacC (2). When the environmental pH is acidic to neutral, PacC is inactive; when the pH becomes alkaline, a signal transduction pathway starts leading to PacC activation by two successive proteolytic steps (3). Activated PacC promotes transcription of most CPC biosynthetic genes (1)

In this work, we removed 366 amino acid residues from the C-terminal end of *A. chrysogenum* PacC to attain the active form of the protein, aiming at increasing the beta-lactam titers, including CPC, regardless of the pH values in the fermentation broth.

Methods. PCR reactions were performed with two sets of primers to obtain two products: 1) a fragment covering the sequence from the ATG codon up to the 971 base pair of the *pacC* gene; and 2) a fragment containing the *pacC* promoter and the *pacC* gene up to position 971. Product 1 was cloned into plasmid pAN52.1, then a fragment containing the *gpdA* promoter attached to the truncated *pacC* gene was amplified by PCR and subcloned into plasmid pLXTtrpC. Product 2 was directly cloned into pLXTtrpC. Resultant plasmids and pLXTtrpC (as control) were introduced in *A. chrysogenum* by polyethylene glycol-assisted protoplast transformation (4). Transformants were tested first on a defined fermentation medium and later on a complex medium.

Results. Most transformants with the *pacC* promoter (P) were significantly (α=0.01) better producers than those with the *gpdA* promoter (S). Both P and S were better producers than the control (X) and WT strain. P17 showed up to 10-fold higher production than the parental and control strains.

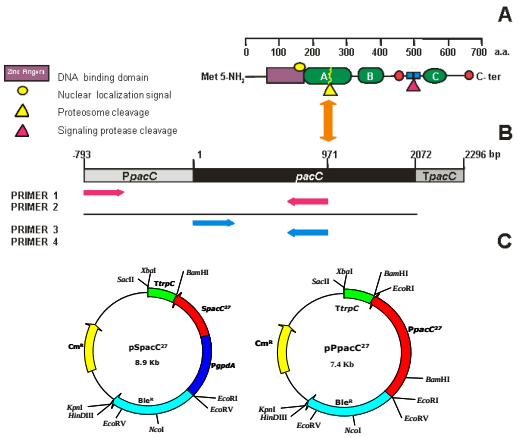

Fig.1 Expression plasmids obtained. A) PacC protein, B) Primer design over *pacC* gene sequence, C) plasmids.

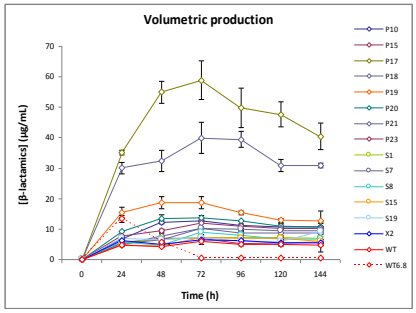

Fig.2 Volumetric production of β-Lactam antibiotics by transformants in complex media fermentation.

Conclusions. The alkaline-mimicking mutation in the *A. chrysogenum pacC* gene led to increased β-lactams production up to 10-fold that of the parental strain. Expression of the truncated *pacC* was better when the own *pacC* gene promoter was used.

Acknowledgments. We acknowledge the financial support from CONACYT (Projects 36801-B, 54662, and 82647)

References

1. Brakhage A. (1998).*Microbiol. Mol. Biol. Rev*. 62: 547-585.
2. Schmitt EK, Kempken R, Kück U. (2001). *Mol. Genet. Genom.* 265: 508-518.
3. Peñalva MA, Tilburn J, Bignell E, Arst HN Jr. (2008). *Trends Microbiol.* 16(6): 291-300.
4. Queener SW, Ingolia TD, Skatrud PL, Chapman JL, KasterKR. (1985). A system for genetic transformation of *Cephalosporium acremonium*. In: *Microbiology*. Leive L. U.S.A. 468-472.