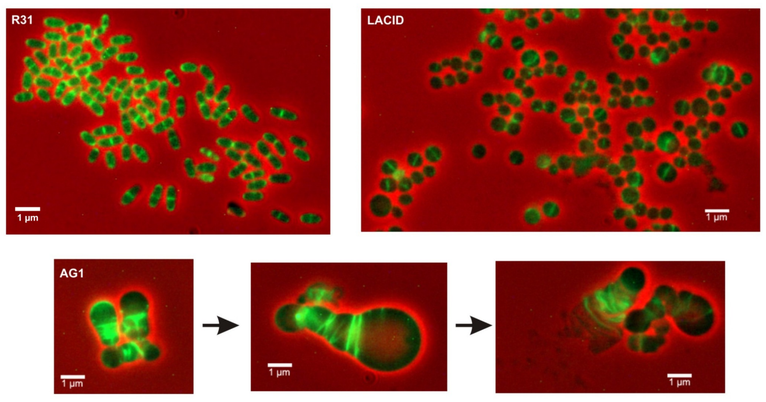
Corynebacterium glutamicum cell growth and cell division

Michal Letek, Efrén Ordóñez, María Fiuza, Luis Mariano Mateos and José A. Gil

Área de Microbiología, Departamento de Biología Molecular, Universidad de León.
24071, León Spain. Email: mletp@unileon.es

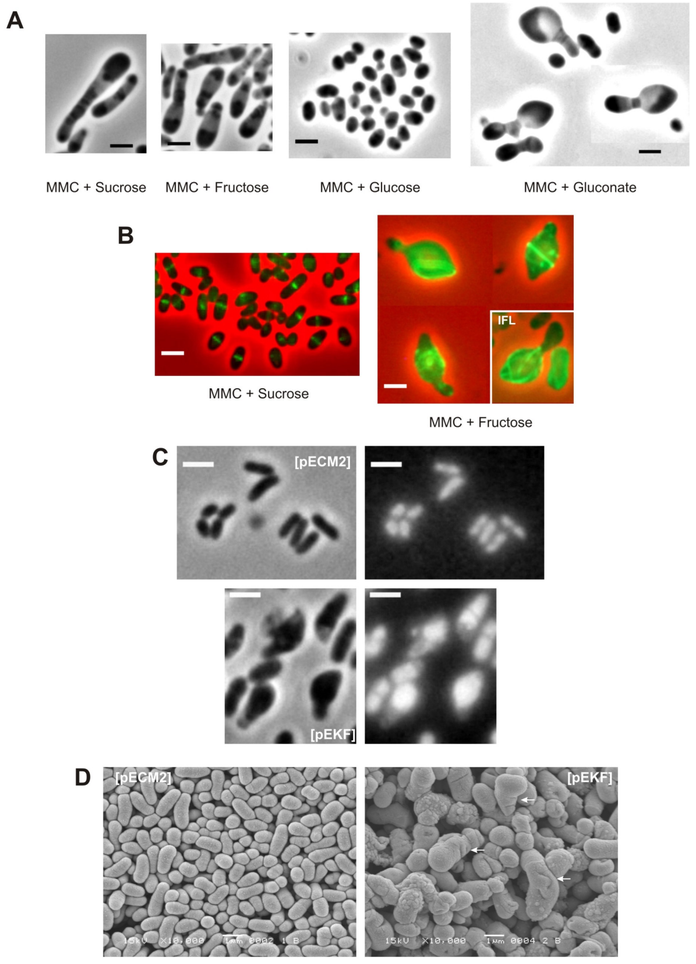
Introduction

In *Escherichia coli* and *Bacillus subtilis* elongation occurs by the insertion of new cell wall material in the cylindrical portion of the cell supported by an actin-like cytoskeleton (MreB/Mbl), while the tubulin-like cytoskeleton (FtsZ) directs cell wall synthesis at the division apparatus (Daniel and Errington, 2003). However, no homologues of the bacterial actin-like proteins MreB/Mbl have been identified in corynebacteria (Ramos *et al.*, 2005), suggesting that cell wall synthesis is organized differently. It is also evident that there are no apparent homologues for positive or negative regulators of cell division, e.g., *ftsA*, *zipA*, *ezrA, noc*, or the *min* system (Ramos *et al.*, 2005). Interestingly, *divIVA*, a component of the *min* system in *B. subtilis*, is also present in corynebacteria, although the DivIVA protein seems to be involved in the regulation of polar growth (Ramos *et al.*, 2003), as is the case in *Streptomyces coelicolor* (Flardh, 2003).



In *Corynebacterium glutamicum* DivIVA is essential for polar cell wall synthesis

Phase contrast and fluorescent vancomycin staining overlay of exponentially growing cells. The typical rod morphology of *C. glutamicum* R31 is converted to a coccoid morphology (no polar growth) in strain LACID which is depleted for DivIVA, or to "ice-cream cone" morphology (strong apical growth) in strain AG1 containing extra copies of *divIVA* by presence of plasmid pEAG1. *C. glutamicum* LACID shows no polar cell wall synthesis, whereas strain AG1 grows apically and forms many apparently incomplete septa.



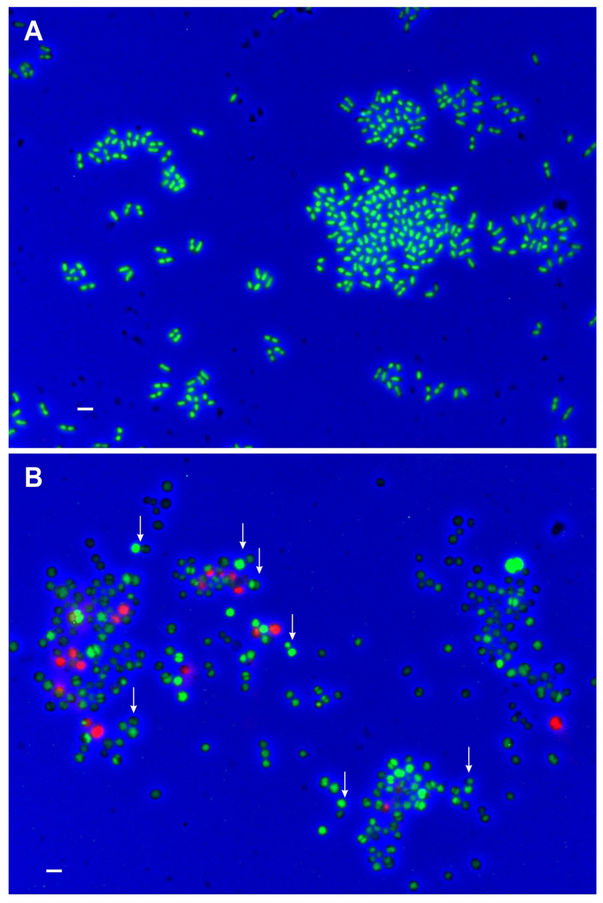
Controlled expression of *ftsZ* in *C. glutamicum* carrying a functional copy of P*gntK ftsZ* in the chromosome (strain KF) or in a high-copy-number plasmid (strains [pEKF] or [pEKFG]).

(A) Phase-contrast microscopy of *C. glutamicum* KF grown in MMC in the presence of different carbon sources. Bars represent 1 µm. Note that under the most repressive conditions (MMC + Sucrose) the cells become filamentous, whereas under conditions yielding the highest expression (MMC + Gluconate) the diameters of the cells are increased.

(B) Expression of *ftsZ* under the control of P*gntK*. Overlays combining phase-contrast and fluorescence microscopy of *C. glutamicum* [pEKFG] (P*gntK-ftsZ-egfp2*) grown in MMC + Sucrose and Fructose. Bars represent 1 µm. Note that under lower repression conditions (MMC + Fructose), the $FtsZ_{CG}$-EGFP2 fused protein detected in *C. glutamicum* [pEKFG] formed spirals or tangles throughout the cells. Identical results were obtained with *C. glutamicum* [pEKF] (P*gntK-ftsZ*) when the native $FtsZ_{CG}$ was detected by immunofluorescence (IFL). In contrast, under high repression conditions (MMC + Sucrose) $FtsZ_{CG}$-EGFP2 localized only at mid-cell.

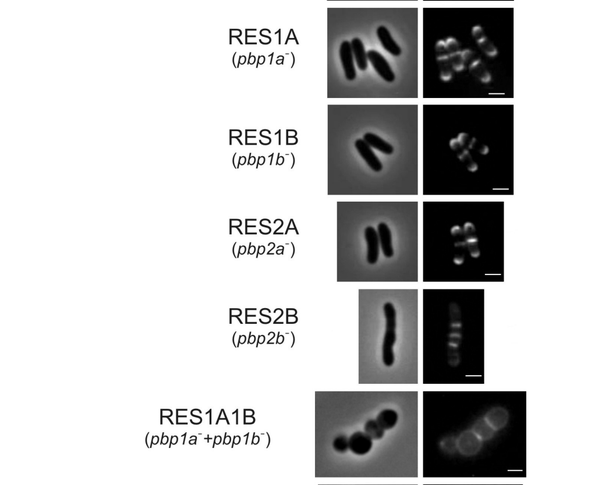
(C) DNA distribution was visualized by phase-contrast (left panels) and DAPI staining (right panels) in *C. glutamicum* [pECM2] and *C. glutamicum* [pEKF]. Both strains were grown in MMC + Fructose. Bars represent 1 µm. Note that nucleoid distribution is altered when FtsZ is overexpressed (pEKF).

(D) SEM of *C. glutamicum* [pECM2] (left) and *C. glutamicum* [pEKF] (right) grown in both cases in MMC + Fructose. Note that *C. glutamicum* [pEKF] presents an irregular cell surface, including small invaginations



DivIVA could be also involved in chromosome segregation

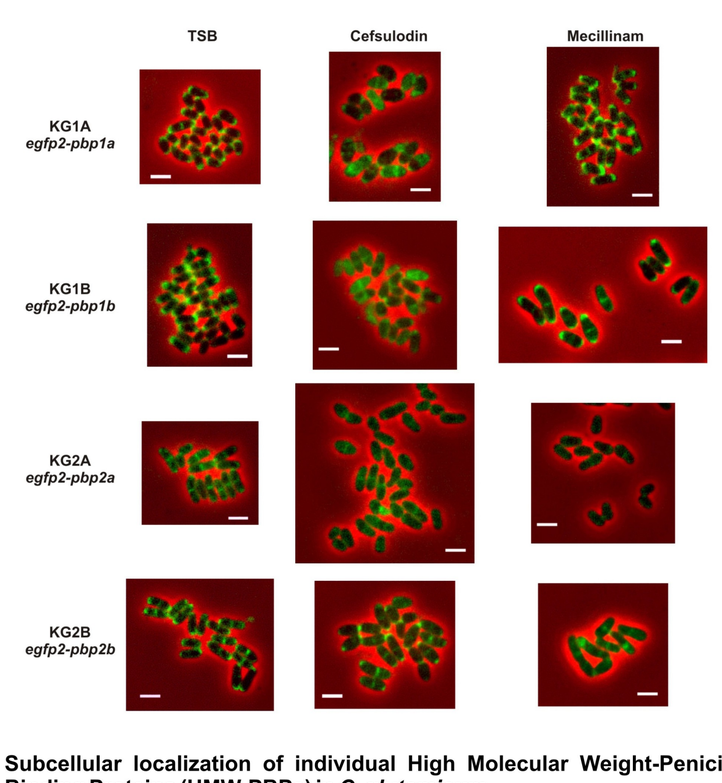
DNA staining with LIVE/DEAD Bac-Light Bacterial Viability Kit of the wild type *C. glutamicum* R31 (A) strain and the DivIVA depleted *C. glutamicum* LACID strain (B). Dead cells stain with propidioum iodide (red) and the DNA content of live cells is visualised using SYTO9 staining (green). Vertical arrows indicated just divided cells with abnormal chromosome segregation. Size bar, 1 µm.



DivIVA localizes at the cell poles, but also at the midcell in *C. glutamicum* after nucleoid segregation and septum cell wall synthesis.

A. Fluorescence microscopy and Van-650/665 staining of *C. glutamicum* R33, carrying an extra chromosomal copy of $divIVA_{CG}$-*egfp2* under the control of P*div* (P*div-*$divIVA_{CG}$*-egfp2*). Arrows indicate different cells in which biosynthesis of new PG (visualized by Van-650/660 staining) is ahead of the septal localization of DivIVA-EGFP2 (EGFP2 fluorescence) at septum once nucleoids are segregatedEGFP2 (EGFP2 fluorescence).

B. Immunofluorescence microscopy using anti-DivIVA antiserum (IFL) and DAPI staining of nucleoids in *C. glutamicum*. Arrows shows that DivIVA localized at septum once nucleoids are segregated.



Subcellular localization of individual High Molecular Weight-Penicillin Binding Proteins (HMW-PBPs) in *C. glutamicum*

Apart from *ftsI* there are four HMW-PBPs in *C. glutamicum*: PBP1a, PBP1b, PBP2a and PBP2b. These proteins are involved in the last step of cell wall synthesis. We show fluorescent images of cells taken from exponentially growing cultures of strains carrying the corresponding gene fusion (*egfp2-pbp1a, 1b, 2a* and *2b*), under the control of P*kan*, integrated in the chromosome. The cells were grown in TSB medium (TSB column), TSB with 1 µg cefsulodin/ml, or TSB with 2 µg mecillinam/ml. Bars represent 1 µm. Note that PBP1a/1b was delocalized from cell poles and septum by cefsulodin treatment, whereas mecillinam affected PBP2a/2b localization, suggesting that susbstrate recognition plays an essential role in their cellular localization.

Microscopy of single and double HMW-PBP mutants of *C. glutamicum*

Phase-contrast images (**A**) of exponentially growing cultures of *C. glutamicum* RES167 (control) and single and double HMW-PBP mutants are paired with the corresponding images of Van-FL-stained cells (**B**). Bars represent 1 µm. Note that in the absence of PBP2b the cells became larger, with multiple septa, suggesting its implication in cell division. In the *pbp1a* + *pbp1b* null mutant, the cells lacked polar growth and thus acquired a coccoid morphology, whereas in the absence of PBP2a and any of the class A HMW-PBPs the cells were apparently smaller.

Protein-protein interactions

Interactions measured by two-hybrid system experiments between HMW-PBPs, RodA, DivIVA, FtsZ, and FtsW from *C. glutamicum*. The results are expressed as fold above background. The cut-off between strong and weak interactions was established at 40 (horizontal line). All combinations between bait (abscises) and prey (legend) are shown. Values are the means of four independent experiments; standard deviations are indicated at the tops of the bars. The results suggest the existence of a cell division "core" composed of FtsZ, FtsW, FtsI and the remaining class B HMW-PBPs (2a and 2b). In contrast, during cell elongation, the main protagonists would be class A HMW-PBPs (required for polar growth) together with DivIVA and RodA.

References

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