Deciphering the function of DivIVA in *Corynebacterium glutamicum*

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Introduction

The amino acid producer *Corynebacterium glutamicum* was used as a model for the identification of new targets for antimicrobial agents against pathogenic corynebacteria. We are studying the *C. glutamicum* essential *divIVA* gene, firstly described in *Mycobacterium tuberculosis* as *wag31* and coding for the highly immunogenic Antigen 84. In *Bacillus subtillis* DivIVA is involved in the correct localization of Z-ring (the scaffold of the division septum), and also in chromosome segregation.

Results

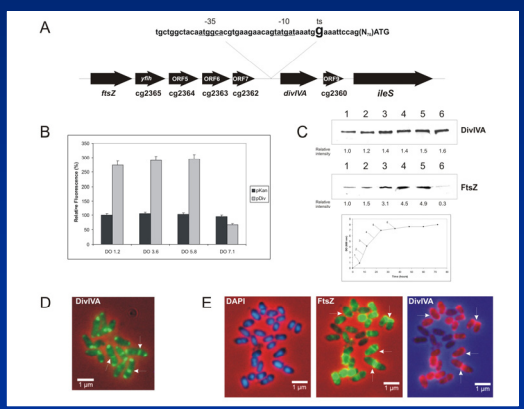

Fig. 1. CHARACTERIZATION OF DivIVA IN *Corynebacterium glutamicum* ATCC 13869.
A. Schematic representation of the chromosomal DNA around *divIVA* and Identification of its transcription start point by RACE.
B. Analysis of the P*div* expression using a promoter probe vector with *gfp* as reporter gen.
C. Quantification of DivIVA and FtsZ during the growth curve of *C. glutamicum* wt.
D. Localization of DivIVA (green) by immunofluorescence (IFL).
E. Nucleoid staining by DAPI, and localization of FtsZ (green) and DivIVA (red) by IFL.

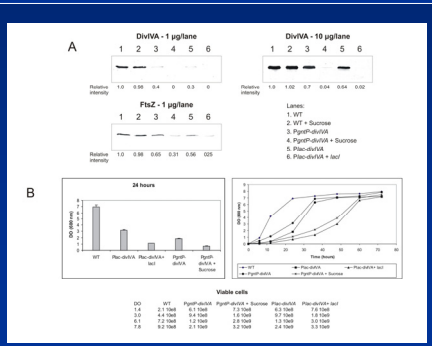

Fig. 2. LOWERING THE LEVEL OF THE ESSENTIAL PROTEIN DivIVA in *C. glutamicum* ATCC 13899 USING REGULATED PROMOTERS.
A. Quantification of DivIVA and FtsZ using anti-DivIVA and anti-FtsZ antibodies.
B. Growth curves and viable cells of different partially DivIVA depleted *C. glutamicum* strains.

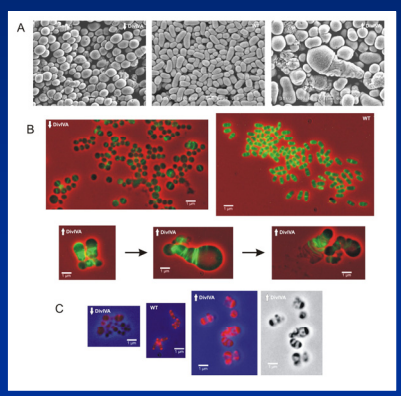

Fig. 3. DIFFERENT MICROSCOPY TECHNIQUES USED TO ANALYZE A PARTIALLY DEPLETED DivIVA MUTANT (↓DivIVA), THE WILD TYPE STRAIN (WT) AND AN OVEREXPRESSED DivIVA STRAIN (↑ DivIVA).
A. The typical morphology of *C. glutamicum* (wt) by Scanning Electron Microscopy (SEM) is converted to a coccoid morphology (no polar growth) in strain ↓DivIVA, or to an "ice-cream cone" morphology (high apical growth) in strain ↑ DivIVA
B. Vancomycin-FL staining, ↓DivIVA grows as the *Streptococcus* model, whereas ↑ DivIVA grows apically and forming many septa (inhibition of cell division).
C. Immunofluorescence of DivIVA.

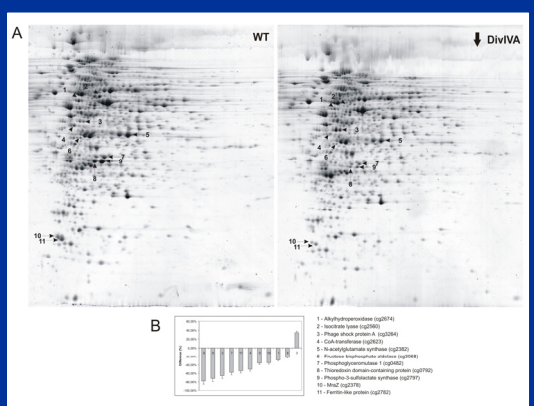

Fig. 4. 2-D GEL ELECTROPHORESIS (IEF-SDS-PAGE) OF TOTAL CYTOPLASMIC PROTEINS OF *C. glutamicum* (WT) and THE PARTIALLY DEPLETED STRAIN (↓DivIVA).
Arrows indicated 11 identified proteins that showed an altered level in the strain ↓DivIVA compared to the normal protein expression profile of the wild type (B).

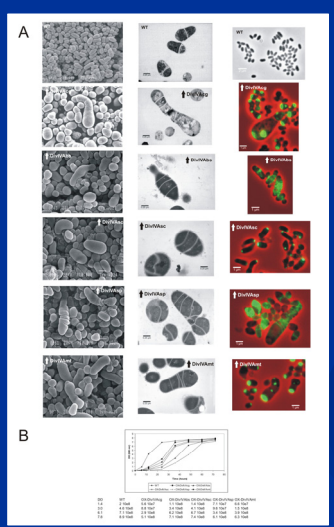

Fig. 5. OVEREXPRESSION OF DivIVA PROTEINS FROM DIFFERENT GRAM POSITIVE BACTERIA IN *C. glutamicum*
A. SEM, TEM and fluorescence microscopy of DivIVA-GFP from *C. glutamicum*, *B. subtilis*, *S. coelicolor*, *S. pneumoniae* or *M. tuberculosis*.
B. Growth curves *C. glutamicum* overexpressing *divIVA* from different microorganisms.

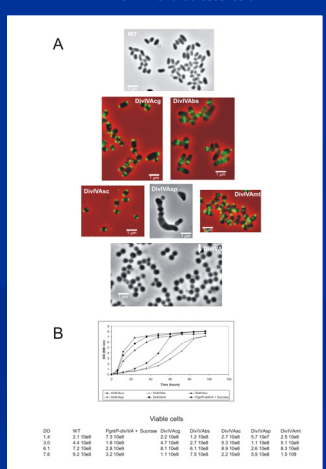

Fig. 6. HOMOLOGOUS AND HETEROLOGOUS COMPLEMENTATION OF A PARTIALLY DEPLETED DivIVA *C. glutamicum* STRAIN.
A. Fluorescence microscopy of the different DivIVA-GFPs in *C. glutamicum*.
B. Growth curves of the partially depleted DivIVA strain complemented with the different DivIVA-GFPs.

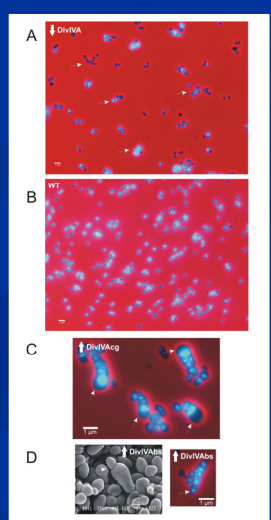

Fig. 7. CHROMOSOME SEGREGATION IN *C. glutamicum* IS DEPENDENT OF DivIVA.
DAPI staining of the partially DivIVA depleted strain (A), the wild type *C. glutamicum* strain (B), *C. glutamicum* strains overexpressing DivIVA from *C. glutamicum* (C) or from *B. subtilis* (D)

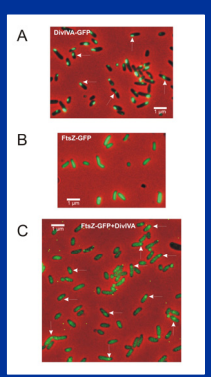

Fig. 8. VISUAL TWO HYBRID SYSTEM TO STUDY PROTEINS WHICH INTERACT WITH DivIVA.
A. DivIVAcg-GFP localizes in *E. coli* at the midcell and after the cell division it remains near the pole.
B. FtsZcg-GFP does not localizes properly in *E. coli*.
C. FtsZ-GFP colocalizes with DivIVA when both are expressed at the same time in *E. coli*.

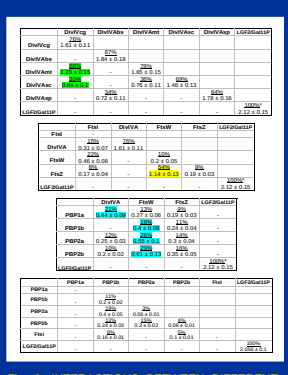

Fig. 9. INTERACTIONS BETWEEN DIFFERENT DivIVA PROTEINS, CELL DIVISION PROTEINS AND PBPs DETECTED BY TWO-HYBRID SYSTEM.

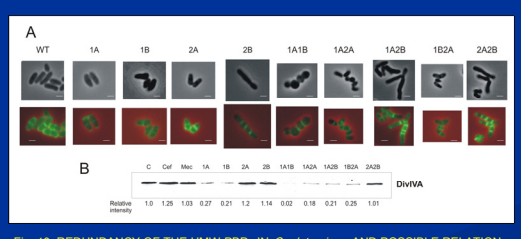

Fig. 10. REDUNDANCY OF THE HMW-PBPs IN *C. glutamicum* AND POSSIBLE RELATION BETWEEN DivIVA AND PBPs.
A. Morphology and Vancomycin-FL staining of different single and double PBP mutants (mutant 1B2B was impossible to obtain).
B. Quantification of DivIVA in the PBP mutants.

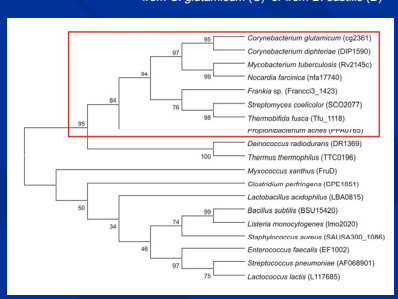

Fig. 11. PHYLOGENETIC ANALYSIS OF DivIVAs FROM DIFFERENT MICROORGANISMS. The DivIVA from *C. glutamicum* is clustered together with Actinobacteria and *Thermus/Deinococcus*, they seem to be different of DivIVAs proteins from Gram positive and the Gram negative *Myxococcus xanthus*.

Conclusions

1. The *divIVA* gene from *C. glutamicum* seems to be expressed from a strong P*div* promoter, which turns off during stationary phase but the cellular concentration of DivIVA is maintained (Fig. 1).
2. DivIVA localizes at the septum once FtsZ is localized and remains there until daughter cells are completely formed (Fig. 1).
3. DivIVA is an essential protein in *C. glutamicum*, no null mutants can be obtained, but it is possible to reduce strongly the level of DivIVA in the cell (Fig. 2).
4. DivIVA is a protein involved in the polar growth of *C. glutamicum* as its partial depletion leads to coccoid morphology; in these coccoid cells growth takes place from the division septa as in *Streptococcus* (Fig. 3B).
5. Immunofluorescence microscopy of DivIVA in strains overexpressing homologous DivIVA suggests that this protein seems to retain a non-DNA compound present at the pole of the cell which is visible by contrast microscopy (Fig. 3C and Fig. 7C).
6. Cell division and central metabolism are lowered in the partially depleted DivIVA mutants (Fig. 2A and Fig. 4).
7. Overexpression of DivIVA from different Gram positive bacteria produces aberrant elongated cells with multiple septa, indicative of an alteration in cell division (Fig. 5).
8. Overexpressed DivIVA-GFP from actinomycetes induces apical growth in *C. glutamicum*, but as single copy in the *C. glutamicum* chromosome reverts the coccoid phenotype of a partially depleted DivIVA strain. On the other hand, overexpressed DivIVA from *B. subtilis* or *S. pneumoniae* localizes at the septum and do not revert the coccoid phenotype when present in the chromosome and they are even toxic (Fig. 5 and Fig. 6).
9. Nucleoids in *C. glutamicum* cells are altered by low or high expression of DivIVA, suggesting a role of DivIVA in chromosome segregation (Fig. 7).
10. DivIVAcg interacts with itself (oligomerizes) and with DivIVA from other actinomycetes. FtsZ interacts with FtsW in *C. glutamicum*, as previously described in *M. tuberculosis*, but also with DivIVA. PBP1a interacts more clearly with DivIVA, and the rest of HMW-PBPs seems to interact more directly with FtsW (Fig. 8 and Fig. 9).
11. Mutant PBP1a1b exhibits coccoid morphology, no polar growth and its level of DivIVA is low (Fig. 10).
12. DivIVAs from actinomycetes are different from other Gram positive and Gram negative DivIVA proteins (Fig. 11).

Acknowledgments

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