ANALYSIS OF *Corynebacterium glutamicum* GLUCONATE GENES AND EFFECTS OF ITS GENE DISRUPTION

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Introduction

Corynebacterium glutamicum, a Gram-positive microorganism traditionally used for amino acids and nucleotide production, is able to metabolize gluconic acid as unique energy and carbon source. Genes *gntK* and *gntP* are involved in the catabolism of gluconic acid in different bacteria. The identification of genes involved in this metabolic pathway in *C. glutamicum* was started by Mateos and col. (1996) using suicide plasmids in corynebacteria; these plasmids can randomly integrate into the chromosome producing mutants by gene disruption. One of these mutants (mutant TRA-8) was unable to grow in minimal medium supplemented with gluconic acid as unique carbon source. The suicide plasmid integrated into the chromosome of mutant TRA-8 was rescued in *Escherichia coli*, sequenced, and analysis of the sequence showed that the suicide plasmid was integrated into the *gntP* gene (Valbuena, 1999). In most of the bacterial genomes analysed, genes involved in the catabolism of gluconic acid are organized as the *gnt* operon (*gntKP*); in contrast, these genes in *C. glutamicum* are dispersed in the chromosome and apparently regulated by catabolic repression . In this work, *gntK* and *gntP* of *C. glutamicum* were disrupted using internal fragments of both genes cloned separately in the suicide conjugative plasmid pK18mob, and the presence of a functional gluconic acid catabolic pathway in *Corynebacterium glutamicum* ATCC13032 was confirmed (Letek, 2002).

Fig. 1. Transmision electron microscopy of *C. glutamicum* cells showing the typical V-shape.

Results

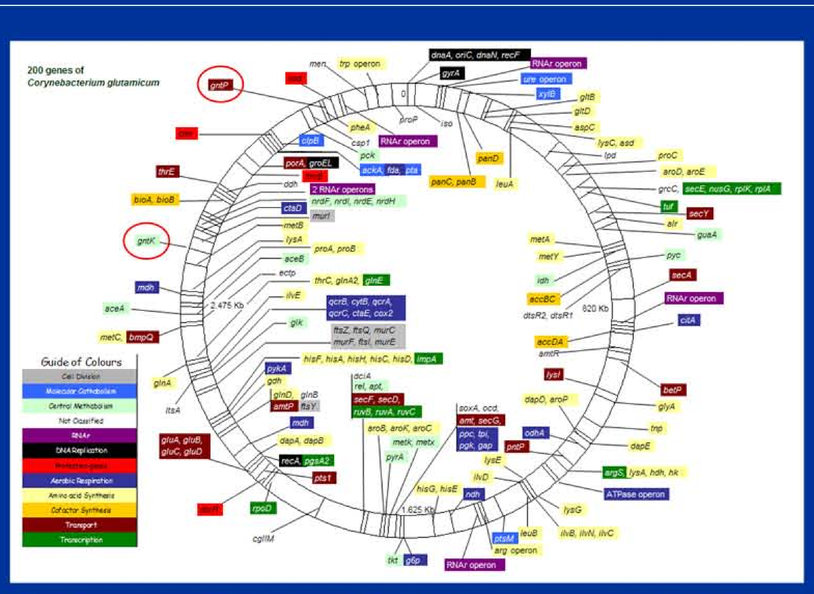
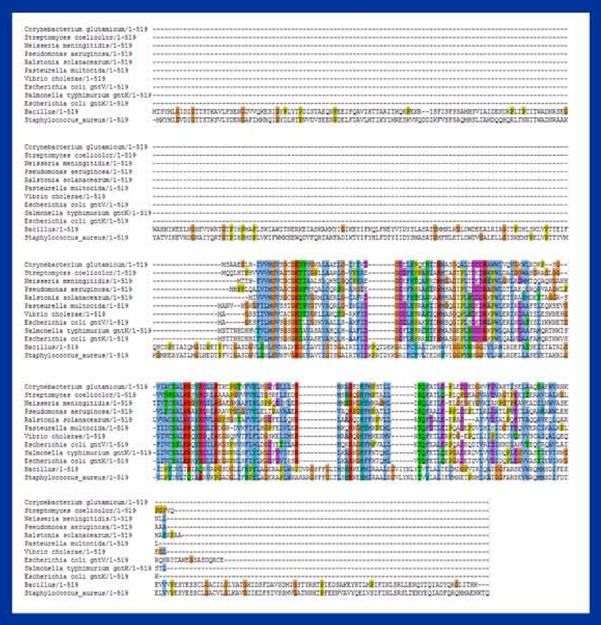


Fig. 2. Genome map of *C. glutamicum* ATCC 13032. The predicted genes involved in gluconate metabolism are indicated (red circles). The identification and position of the genes was achieved using the program BLAST-P specific for the genome of *Corynebacterium glutamicum* (Accession number: NC_003450).



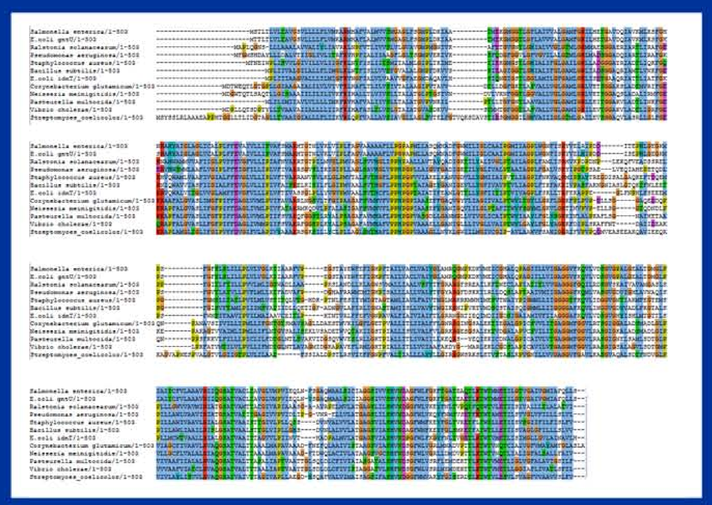


Fig. 3. Clustal-W for GntK (left) and GntP (right). Surprisingly GntK from *C. glutamicum* is more related to their equivalent proteins in Gram-negative bacteria. In contrast, GntP is conserved in both Gram-positive and Gram-negative bacteria.

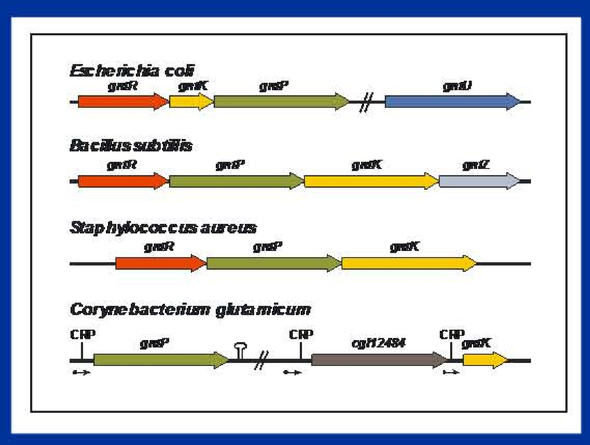
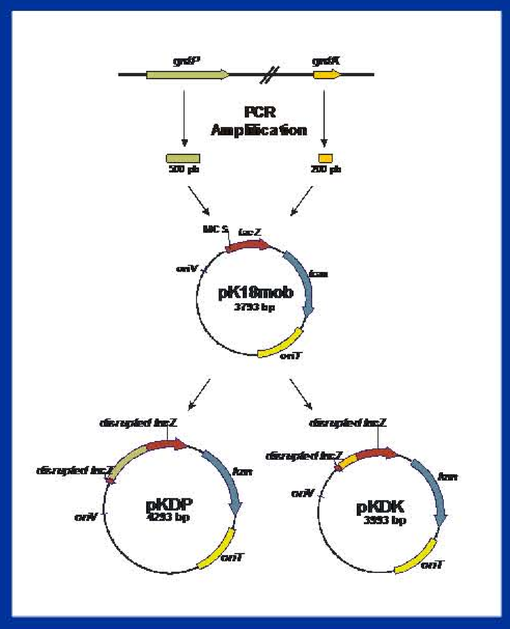


Fig. 4. Choromosomal location of genes involved in the catabolism of gluconic acid (*gntRKP* operon) in different bacteria. In *C. glutamicum gntP* is isolated in the chromosome flanked by its own promoter with a Cathabolic-Repression-Protein (CRP) recognition site, and an strong terminator. However *gntK* seems to be linked with *cgl1 2484* that encoded a glucuronate isomerase activity. Both genes have their own promoter sequence with a CRP recognition site (De Crombrugghe et. al., 1984).



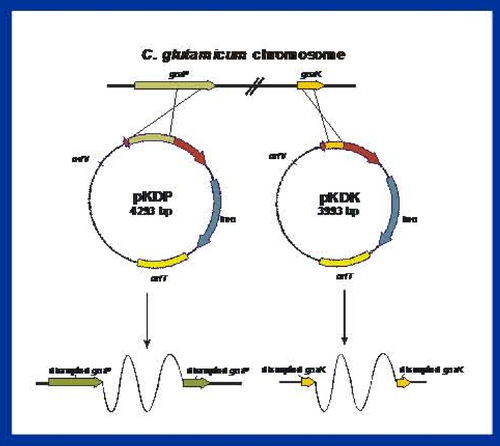


Fig. 5. Disruption of *gntK* and *gntK*. The relevant part of the *C. glutamicum* ATCC 13032 chromosome is represented together with the interpretation of the possible integration results. Internal fragments of both genes were subcloned in pK18mob, a suicide conjugative plasmid for corynebacteria (Schafer and col., 1994). Plasmids pKDP and pKDK were introduced into *C. glutamicum* by conjugation. Kanamycin resistant transconjugants will arose by single recombination between the internal fragment present in the plasmid and the chromosomal gene. The disruption of both genes was confirmed by Southern blot analysis.

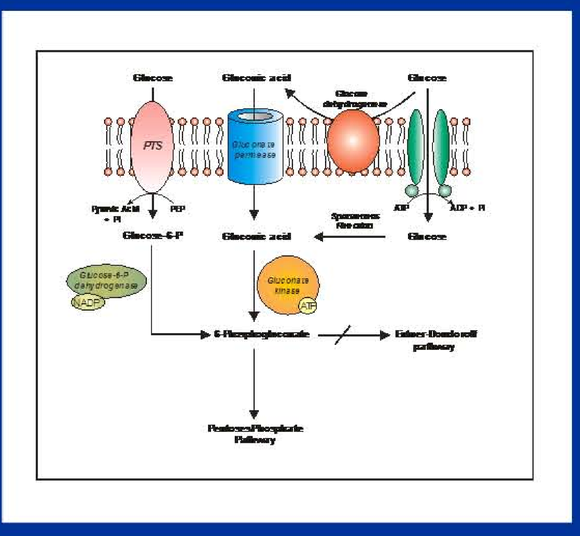


Fig. 6. Glucose uses three different ways to enter into *C. glutamicum*. The molecules of glucose can enter into the cell by active transport, group translocation (PTS transport) or by extracellular conversion to gluconate.

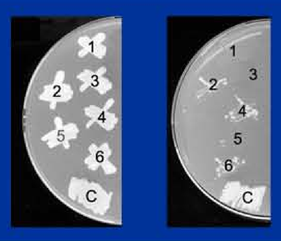


Fig. 7. Mutants obtained by the disruption of *gntK* and *gntP* were unable to grow in mimimal medium with gluconic acid as the unique carbon source: 1, 2 and 3 are *C. glutamicum* mutants disrupted in *gntP*; 4, 5 and 6 are *C. glutamicum* mutants disrupted in *gntK*; C is *C. glutamicum* ATCC 13032 used as positive control.

Bibliography

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