



# X CONGRESO ARGENTINO DE MICROBIOLOGIA GENERAL SAMIGE

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of *B. subtilis* Natto are affected by the agar concentration used and depends on Spo0A, the global transcription regulator of sporulation. Spo0A integrates environmental signals related to starvation or stress conditions and activates various developmental pathways. Examination of strains with reduced biofilm structure formation in *B. subtilis* resulted in the identification of *bslA* gene required for biofilm development. BslA is a small secreted protein that forms a hydrophobic layer on the surface of *B. subtilis* biofilms and increases liquid repellency. Transcription of *bslA* is regulated by several global regulators and shows a spatiotemporal expression pattern during the development of complex colonies. Interestingly, we detected altered expression of *bslA* gene next to the genes related to biofilm formation in our microarray experiments where we examined the sliding behaviour of *B. subtilis* Natto and *B. subtilis* Marburg strains under sliding restrictive compared to permissive conditions (using *spo0A* mutant strain or higher agar concentration). Introduction of the *bslA* mutation into *B. subtilis* reduced sliding motility. Further, our experiments show that the production of exopolysaccharide is also needed for sliding of *B. subtilis*, while the protein component of the biofilm matrix, the amyloid fibers and the presence of flagella are not required for sliding. BslA, therefore next to protecting the biofilm community against various stresses, contributes to surface spreading. Our results point to presence of shared regulators (some of them presented herein) and genes for distinct surface-dependent growth of *B. subtilis*.

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### **ANÁLISIS GENÓMICO DEL CLUSTER BIOSINTÉTICO DE VITAMINA B<sub>12</sub> (COBALAMINA) EN *Lactobacillus coryniformis* CRL 1001**

#### **GENOMIC ANALYSIS OF VITAMIN B12 (COBALAMIN) BIOSYNTHETIC CLUSTER IN *Lactobacillus coryniformis* CRL 1001**

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The vitamin B12 (cobalamin-CBL), a very complex non-polymeric macromolecule synthesized only by some bacteria and archaea, and essential to humans and animals. The highly complicated CBL biosynthesis involves about 30 synthesis steps.

We demonstrated that cell extract of *Lactobacillus coryniformis* CRL 1001 is able to correct the coenzyme B12 requirement of *Salmonella enterica* serovarTyphimurium in minimal medium. The aim of this study was the sequencing of CRL1001 genome and the molecular characterization of CBL biosynthesis in this strain.

*L. coryniformis* CRL 1001 genome was sequenced by a whole-genome shotgun (WGS) strategy with an Ion Torrent personal genome machine based upon libraries created using NEBNext DNA library kits. Genomic analysis was done using the RAST annotation Server, Blast algorithms, ISGA and KEGG databases. The draft genome sequence consists of 2,829,178 bases with a mean GC content of 42%. A total of 3,341 coding sequences (CDS) and 82 structural RNAs (58 tRNAs) were predicted.

RAST analysis evidenced the presence of at least 30 genes (*cob* genes) involved in the CBL biosynthesis in CRL 1001 strain. This finding is the first evidence for cobalamin biosynthesis genes in this species.

Comparative studies among vitamin B12 producer strains demonstrated that the genetic organization of *cob* operon is conserved in this strain and these genes are adjacent to the *pdu* operon and *pocR* gene. The *hem* genes (*hem A, C, B y L*) present in *L. coryniformis* CRL 1001 genome are located among *cob* operon in similar way to anaerobic microorganisms. Interestingly, the *cbIT* y *cbIS* genes were identified in CRL1001 strain genome. These genes encode a putative protein kinase and a  $\alpha$ -ribasol transporter, respectively. The *cbIT* y *cbIS* genes are present in CBL-producers *Listeria sp.* strains but absent in CBL-producers *L. reuteri* strains.

The knowledge of *cob* genes and their regulation in vitamin producer lactic strains constitutes an interesting biotechnological alternative for developing fortified foods.