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MI-P21

EXPRESSION AND PURIFICATION OF *Salmonella typhimurium* RcsCDB SYSTEM PROTEINS

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The Rcs phosphorelay system involves the sensor protein RcsC, the cognate response regulator RcsB, and the histidin-containing phosphotransfer protein RcsD, which serve as an intermediary in the phosphoryl transfer from RcsC to RcsB. Previously, we reported that in the double mutant *rcsD rcsC*, the overproduction of RcsB regulator can not promote the Rcs system activation. These results suggested that only RcsB-P, the RcsB active form, is able to induce the RcsB-dependent genes modulation. We are interested to determine if RcsC or RcsD can independently transfer the phosphate group to RcsB, or if in this process is necessary that both proteins act together. In order to obtain soluble proteins, the full length *rcsB* gene and the sequences encoding the cytoplasmic domain of RcsC and RcsD, labeled with a His6 tag, were cloned into pT7-7 vector. The recombinant plasmids obtained were sequenced and the RcsB, RcsCcyt and RcsDcyt were expressed in *E. coli* BL21 DE3 strain. In the present work we performed the proteins purification step using Ni^{2+} affinity chromatography. The quality and quantity of the purified proteins were monitored by SDS-PAGE and BSA assay. The soluble proteins will be used an in vitro phosphorylation assays to determine the phosphorelay mechanism of the Rcs system.

MI-P22

SCREENING OF DesK MUTANTS IN *Bacillus subtilis* EXPLOITING A NOVEL REPORTER GENE ASSAY

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The histidine kinase DesK from *B. subtilis* is a membrane thermosensor that sense a decrease in membrane fluidity. Together with the response regulator DesR, constitutes a two-component system that regulates the transcription of the *des* gene, coding for the acyl lipid desaturase delta5-Des. To uncover the mechanism of membrane fluidity sensing we performed a random mutagenesis approach of the transmembrane segments of DesK. The phenotypic selection of mutants was carried out using the screening system that we have previously developed in *B. subtilis*, based on sporulation as reporter phenotype. So, as sporulation is impaired when RapA is expressed without its inhibitor, PhrA, we used a *rapA-phrA* null mutant expressing ectopically P*des-rapA* to build a derivative *des* and *desK* null mutant that expresses isotopically *desR* under P*xyl* promoter. The resulting strain, ARDCM2, showed Spo+ phenotype in sporulation medium after a temperature downshift to 23ºC. A vector carrying P*xyl-desK*C-end was built, which is suitable to clone *desK*N-end alleles upstream to *desK*C-end domain, regenerating full-length *desK*. ARDCM2 transformed with the vector expressing DesKwt showed reversion to Spo- phenotype. ARDCM2 transformants expressing mutated versions of DesK that showed Spo+ phenotype at 23ºC or Spo- phenotype at 37ºC were isolated.

MI-P23

TRANSCRIPTIONAL REGULATION OF *gbdR* THAT ENCODES A REGULATOR OF CHOLINE METABOLISM IN *P. aeruginosa*

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Choline is present in high concentrations in different tissues (lung, corneal and urinary epithelium) where *P. aeruginosa* causes infections. It is found as phosphatidylcholine, phosphorylcholine, acetylcholine or as choline-free and, in this way, it can be used as a source of carbon (C) and nitrogen (N) and favors the colonization of this pathogen. GbdR, a regulator that belongs to AraC/XylS family, is essential for the metabolism of choline and also NtrC and CbrB are involved. The aim of this work was to know the transcriptional regulation of *gbdR*. For that, we investigated if the metabolites related or not to choline affected the expression of *gbdR* and if its expression depended on other global regulators. To carry out this study, transcriptional fusions to *lacZ* were performed and inserted into the chromosome of *P. aeruginosa* WT, *ΔntrC, ΔcbrB* and *ΔrpoN* strains. The nitrogen depletion in the culture medium increased the reporter activity 3-fold times. The increase was higher (from 4 to 12 times) if histidine or choline were added as supplementary nitrogen source, respectively. Choline (no betaine or DMG) was the real inductor of *gbdR* expression. This expression also depended on the presence of RpoN and NtrC. The contributions made in this study will allow progress in molecular knowledge of the specific and global transcriptional regulation of choline metabolism in *P. Aeruginosa*.

MI-P24

SUNLIGHT RESPONSE OF *S. enterica* SEROVAR *Typhimurium* ATCC14028 AND LT2 STRAINS: ROLE OF RpoS

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Virulent ATCC14028 and non-virulent LT2 strains of *Salmonella enterica* serovar Typhimurium are closely related but, as it was reported, they exhibit different environmental stress tolerance. Whilst sensitivity to DNA damage and acid stress are increased in LT2, the effects of starvation and oxidative stress are similar in both strains. We recently described that kinetics of sunlight induced cell death was similar for ATCC14028 and LT2, except for a slightly increased sensitivity found in LT2, which could be ascribed to a low level of RpoS protein. To further analyze the role of *rpoS* in sunlight response, it was inactivated in both strains by introducing the mutation *rpoS*::Ap^R. Survival curves of stationary phase cells of the parental strains and their *rpoS* defective derivatives were obtained during natural sunlight exposures at 25-26°C with an irradiance of 860-868 Wm^2. In both genetic contexts, *rpoS* inactivation increased sensitivity, but at start of the irradiation the loss of viability was delayed in cells derived from ATCC14028. Since differences in the cell death kinetics of the mutants could not be explained by differences in their RpoS levels, the results suggest that the sunlight response of *S. Typhimurium* involves a mechanism beyond the RpoS control, and this mechanism is modified in LT2 strain with respect to ATCC14028.