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Expression of the translocator protein (TSPO) from *Pseudomonas* fluorescens Pf0-1 requires the stress regulatory sigma factors AlgU and RpoH.

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The translocator protein (TSPO), previously designated as peripheral-type benzodiazepine receptor, is an evolutionary conserved protein that is found in many Eukarya, Archae and Bacteria, in which it plays several important functions including membrane biogenesis, signaling and stress response. A *tspo* homologue gene has been identified in several members of the *Pseudomonas* genus, among which the soil bacterium *P. fluorescens* Pf0-1. In this bacterium, the *tspo* gene is located in the vicinity of a putative hybrid histidine kinase-encoding gene.

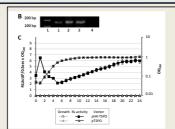
Since *tspo* has been involved in water stress related response in plants, we explored the effects of hyperosmolarity and temperature on *P. fluorescens* Pf0-1 *tspo* expression using a strategy based on *lux*-reporter fusions.

The tspo gene (Pfl01_2811) of P. fluorescens Pf0-1 forms an operonic structure with Pfl01_2810 and is expressed transiently during the bacterial growth.



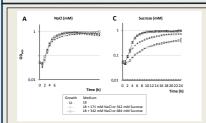
(A) Schematic representation of the genomic environment of tspo and of transcriptional fusions pTSPO and pHK-TSPO. Grey bars represent the beginning of the *luxCDABE* reporter cassette of the promoterless pAB133 vector.

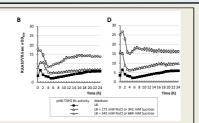
The position of the studied promoter region is indicated relatively to the translational initiation start of each ORF.



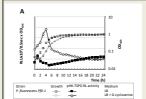
- (B) Co-transcription of Pfl01_2810 and tspo by RT-PCR assay. RT-PCR was assayed on tspo (1), Pfl01_2810 (2) and on the putative operonic structure Pfl01_2810 tspo (3), using primers located into tspo (1) or Pfl01_2810 (2) ORFs, or into both tspo and Pfl01_2810 ORFs (3). RT-PCR achieved on total RNA did not lead to a PCR fragment (line 4, negative control). L: Ladder 1kb+ (Biorad®),
- (C) Growth curves in microtitre wells in LB medium at 28 C and relative luminescence (RL) activity of pTSPO and pHK-TSPO in *P. fluorescens* Pf0-1.

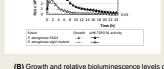
Expression of tspo is increased in response to hyperosmolarity at least partly via the extracytoplasmic function sigma factor AlgU activity.





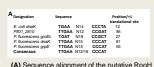
Growth curves (\mathbf{A}, \mathbf{C}) and transcriptional activity (\mathbf{B}, \mathbf{D}) of P. fluorescens Pf0-1 in microtiter wells in LB medium supplemented or not with 171 mM or 342 mM NaCl (\mathbf{A}, \mathbf{B}) or with 342 mM or 684 mM sucrose (\mathbf{C}, \mathbf{D}) .



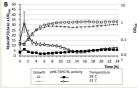


(A) The growth of *P. fluorescens* Pf0-1 containing pHK-TSPO and the relative bioluminescence levels of pioluminescence levels generated by pHK-TSPO *P. aeruginosa* PAO1 and its isogenic *algU* mutant are shown when bacteria were grown in LB with or without D-cycloserine.

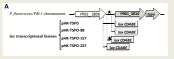
A putative RpoH-consensus binding site is located in PfI01_2810-tspo promoter region: effect of temperature on growth and TSPO expression.



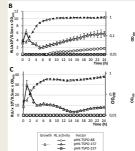
(A) Sequence any imment on the plutative Apolibinding sites identified by MEME in the regions located upstream of dnaK in E. coli and groEL, dnaK, grpE and Pfl01_2810 in P. fluorescens Pfl0-1, relatively to the E. coli RpoH consensus sequence. Localization of this sequence is given relatively to the +1 translational start site.

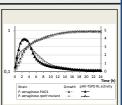


(B) Growth and transcriptional activity of *P. fluorescens* Pf0-1 containing pHK-TSPO reporter fusion in microtiter plates cultured at 28 C or 32 C.



(A) Scientific tepsel flation to the 4 transcriptorial fusions. RpoH binding sequence (black star).
(B, C) Growth in microtiter plates at 28 C (B) or 32 C (C), and transcriptional activity of P. fluorescens Pf0-1 containing pHK-TSPO-88, pHK-TSPO-157 and pHK-TSPO-227 plasmids.





Growth in microtiter plates at 37 C and transcriptional activity of *P. aeruginosa* PAO1 and *rpoH* mutant containing pHK-TSPO-157.

Conclusions

We show that the two genes Pfl01_2810 and tspo are co-transcribed forming a transcription unit. The expression of this operon is growth phase-dependent and is increased in response to high concentrations of NaCl, sucrose and to a D-cycloserine treatment, which are conditions leading to activity of the major cell wall stress responsive extracytoplasmic sigma factor AlgU. The promoter region activity is strongly lowered in a P. aeruginosa algU mutant, suggesting that AlgU may be involved at least partly in the molecular mechanism leading to Pfl01_2810-tspo expression. In silico analysis of this promoter region failed to detect an AlgU consensus binding site; however, we detect a putative binding site for the heat shock response RpoH sigma factor. Accordingly, the promoter activity of the region containing this sequence is increased in response to high growth temperature and slightly lowered in a P. aeruginosa rpoH mutant strain. Taken together, our data suggest that P. fluorescens tspo gene may belong at least partly to the cell wall stress response.