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

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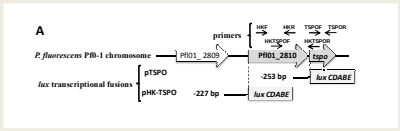
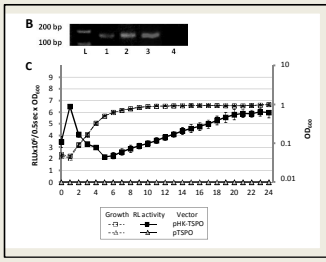
The translocator protein (TSPO), previously designated as peripheral-type benzodiazepine receptor, is an evolutionary conserved protein that is found in many Eukarya, Archae and Bacteria, in which it plays several important functions including membrane biogenesis, signaling and stress response. A *tspo* 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.



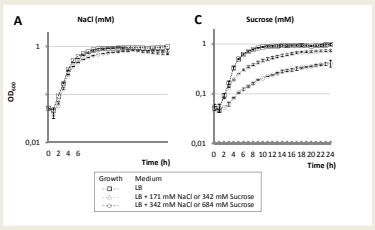
(A) Schematic representation of the genomic environment of *tspo* and of transcriptional fusions pTSPO and pHK-TSPO. Grey bars represent the beginning of the *luxCDABE* reporter cassette of the promoterless pAB133 vector.
The position of the studied promoter region is indicated relatively to the translational initiation start of each ORF.

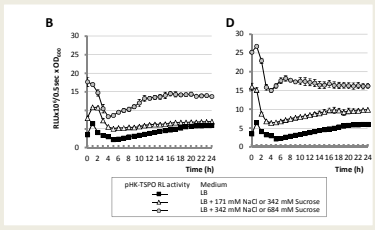


(**B**) Co-transcription of Pfl01_2810 and *tspo* by RT-PCR assay. RT-PCR was assayed on *tspo* (1), Pfl01_2810 (2) and on the putative operonic structure Pfl01_2810-*tspo* (3), using primers located into *tspo* (1) or Pfl01_2810 (2) ORFs, or into both *tspo* and Pfl01_2810 ORFs (3). RT-PCR achieved on total RNA did not lead to a PCR fragment (line 4, negative control). L: Ladder 1kb+ (Biorad®),

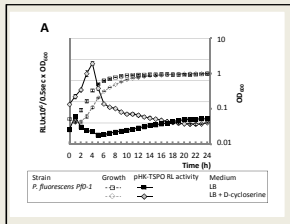
(C) Growth curves in microtitre wells in LB medium at 28 C and relative luminescence (RL) activity of pTSPO and pHK-TSPO in *P. fluorescens* Pf0-1.

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

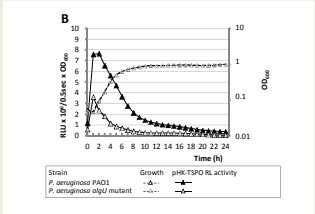




Growth curves **(A, C)** and transcriptional activity **(B, D)** of *P. fluorescens* Pf0-1 in microtiter wells in LB medium supplemented or not with 171mM or 342 mM NaCl (**A, B**) or with 342 mM or 684 mM sucrose (**C, D**).



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



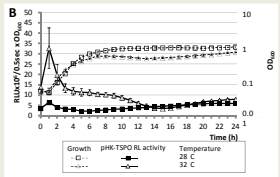
(B) Growth and relative bioluminescence levels of *P. aeruginosa* PAO1 and its isogenic *algU* mutant containing pHK-TSPO in LB in microtiter wells.

A putative RpoH-consensus binding site is located in Pfl01_2810-*tspo* promoter region: effect of temperature on growth and TSPO expression.

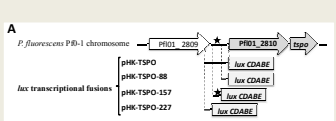
A

Designation	Sequence		Position/+1 translational site
E. coli dnaK	TGAA	N14	CCCTA 12
Pfl01_2810	TTGAA	N12	CCGAT 95
P. fluorescens groEL	TGAT	N18	CCGCT 27
P. fluorescens dnaK	TTGAA	N15	CCCAT 81
P. fluorescens grpE	TTGAA	N15	CCCAT 55
Consensus	TTGAA	N12/18	CCCAT

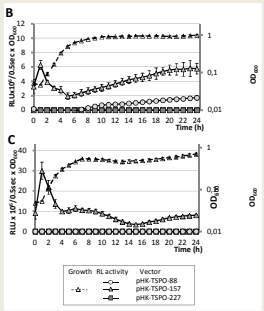
(A) Sequence alignment of the putative RpoH binding sites identified by MEME in the regions located upstream of *dnaK* in *E. coli* and *groEL*, *dnaK*, *grpE* and Pfl01_2810 in *P. fluorescens* Pf0-1, relatively to the *E. coli* RpoH consensus sequence. Localization of this sequence is given relatively to the +1 translational start site.

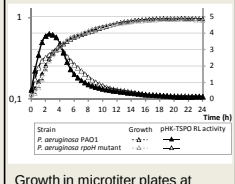


(B) Growth and transcriptional activity of *P. fluorescens* Pf0-1 containing pHK-TSPO reporter fusion in microtiter plates cultured at 28 C or 32 C.



(A) Schematic representation of the 4 transcriptional 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.