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Expression of the *oprQ* gene, encoding a porin of the OprD family, in response to iron limitation in *Pseudomonas protegens* Pf-5.

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Porins form water-filled channels in the outer membrane of Gram-negative bacteria which allow solutes passage or contribute to the stability of the bacterial envelope. Porins can be classified into non-specific large general porins and substrate-specific porins. The outer membrane of *Pseudomonas* spp. has lower permeability and larger exclusion limit than that of Enterobacteriaceae. This can be explained by the fact that *Pseudomonas* species do not possess large general porins and have several substrate-specific porins for the uptake of small molecules. Previous results from our research group, demonstrated that the cellular content of the OprQ porin increased during the exposure of *Pseudomonas protegens* Pf-5 to the mycotoxin fusaric acid. Fusaric acid, a secondary metabolite produced by several fungi of the *Fusarium* genus, is able to bind iron by high affinity. Taking this result into account, the hypothesis of this work was that iron limitation conditions increase the expression of the *oprQ* gene. To test this hypothesis, the *oprQ* promoter sequence was cloned upstream of the mcherry reporter gene of plasmid pSEVA237R and introduced into *P. protegens* Pf-5. Growth was evaluated by measuring the optical density at 600 nm in four different conditions: without the addition of iron salts into the growth medium (iron limitation), with the addition of 100 µM iron into the medium (iron excess), with the addition of 10 µM iron and fusaric acid, and with the addition of 10 µM iron and absence of fusaric acid. The expression of *oprQ* was monitored by measuring mCherry fluorescence (?exc=576 nm, ?emm=621 nm) in a spectrofluorometer. In addition, the production of the siderophore pyoverdine was also analyzed by monitoring pyoverdine fluorescence (?exc=420 nm, ?emm=520 nm). By comparing the results obtained under iron limitation and iron excess, we observed that although the specific growth rate of *P. protegens* Pf-5 was not affected by iron limitation, the cultures grown under this condition showed an early entrance into the stationary phase and, as a consequence, a much lower final biomass than the iron excess-condition. The *oprQ* expression levels were significantly higher under iron limitation compared to iron excess when iron limited cultures entered the stationary growth phase. The same was observed for pyoverdine production. The addition of fusaric acid into the growth medium lowered the specific growth rate and increased *oprQ* expression and pyoverdine production levels throughout the growth curve. These results show that *oprQ* expression respond to iron availability in *P. protegens* Pf-5 and demonstrate that the OprQ porin possesses an important role in the adaptation

to low iron environments.

Palabras clave: iron limitation-adaptation-porin-soil bacteria