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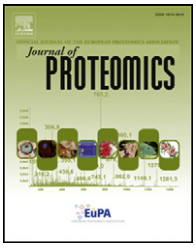

Proteogenomic insights into salt tolerance by a halotolerant alpha-proteobacterium isolated from an Andean saline spring☆

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ABSTRACT

Tistlia consotensis is a halotolerant *Rhodospirillaceae* that was isolated from a saline spring located in the Colombian Andes with a salt concentration close to seawater (4.5%w/vol). We cultivated this microorganism in three NaCl concentrations, *i.e.* optimal (0.5%), without (0.0%) and high (4.0%) salt concentration, and analyzed its cellular proteome. For assigning tandem mass spectrometry data, we first sequenced its genome and constructed a six reading frame ORF database from the draft sequence. We annotated only the genes whose products (872) were detected. We compared the quantitative proteome data sets recorded for the three different growth conditions. At low salinity general stress proteins (chaperons, proteases and proteins associated with oxidative stress protection), were detected in higher amounts, probably linked to difficulties for proper protein folding and metabolism. Proteogenomics and comparative genomics pointed at the CrgA transcriptional regulator as a key-factor for the proteome remodeling upon low osmolarity. In hyper-osmotic condition, *T. consotensis* produced in larger amounts proteins involved in the sensing of changes in salt concentration, as well as a wide panel of transport systems for the transport of organic compatible solutes such as glutamate. We have described here a straightforward procedure in making a new environmental isolate quickly amenable to proteomics.

Biological significance
The bacterium *Tistlia consotensis* was isolated from a saline spring in the Colombian Andes and represents an interesting environmental model to be compared with extremophiles or other moderate organisms. To explore the halotolerance molecular mechanisms of the bacterium *T. consotensis*, we developed an innovative proteogenomic strategy consisting of i) genome sequencing, ii) quick annotation of the genes whose products were detected by mass spectrometry, and iii) comparative proteomics of cells grown in three salt conditions. We highlighted in this manuscript how efficient such an approach can be compared to

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time-consuming genome annotation when pointing at the key proteins of a given biological question. We documented a large number of proteins found produced in greater amounts when cells are cultivated in either hypo-osmotic or hyper-osmotic conditions.
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1. Introduction

Halotolerant organisms isolated from moderate saline environments are able to grow in the presence of relatively high salt concentrations but also in the absence of salt [1]. As a result of long periods of drought or rain, external changes in osmolarity are frequent in the environment. Therefore, halotolerants have the ability to grow in a wide range of salinities whereas halophiles require a saline environment for growth. Only the extremely halophilic archaea and very few species of halophilic bacteria accumulate inorganic ions, mainly K^+ and Cl^- inside cytoplasm, whereas most moderate halophilic or halotolerant bacteria accumulate compatible solutes, by uptake or *de novo* synthesis, such as polyols and derivatives, sugars and derivatives, amino acids and derivatives, betaines, and ectoines [2,3].

Osmoregulation mechanisms from extreme halophiles such as *Halobacterium salinarium* [4,5] and *Haloferax volcanii* [6], as well as from moderate halophiles, namely *Halobacillus dabanensis* [7–9], *Chromobacter salexigens* [10] and *Halomonas elongata* [11,12] have been extensively studied. However, only little information has been reported on the proteins involved in osmoregulation mechanisms of halotolerant microorganisms. Studies on the molecular mechanisms used by these microorganisms to adapt to osmotic stress have been reported both at genetic and biochemical levels for bacteria such as *Escherichia coli* [13], *Bacillus subtilis* [14], *Listeria monocytogenes* [15], *Staphylococcus xylosus* [16], *Sinorhizobium melitoti* [17] and *Arthrobacter globiformis* [18]. Within the *Rhodospirillaceae* family belonging to Alphaproteobacteria, many genera include halophilic strains isolated from marine environments, such as *Thalassobaculum* [19], *Nisaea* [20], *Thalassospira* [21], *Rhodospira*, *Roseospira*, *Rhodovibrio*, *Rhodospirillum* [22], *Marispirillum* [23], *Oceanibaculum* [23] and *Pelagibius* [24]. It also includes halotolerant strains isolated from sludge, cavity within white rock and saline spring, as exemplified with strains from the genera *Caenispirillum* [25], *Inquilinus* [26], *Constrictibacter* [27] and *Tistlia* [28]. Although this family includes numerous genera related to halotolerant and halophilic microorganisms, no detailed proteomic study related to the osmoregulation mechanisms used by these microorganisms has been yet reported.

T. consotensis is a Gram-negative, aerobic, mesophilic non-spore forming and nitrogen-fixing bacterium, identified as a new species of the *Rhodospirillaceae* family distantly related to the genus *Thalassobaculum* (90% identity at the level of their 16S rRNA gene sequences). *T. consotensis* was isolated from a saline spring located in the Colombian Andes. This spring is characterized by a high content of Ca^{2+}, Na^+ and Cl^-, a salt concentration close to that reported for seawater, *i.e.* 4.5% (w/vol), and has a neutral pH [28]. Resultant of its specific environment, *T. consotensis* was characterized as being a halotolerant bacterium with an optimum growth at 0.5% (w/vol) NaCl concentration and range growth between 0.0 and 4.0% (w/vol) NaCl. Previous studies have shown that this microorganism is able to accumulate 3-polyhydroxyalkanoates (PHAs) and interesting secondary metabolites with potential biological activity [28].

Current high-throughput proteomic tools based on high-resolution mass spectrometers and shotgun strategies provide the opportunity to study the physiology of a bacterium at high resolution [29,30]. It is of great interest to further characterize with such tools the general mechanisms used by halotolerant microorganisms to withstand changes in external conditions of salinity. Here, we investigated the proteomic changes of *T. consotensis* when cells were grown in different concentrations of salinity: without salt (0.0% NaCl w/vol), optimal salt (0.5%) and high salt concentration (4.0%). We first carried out the sequencing of *T. consotensis* genome in order to obtain the most appropriate protein database for proteogenomic detection of the cellular players [31]. Then, the intracellular proteome was analyzed using a massive shotgun nanoLC–MS/MS approach consisting in the analysis of 72 samples. Data comparisons highlighted the key factors involved in the osmoregulation mechanisms used by the halotolerant bacterium *T. consotensis* under hypo- and hyper-osmotic conditions. This study is the first proteomic analysis of the cellular response to different concentrations of NaCl reported for a member of the *Rhodospirillaceae* family, widely present in the environment.

2. Experimental procedures

2.1. Strain, media and culture conditions

T. consotensis — USBA 355^T from our culture collection was cultured in basal medium (BM) 1 g NH_4Cl, 0.3 g K_2HPO_4, 0.3 g KH_2PO_4, 3 g $MgCl_2.6H_2O$, 0.1 g $CaCl_2.2H_2O$, 0.1 g KCl and 10 ml trace element solution SL-10) supplemented with 2 g yeast extract, and 20 mM D-glucose as the sole carbon source (L^{-1}). The pH of the medium was adjusted to 6.8 with 5 N NaOH [28]. Three different growth conditions based on NaCl content were evaluated, *i.e.* 0.0, 0.5 and 4.0% (w/v) NaCl, which approaches the minimum, optimum and maximum NaCl concentrations for growth, respectively. For each NaCl concentration, three flasks (independent biological triplicates) containing 100 ml of BM medium were inoculated with *T. consotensis* cells previously grown on agar plate. The cultures were incubated in the dark at 30 °C in shaker at 200 rpm and the growth was monitored by OD_{600nm}. The nine cultures were harvested by centrifugation at 4000 rpm when the mid exponential phase (OD_{600} = 0.4) was reached. The pellets were quickly washed twice in 50 mM Tris–HCl buffered at pH 8.0.

2.2. Proteome sample preparation and trypsin in-gel proteolysis

Cell pellets (41–56 mg, wet weight) were dissolved in 100 μL lithium dodecyl sulfate-β-mercaptoethanol protein gel sample