Metabolic dynamics of Paenibacillus and Bacillus in response to tellurite

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Abstract

The increased demand of Tellurium (Te) has led to the rise of soluble free forms of Te in environmental niches occupied by living organisms. Considering that new isolates can be a source of new genetic and metabolic strategies that are selected on organisms colonizing new contaminated environments. The objective of this study was to find new isolates from metal-contaminated sites that reduce tellurite ion (Te (IV)) to metallic Te, and, by combining genome sequencing and proteomic analysis, to understand the effect of Te (IV) on these organisms at the proteomic level. Cultivation in the presence of Te (IV), lead to the overexpression in *Bacillus altitudinis* 3W19 of the proteins from *ter* and the *ars* operons. In *Paenibacillus pabuli* ALJ109b, marker proteins for stress-response as phage shock protein and the chaperon FloT were overexpressed. The overexpression of specific pathways was characteristic for each strain and only showed some similarity to described proteomics analysis to comparable metal ions. The quantification of cell ROS and metabolic activity by MTT supported the proteomic results.

The work shows the importance of environmental dynamism on the diversity of genomes, and the existence of different genetic rearrangements resulting in diverse metabolic pathways in microorganisms living under metal stress.

Cell growth and Te (IV) reduction

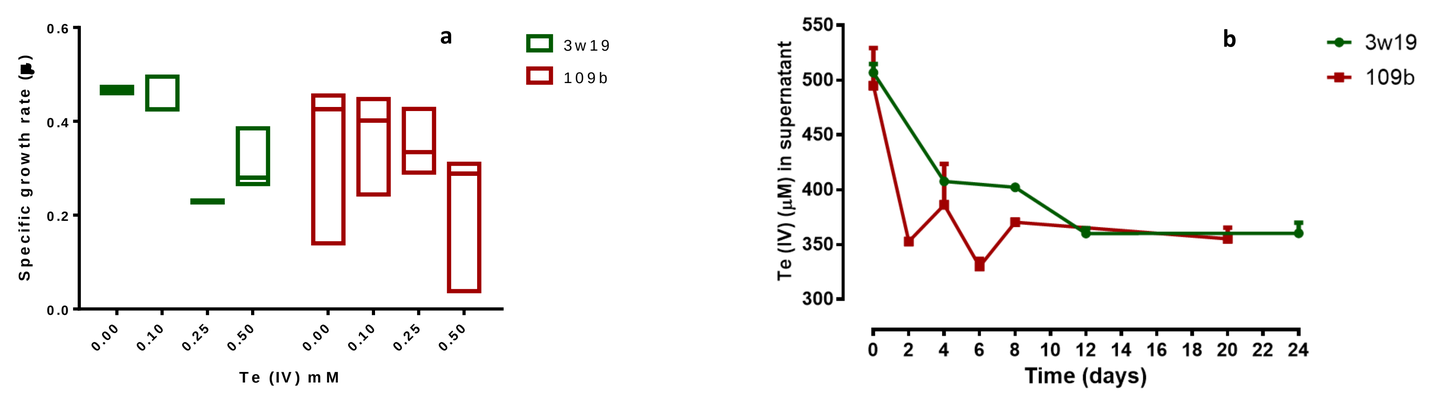

Figure1: a) Specific growth rate, calculated from growth of *Bacillus altitudinis* 3W19 (green) and *Paenibacillus pabuli* ALJ109b (red), in LB with increasing Te (IV) concentrations. b) Te (IV) depleation by strains 3W19 and ALJ109b, over time, determined by DDTC colorimetric assay.

Metabolism and oxidative stress

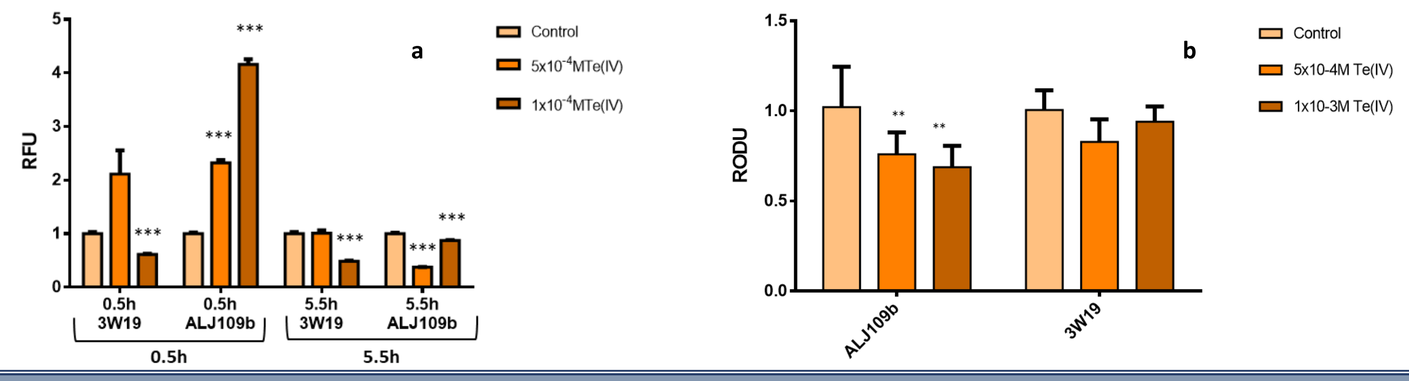

Figure2: a) Reactive oxygen species determination in Control, $1x10^{-3}$ M Te(IV) and $5x10^{-4}$ M Te(IV) conditions, at times 30 min and 5.5 h, of incubation with HDCMA probe. RFU express relative fluorescent units with Control situation normalized to 1. b) Metabolic activity determined by MTT assay in Control, $1x10^{-3}$ M Te(IV) and $5x10^{-4}$ M Te(IV) conditions. RODU expresses relative optic units with Control situation normalized to 1.

Results

Both strains tested were able to grow in the presence of Te (IV) up to $5x10^{-4}$ M, Figure 1a. *B. altitudinis* 3W19 decreased its specific growth rate, mostly from a concentration of $2.5x10^{-4}$ M of Te (IV), by 0.12. *P. pabuli* ALJ109b decreased its specific growth rate from the control situation to highest metal concentration by the same amount as 3W19 but at a constant rate. Both strains are able to reduce the concentration of soluble Te(IV) in the media from approximately $5x10^{-4}$ M to $3.5x10^{-4}$ M in 12 h, strain 3W19, and 20 h, strain ALj109b, Figure 1b.

Response to oxidative stress, determined by tracking ROS, is effective in both strains as is seen in Figure 2a. In 30 min ROS production is higher at the concentration of $1x10^{-3}$M and $5x10^{-4}$ M of Te (IV) for 3W19 and ALJ109b strains, respectively. In both strains ROS levels match control situation after 5.5 h. Metabolic activity determined by MTT assay decreases, up to 14 %, in *P. pabuli* ALJ109b in the presence of $5x10^{-4}$ M of Te (IV), wile in *B. altitudinis* 3W19 this variations is not significant, Figure 2b.

Proteomic analysis reveals the highest fold change in proteins over/down expressed in the presence of $5x10^{-4}$ M of Te (IV), Figure 3. In *B. altitudinis* 3W19 highest protein overexpression in Te (IV) resistance related proteins TerD and ArsC as well as stress response related proteins PspA and HslO, Figure 3a, for the most part the increased proteins corresponding genes are grouped in gene clusters with functionally linked genes, Figure 3b. In *P. pabuli* ALJ109b highest protein overexpression is seen in stress response related proteins PspA and FloT, Figure 3c.

Pathways expression, induced by Te (IV), reveals the down expression of carbohydrate metabolism and the over expression of amino acid metabolism in strain 3W19. In strain ALJ109b only membrane transport is affected by Te (IV), Figure 4.

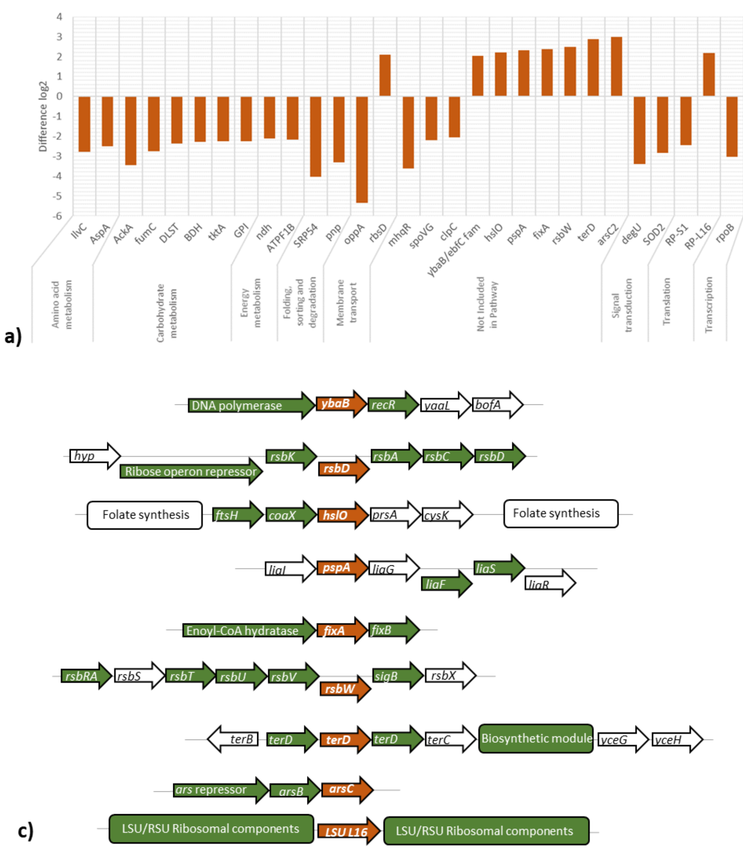

Figure 3: Representation of proteins with higher than log2 2.0 (absolute value) in both strains of *Bacillus altitudinis* 3W19 a) and *Paenibacillus pabuli* ALJ109b b). Protein were grouped according to metabolic function, KEGG functional level 2 for strain 3W19and levels 2 and 3 for strain ALJ109b. Schematic representation of the coding gene (orange arrows) for each overexpressed protein, represented in a) and b), in strain 3W19 c) and strain ALJ109b d), including the close genetic neighborhood. Genes whose relative position is conserved in at least four other species, according to RAST, are functionally coupled and are represented in green arrows.

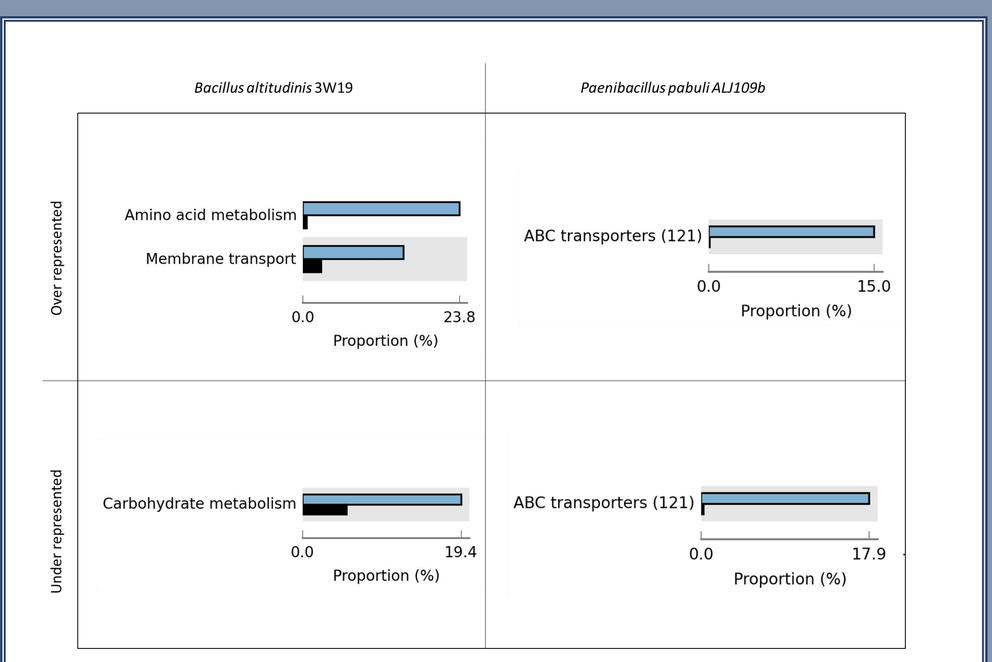

Figure 4: SCA proteins were mapped with subsystems classifications from KEGG. Level 2 and 3 KEGG pathway were analyzed for regulation using a Fisher's exact test. FDR adjusted p-values are presented for each pathway, p-values equal or under 0,05 were considered for determining significant pathways. Black bars display pathway size compared to the size of reference proteome, which can be grouped in pathways. Blue bars display the ratio of SCA proteins in the pathway compared to the total amount of SCA proteins in pathways.

Conclusions

Bacillus altitudinis 3W19 and *Paenibacillus pabuli* ALJ109b are two heterotrophic bacteria with high Te (IV) resistance and reducing ability. The genome sequencing analysis and differential proteomics revealed specific metabolic response to Te(IV) in *B. altitudinis* 3W19, and in *P. pabuli* ALJ109b for the first time. ROS determination showed that Te (IV) induces oxidative stress in both organisms. Both strains resolved their cells' oxidative stress by activating unique proteins and metabolic pathways. The overall over/down representation of metabolic pathways is, mostly, in accordance between *B. altitudinis* 3W19 and *P. pabuli* ALJ109b. In both strains, membrane transport was over-represented and carbohydrate metabolism was down represented.

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