TRANSCRIPTIONAL ANALYSIS OF *Acinetobacter sp. neg1* CAPABLE OF DEGRADING OCHRATOXIN A

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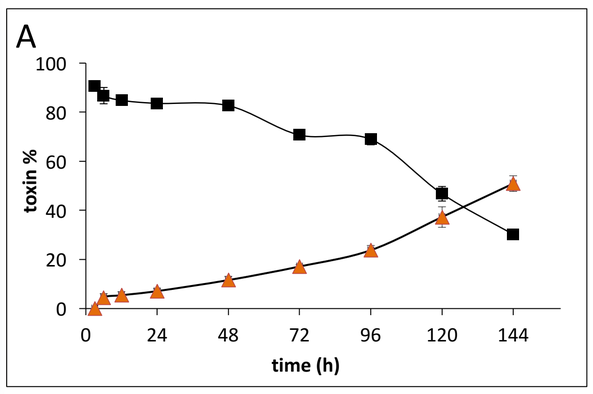
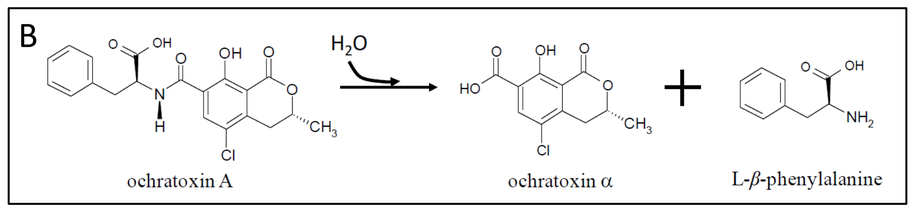
Ochratoxin A (OTA) is a fungal secondary metabolite produced by several species of *Aspergillus* and *Penicillium*. This mycotoxin contaminates grapes, grains and other plant products such as red wine, coffee beans, as well as livestock products, such as milk and pork meat. It is primarily known for its nephrotoxic effects, but mutagenic, teratogenic and carcinogenic properties have also been demonstrated.

We recently isolated from soil a novel free-living *Acinetobacter* strain, named *Acinetobacter sp. neg1* (1), able to degrade OTA into the not toxic catabolic product OTalpha (OTα), suggesting that the degradation reaction proceeds via peptide bond hydrolysis and phenylalanine release. In order to identify enzymes and pathways involved in toxin degradation we performed a comparative transcriptional analysis of bacterial cultures grown in media supplemented with OTA.

Acinetobacter sp. neg1 OTA degrading activity

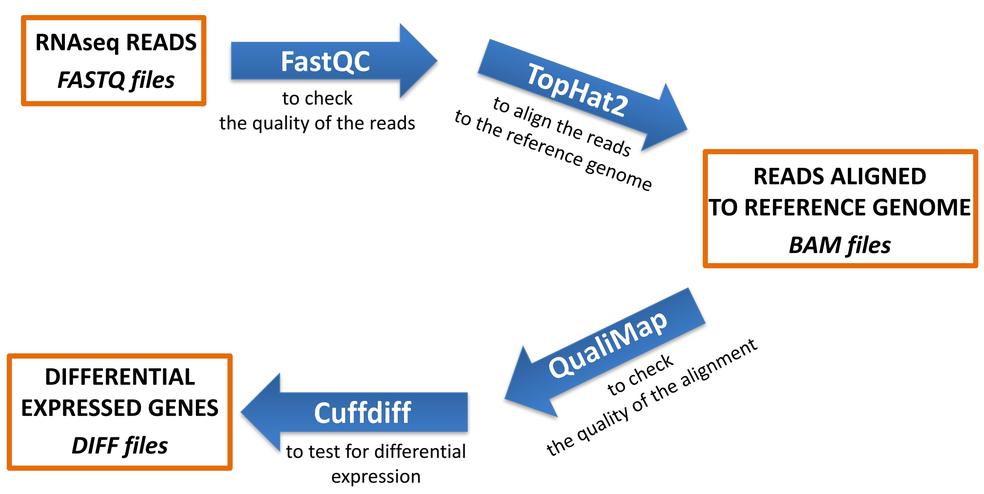




A) Bacteria were grown in the presence of OTA (1µg/ml) and the changes in OTA (squares) and OTα (triangles) concentrations were monitored. OTA level decreased rapidly after 6 h of incubation, then the degrading activity remained stationary until 48 h. The maximum rapidity of OTA decrease was measured between 48-72h and 96-144h. At 144 h more than 70% of OTA was degraded to Otα.

B) The microbial degradation reaction proceeds via the hydrolysis of the amide bond with phenylalanine release. Our analyses did not reveal the presence of L-β-phenylalanine in the medium, thus suggesting that it might be immediately used or further degraded by bacterial cells.

Differential expression analysis

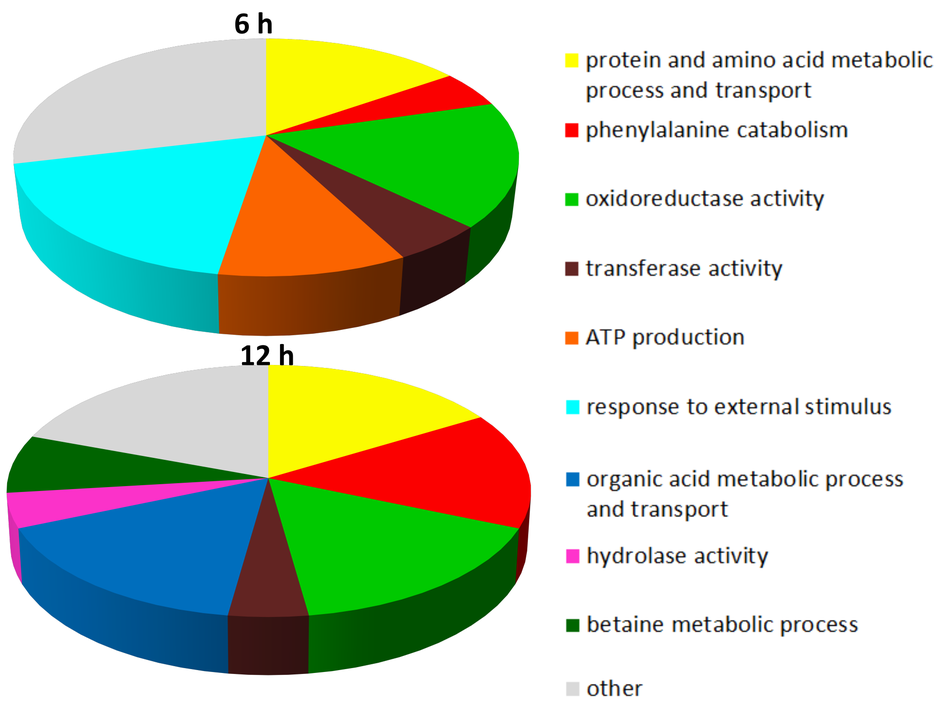


RNA was isolated from bacteria grown in the presence or in the absence of OTA for 6 and 12 hours and sequenced with the Illumina MiSeq system, obtaining 4 x 10^6 paired-end reads. The RNA seq data were analyzed in the discovery environment of *iPlant* (2). Reads were aligned to the reference genome using *TopHat2*. Then, *Cuffdiff* was used to calculate FPKM and detect significant differences between the two conditions. This analysis revealed 107 and 135 genes from 6 h-samples and 12 h-samples respectively with a FDR-adjusted p-value < 0.05. Among them, we found 4 genes coding for peptidases or hydrolases that could be involved in the reaction of degradation: 2 serine-type endopeptidases were up-regulated at 6 hours, while one aminopeptidase and one hydrolase were up-regolated at 12 hours.

GENE NAME	MOLECULAR FUNCTION	EXPRESSION IN THE PRESENCE OF OTA
PJ15_1852	aminopeptidase	up-regulated at 12 h
PJ15_2037	hydrolase	down-regulated at 6 h up-regulated at 12 h
PJ15_2954	serine-type endopeptidase	up-regulated at 6 h
PJ15_2971	serine-type endopeptidase	up-regulated at 6 h

Pathway analysis

An enrichment analysis of the differentially expressed genes for Gene Ontology terms was completed using the Java-based tool Bingo (3), implemented as a plugin for Cytoscape. It revealed the over-representation of a total of 59 pathways in 6h-grown bacteria and 67 in 12h-grown bacteria. The dysregulated pathways were grouped in 10 functional categories on the basis of the Gene Ontology relations between terms. In the pie charts the arc length of the slices is proportional to the percent of dysregulated pathways of each category.



At 6 hours, activities and processes related to the category *response to external stimulus* are dysregulated with respect to the control, suggesting that the bacterium is sensing the OTA presence as a stressing condition. We also found the over-representation of processes belonging to the *protein and amino acid metabolic process and transport* category both at 6 and 12 hours. This category includes transmembrane transporters and specific amino acid transporters which are downstream the phenylalanine catabolic pathway. Accordingly, among the gene sets that displayed significant OTA-related dysregulation at 12h we found 10 pathways involved in phenylalanine catabolism. This suggests that Phe is an energy source for the bacteria and the degrading reaction is followed by the modulation of further catabolic activities.

CONCLUSION

Our results demonstrate that *Acinetobacter sp. neg1* degrades OTA in L-β-phenylalanine and Otα through the hydrolysis of the amide bond. Transcriptional analysis of bacteria grown in the presence of OTA revealed 4 genes coding for peptidases or hydrolases that could be involved in the reaction of degradation. Further analysis are ongoing to verify the activity of the candidate genes. The absence of L-β-phenylalanine in the medium suggests that it is immediately used by the bacterial cells. The enrichment analysis of the differentially expressed genes confirms that the degradation reaction induces the activation of pathways involved in the phenylalanine catabolism.

References

1. Fanelli F, Chiara M, Liuzzi VC, Haidukowski M, Tristezza M, Caterina M, D'Erchia AM, Pesole G, Horner DS, Mule G, Draft genome sequence of *Acinetobacter sp. neg1* capable of degrading ochratoxin A. FEMS Microbiol Lett. 2015; 362(7);
2. Goff SA et al., The iPlant Collaborative: Cyberinfrastructure for Plant Biology; http://www.iplantcollaborative.org. Front Plant Sci. 2011; 2:34;
3. Maere S, Heymans K, Kuiper M, BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. Bioinformatics. 2005; 21(16):3448-9.

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