

Detection and Profiling of Diarrhetic Marine Biotoxins in Shellfish by mRNA Analysis of Exposed Caco-2 Cells Using qRT-PCR and Multiplex Magnetic Bead-Based Assays

Supplementary Data

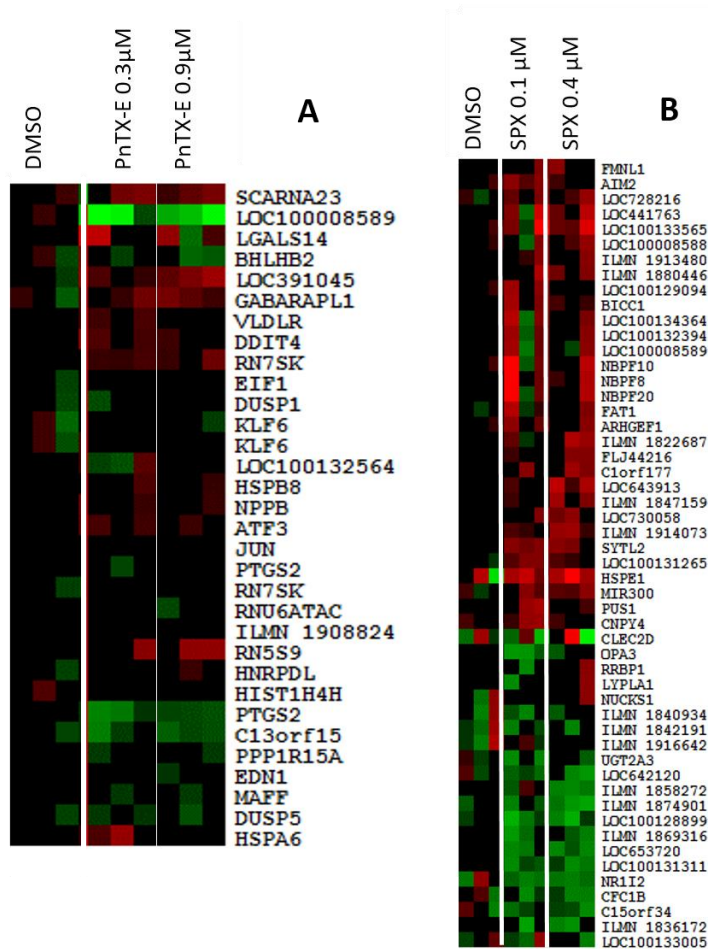


Fig. S1: Hierarchical clustering of responsive genes in Caco-2 cells from Illumina microarrays using the programs Cluster (uncentered correlation, average linkage clustering) and Treeview (Eisen et al., 1998)
 (A) Exposure to PnTX-E. Gene selection is based on an average fold change of ≥ 2 (\log_2 ratio $\geq |1.0|$) in at least 3 of 15* microarrays. Red indicates upregulation, green indicates downregulation and black not affected. A maximal red or green color indicates 2 times up or down regulation versus the control. *Six Microarrays of the non-related compound palytoxin (PITX) were deleted from the figure, as this compound is already cytotoxic at low concentrations (pM range) resulting in strong upregulation of cognate genes. (B) Exposure to SPX. Gene selection is based on an average fold change of ≥ 1.4 (\log_2 ratio $\geq |0.5|$) in at least 2 of 9 microarrays. A maximal red or green color indicates 1.4 times up or down regulation.

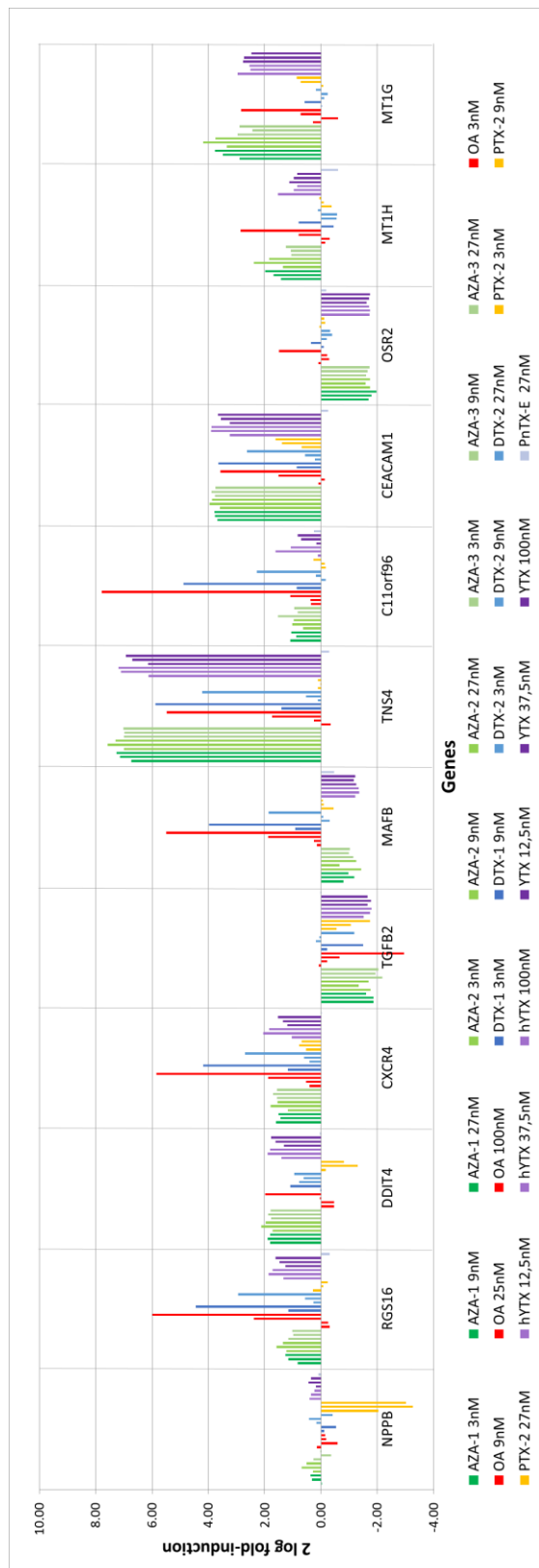


Fig. S2: Multiplex magnetic bead-based assay

Caco-2 cells were exposed to OA, DTX-1, DTX-2, AZA-1, AZA-2, AZA-3, YTX, hYTX, PTX-2, and PnTX-E. Bars represent log₂ of fold-induction values of each of the 12 genes. Positive values represent upregulation, negative values represent downregulation.

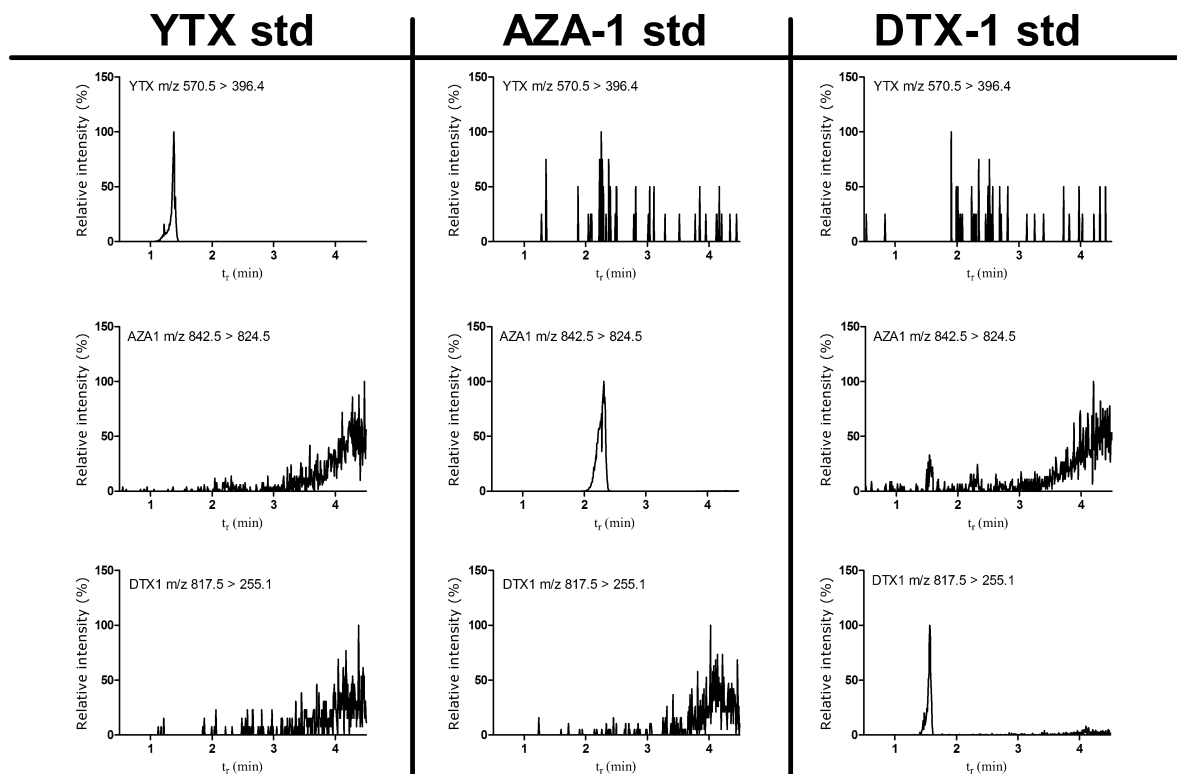


Fig. S3: Chromatograms obtained by LC-MS/MS analysis (Gerssen et al., 2010) of the individual YTX, AZA-1, and DTX-1 standards in DMSO

Caption Figure S6. 20 Additional blank samples tested in the multiplex magnetic bead-based assay. Bars represent log₂ of fold-induction values of each of the 14 genes. Positive values represent upregulation, negative values represent downregulation.

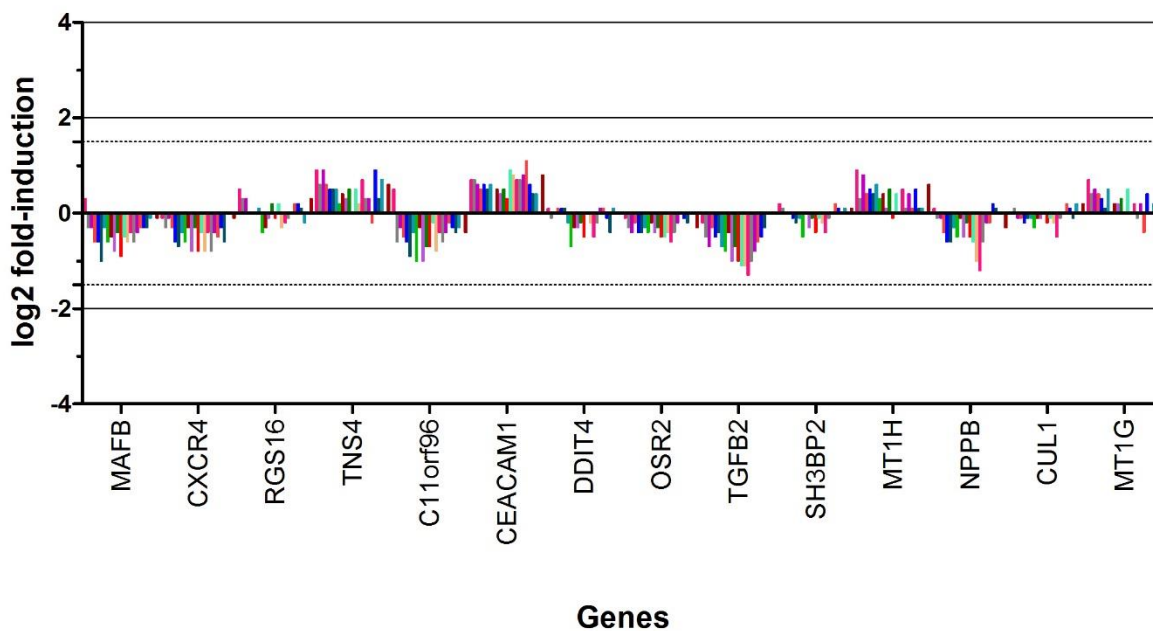


Fig. S4: 20 Additional blank samples tested in the multiplex magnetic bead-based 674 assay

Bars represent log₂ of fold-induction values of each of the 14 genes. Positive values represent upregulation, negative values represent downregulation.

Tab. S1: Expected vs obtained results for qPCR with analogues (expected results are the same included in Table 2)

Red arrows represent genes that are upregulated with log2 values higher than 0.7; green arrows are genes downregulated with log2 values lower than -0.7. (*) Represent up regulation higher than log2 value 2.0 or down regulation lower than -1.5. (-) Represent log2 values between -0.4 and 0.4, which is considered no effect.

Gene	Toxin analogue				
	AZA-1	OA	DTX-1	YTX	PTX-2
NPPB	-	-	↓*	-	↓*
RGS16	-	↑	↑*	-	-
DDIT4	↑*	↑	↑	↑	↓
CXCR4	-	↑*	↑*	-	-
TGFB2	↓	↓	↓	-	-
TMEM179B	-	-	-	-	-

PCR	Toxin analogue								
	AZA-1	AZA-2	AZA-3	OA	DTX-1	DTX-2	YTX	hYTX	PTX-2
NPPB	-	-	-	-	-	-	-	-	↓*
RGS16	-	-	-	↑*	↑*	-	-	-	-
DDIT4	↑*	↑*	↑*	-	-	-	-	↑*	↓
CXCR4	↑*	↑*	↑*	↑*	↑*	-	↑*	↑*	-
TGFB2	↓*	↓*	↓*	↓*	↓*	-	↓*	↓*	↓*
TMEM179B	-	-	-	-	-	-	-	-	-

Tab. S2: Expected vs obtained results for multiplex magnetic bead-based assay (expected are the same in Table 2)

Red arrows represent genes that are upregulated with log2 values higher than 0.7; green arrows are genes downregulated with log2 values lower than -0.7. (*) Represent up regulation higher than log2 value 2.0 or down regulation lower than -1.5. (-) Represent log2 values between -0.4 and 0.4, which is considered no effect.

Gene	Toxin analogue				
	AZA-1	OA	DTX-1	YTX	PTX-2
NPPB	-	-	↓*	-	↓*
RGS16	-	↑	↑*	-	-
DDIT4	↑*	↑	↑	↑	↓
CXCR4	-	↑*	↑*	-	-
TGFB2	↓	↓	↓	-	-
MAFB	-	↑*	↑*	-	-
TNS4	↑	-	↑	-	-
C11orf96	-	↑*	↑*	ND	ND
CEACAM1	↑	↑	↑	↑*	-
OSR2	↓	-	↑	-	-
MT1H	↑*	↑	↑	-	-
MT1G	↑*	-	↑	↑	-
TMEM179B	-	-	-	-	-
CUL1	-	-	-	-	-
SH3BP2	-	-	-	-	-

Gene	Toxin analogue									
	AZA-1	AZA-2	AZA-3	OA	DTX-1	DTX-2	YTX	hYTX	PTX-2	PnTX-1
NPPB	-	-	-	-	-	-	-	-	↓*	-
RGS16	-	-	-	↑*	↑*	-	-	-	-	-
DDIT4	-	↑*	-	-	-	-	-	-	-	-
CXCR4	-	-	-	-	↑*	-	-	-	-	-
TGFB2	↓*	-	↓*	-	↓*	-	↓*	↓*	-	-
MAFB	-	-	-	-	↑*	-	-	-	-	-
TNS4	↑*	↑*	↑*	-	↑*	-	↑*	↑*	-	-
C11orf96	-	-	-	-	↑*	-	-	-	-	-
CEACAM1	↑*	↑*	↑*	-	↑*	-	↑*	↑*	-	-
OSR2	↓*	↓*	↓*	-	-	-	↓*	↓*	-	-
MT1H	-	↑*	-	-	-	-	-	-	-	-
MT1G	↑*	↑*	↑*	-	-	-	↑*	↑*	-	-

References

- Eisen, M. B., Spellman, P. T., Brown, P. O. and Botstein, D. (1998). Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci U S A* 95, 14863-14868. doi:10.1073/pnas.95.25.14863
- Gerssen, A., van Olst, E. H., Mulder, P. P. and de Boer, J. (2010b). In-house validation of a liquid chromatography tandem mass spectrometry method for the analysis of lipophilic marine toxins in shellfish using matrix-matched calibration. *Anal Bioanal Chem* 397, 3079-3088. doi:10.1007/s00216-010-3886-2