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Sodium hypochlorite stimulates biofilm formation in *Pseudomonas aeruginosa*

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Introduction

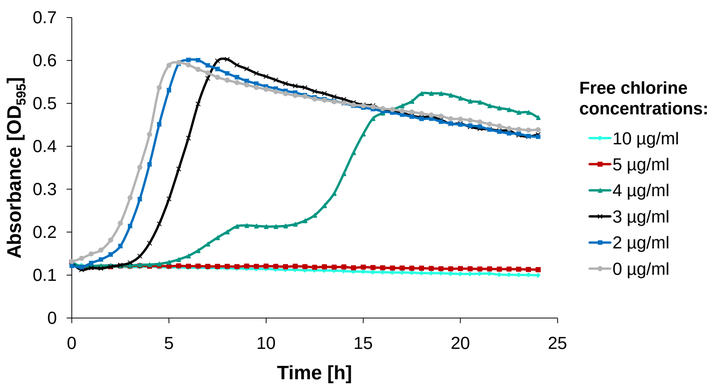
Pseudomonas aeruginosa is an important opportunistic human pathogen which is involved in about 10 % of hospital infections and is the major cause of chronic lung infections in cystic fibrosis patients. This motile Gram-negative bacterium is able to survive under a variety of often harmful environmental conditions due to a multitude of intrinsic and adaptive resistance mechanisms, including biofilm formation as one important defense strategy [1].
It has been shown recently that *P. aeruginosa* biofilm formation can be both stimulated and inhibited by sublethal concentrations of specific antimicrobial substances such as aminoglycosides or antimicrobial peptides [2;3]. The aim of this study was to investigate stress response and biofilm development of *P. aeruginosa* to the commonly used disinfectant sodium hypochlorite which is frequently utilized for surface sterilization and drinking water treatment.

Conclusions

- Sublethal concentrations of sodium hypochlorite stimulate biofilm formation in *P. aeruginosa* PAO1.
- Increase in biomass of pregrown *P. aeruginosa* biofilms could be observed after incubation with sodium hypochlorite.
- Microarray analysis shows upregulation of genes involved in biosynthesis of flagella, pili and exopolysaccharides which are important for initial attachment and subsequent biofilm formation.

Results & Methods

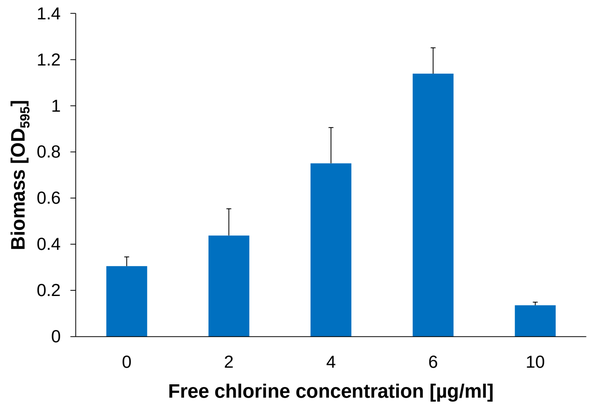
Growth of *P. aeruginosa* in the presence of sodium hypochlorite



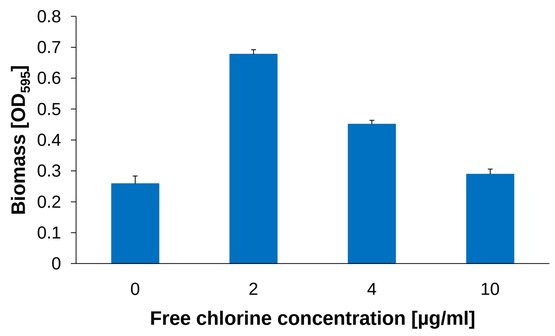
P. aeruginosa PAO1 cultures were grown in 96-well microtiter plates under shaking at 37°C in minimal medium BM2 containing different concentrations of sodium hypochlorite.

Sodium hypochlorite treatment increases biomass of pregrown biofilms

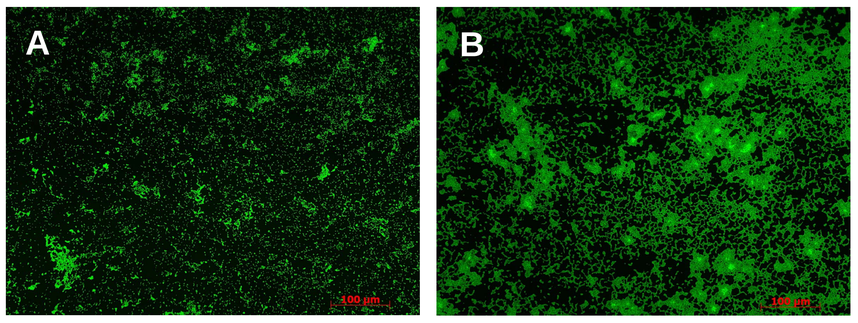
P. aeruginosa PAO1 biofilms were pregrown in 96-well microtiter plates for 24 h at 37°C followed by the addition of sodium hypochlorite in fresh BM2 medium and an additional 24 h incubation time. Biofilm formation was determined by crystal violet staining.



Subinhibitory concentrations of sodium hypochlorite stimulate attachment of *P. aeruginosa* PAO1

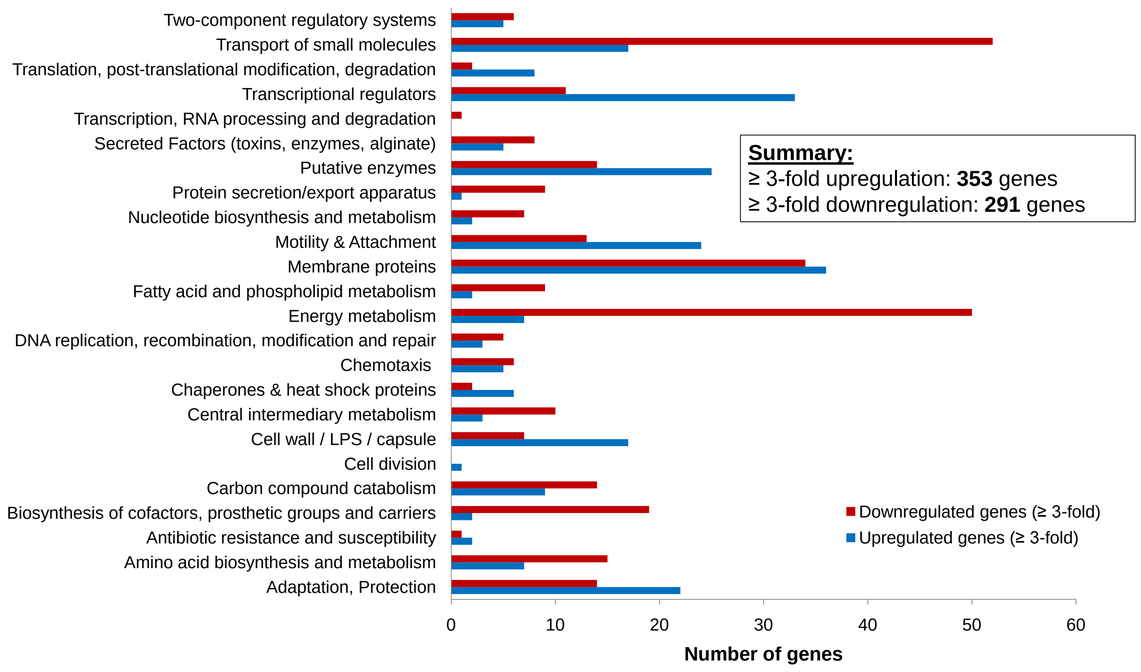


Overnight cultures of *P. aeruginosa* PAO1 grown in minimal medium BM2 were washed and diluted to an optical density (OD_{595}) of 0.2 followed by incubation in 96-well microtiter plates for 2 h at 37°C with different concentrations of sodium hypochlorite. Early biofilm formation was subsequently visualized by crystal violet staining.



P. aeruginosa PAO1 cells attached to a glass microscope slide during 2 h of incubation at 37°C under static conditions in BM2 medium without (A) or with the addition of sodium hypochlorite at free chlorine concentration of 2 µg/ml (B). After a washing step the cells attached to the surface were fixed with 3 % formaldehyde and stained with SYTO9 followed by visualization using fluorescence microscopy.

Microarray analysis shows altered gene expression after incubation with sodium hypochlorite



Selected upregulated genes in the presence of subinhibitory concentrations of sodium hypochlorite

Public ID	Gene designation	Fold change	Gene function
PA1077 – PA1084 PA1098 – PA1099 PA1100 – PA1101 PA1443 – PA1445 PA1452 – PA1453	*flgB, flgC, flgD, flgE, flgF, flgG, flgH, flgI* *fleS, fleR* *fliE, fliF* *fliM, fliN, fliO* *flhA, flhF*	4 – 11 6 – 15 7 – 11 3 – 4 12 – 13	Flagella assembly
PA4550 – PA4553	*fimU, pilV, pilW, pilX*	3 – 5	Type IV pili biosynthesis (twitching motiliy)
PA4304 – PA4306	*rcpA, rcpC, flp*	3 – 5	Type IVb pili assembly
PA0762 – PA0764 PA5261 PA5483	*algU, mucA, mucB* *algR* *algB*	4 – 6 4 4	Regulation of alginate production
PA2849 – PA2850 PA2825	*ohrR, ohr* *ospR*	66 – 100 80	Transcriptional regulators; response to oxidative stress

P. aeruginosa PAO1 cultures in BM2 medium containing sodium hypochlorite (2 µg/ml free chlorine) were incubated for 1 h at 37°C under static conditions. RNA was isolated and used for microarray analysis; RNA of untreated bacteria served as the control. The figure shows genes with a greater than three-fold up- or downregulation categorized according to their functional classes. Genes with unknown function are not displayed.

References:
[1] *Drug Resist Updat* (2000);3(4):247-255.
[2] *Nature* (2005); 436(7054):1171-5.
[3] *Infect Immun* (2008);76(9):4176-82.

Acknowledgements:
The Research Group *Bacterial Stress Response and Process Engineering* is financed by the concept for the future of the Karlsruhe Institute of Technology (KIT) within the German Excellence Initiative.

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