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SPECIFIC AND GLOBAL STRESS RESPONSES IN BACTERAL POPULATIONS

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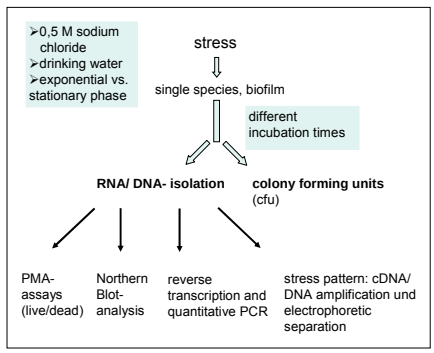
BACKGROUND AND METHODIC APPROACH

Bacteria are exposed to various stresses in the natural environment. The aim of our work was to study specific and global stress responses to starvation, osmotic changes and increasing densities of hygienic relevant bacteria.
Generally, we investigated global parameters like total RNA amount, viability via counts and DNA contents from living bacteria.
More specifically gene expression was quantificated during stress by Real-Time-PCR and Nothern Blot analysis in reference bacteria. The sigma factor *rpoS* of *P. aeruginosa*, *rcsA*, a member of the Rcs phosphorelay of *E. coli* and the penicillin binding protein (*pbp5*) of *E. faecium* and *E. faecalis* participating in peptidoglycan synthesis are the selected gene targets. The expression pattern of these genes was compared with the expression of *usp* (universal stress protein) as a common putative marker of stress.
As fingerprint technique for detection of non gene directed stress patterns we used RAPD-PCR (randomly amplified polymorphic DNA-PCR).

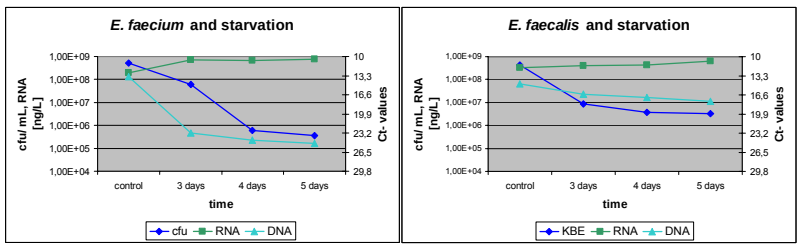


RESULTS

A) Physiology

➢ Enterococci showed an **increase in total RNA** (2-4 fold) but **a decrease in colony counts (cfu)** (1-3 fold) during starvation and osmotic shock.

➢ Decreasing numbers of cultivable *E. faecalis* and nearly constant DNA contents from living bacteria of the culture indicate a **VBNC-state** (viable but nonculturable).



B) Species specific and universal stress genes

	stationary phase		starvation		osmotic shock	
	usp	specific	*usp*	specific	*usp*	specific
P. aeruginosa	induction	√ (*rpoS*)	induction	√ (*rpoS*)	induction	√ (*rpoS*)
E. coli	induction	√ (*rcsA*)	repression	~~√ (*rcsA*)~~	induction	√ (*rcsA*)
E. faecalis	induction	√ (*pbp5*)	repression	√ (*pbp5*)	repression	√ (*pbp5*)

A) Species specifc genes

➢ **Induction** of the master regulator of stress, ***rpoS***, and ***rcsA*** which is involved in biofilm formation during all stress exposures.

➢ **Repression of *pbp5***, which might be a marker for VBNC-state during starvation and osmotic stress, but an **induction during stationary growth phase.**

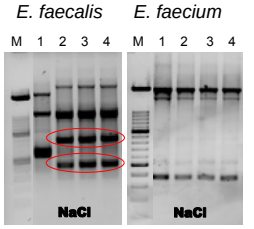
➢ During **biofilm formation** there was an induction of ***rpoS*** and ***rcsA*** participating in EPS synthesis (extracellular polymeric substances) in biofilms.

B) Universal stress protein (*usp*)

➢ ***usp*** seems to be a **suitable stress marker** with one exception (see *rcsA* and starvation).

D) Global stress response (RAPD-PCR)

For differentiation of physiological states we used cDNA and DNA as template.



NaCl: sodium chloride, DW: drinking water, M: marker, control, 2: after 3 days, : after 4 days, 4: after 5 days

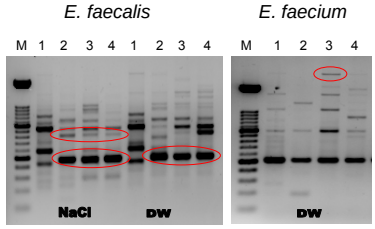
Transcriptom analysis

➢ **Gram- negative bacteria** showed distinctions after treatment with **sodium chloride** (data not shown).

➢ *E. faecalis* had a stress response on osmotic shock and starvation while *E. faecium* showed different band patterns only at starvation, indicating **a stronger stress adaption of *E. faecium*** (Fig. right).

Genome analysis

➢ These findings were corroborated by using DNA as a template indicating that ***E. faecalis*** is **more sensible to osmotic stress than *E. faecium*** (Fig. left).



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CONCLUSION AND OUTLOOK

Impacts on defined bacterial populations and their responses to starvation and osmotic shock were detected with the demonstrated methods. RAPD-PCR seems to be a promising technique for detection of stress patterns without investigation of specific genes. Further investigations of natural bacterial populations will lead to assessment concept of physiological states and to potential measures for manipulating biofilms in technical processes.