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Karlsruhe Institute of Technology

Institute of Functional Interfaces (IFG)
Microbiology of Natural and Technical Surfaces Department

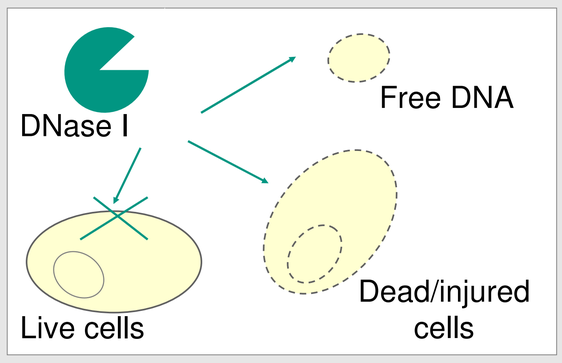
Live/Dead Discrimination of Bacteria via DNase/Proteinase Treatment

Jéssica Varela Villarreal, Christina Jungfer, Ursula Obst, and Thomas Schwartz

E-mail: jessica.villarreal@kit.edu

INTRODUCTION

DNA-based molecular biology techniques are very sensitive, but have some limitations to discriminate DNA coming from **live**, **injured,** and **dead** cells as well as **extracellular DNA** (eDNA) in natural and technical systems. DNase I is an endonuclease that non-specifically cleaves single and double stranded DNA.
DNase I combined with proteinase K (PK) treatment (DNase/PK) was tested in order to analyze its capacity of digesting available DNA (eDNA and DNA from cells with damaged cell membranes), leaving DNA from live and VBNC cells unaffected and available for DNA-based methods.



POSSIBLE APPLICATIONS

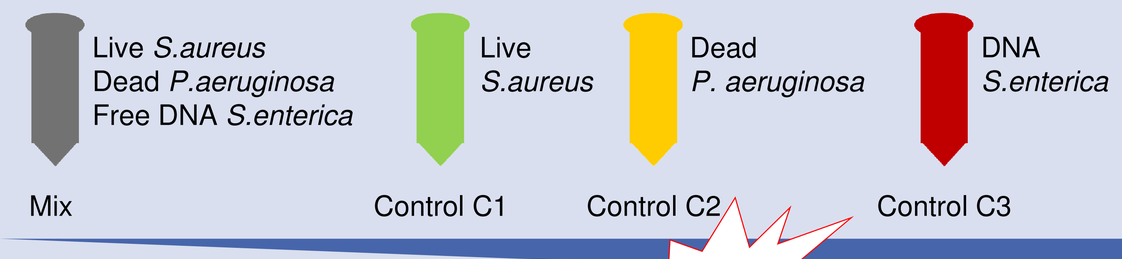
Analysis of live bacterial fraction at the: • food industry, • biomedical industry, • pharmaceutics industry, • cosmetic industry, and for the • analysis of clinical samples.

CONCLUSIONS

- DNA from dead/injured bacteria and eDNA was blocked or digested by propidium monoazide (PMA) or DNase/PK treatment, respectively.
- DNase/PK treatment demonstrated a more distinct effect on live/dead differentiation as PMA, due to **no loss of sample**.
- DNase/PK was successfully applied to characterize **live** bacteria from drinking water biofilms at a German waterworks.

EXPERIMENTAL APPROACH

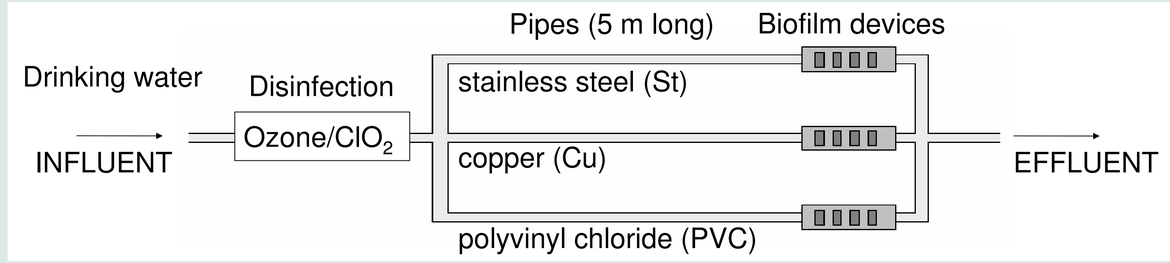
LIVE/DEAD DISCRIMINATION ASSAY



Treatment	No treatment	DNase⁺/PK	PMA⁺	DAPI	R2A
		1. Digestion of DNA: Addition of DNase I ; 37 °C x 10 min 2. DNase I inactivation: Addition of proteinase K; 57 °C x 1 h 3. Proteinase K inactivation: 90 °C x 10 min	1. DNA intercalation: Addition of PMA; 21 °C x 15 min (dark) 2. Covalent crosslinking: 8 min light exposure 3. 2 x wash step (or DNA purification)		
Detection methods	q-PCR and PCR-DGGE			Microscopy	Plating technique
Physiological stage	Live + Dead + DNA	Live	Live	Live + Dead	Cultivable

No loss of sample!

APPLICATION AT GERMAN WATERWORKS



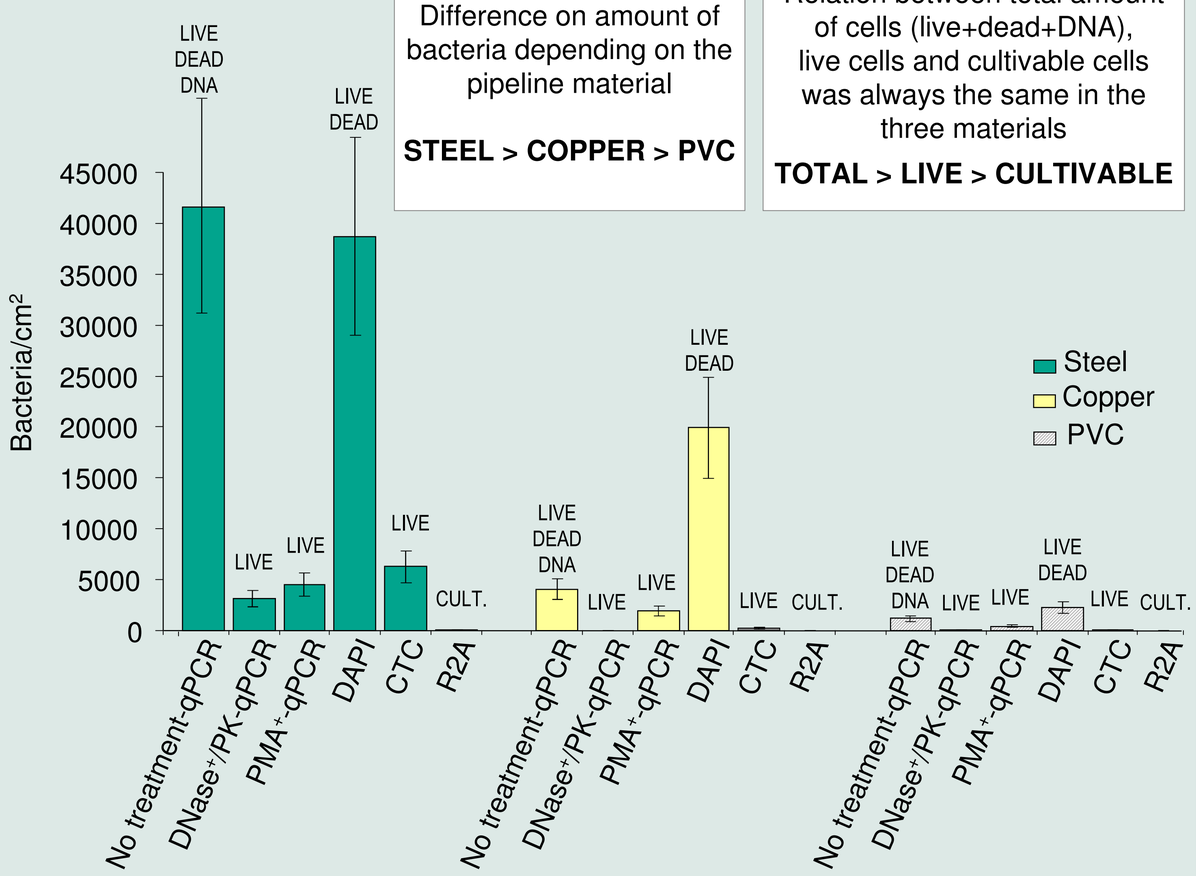
RESULTS

PCR-DGGE

	S. aureus (live)		*P. aeruginosa* (dead)		*S. enterica* (free genomic DNA)	
	theory	empiric	theory	empiric	theory	empiric
Mix - No treatment	+	+	+	+	+	+
	NO LIVE bacteria determined !!! ☹					
Mix - PMA	+	-	-	-	-	-
					Loss of DNA !!! ☹	
Mix - Control w.o. PMA	+	+	+	+	+	-
	Only LIVE bacteria determined !!! ☺					
Mix - DNase/PK	+	+	-	-	-	- ✓
	NO LOSS of sample !!! ☺					
Mix - Control w.o. DNase	+	+	+	+	+	+ ✓
C1 live - No treatment	+	+	-	-	-	-
C2 dead - No treatment	-	-	+	+	-	-
C3 DNA - No treatment	-	-	-	-	+	+

DNase/PK treatment was the best method for live/dead differentiation of bacteria in drinking water !

w.o.: without



Difference on amount of bacteria depending on the pipeline material

STEEL > COPPER > PVC

Relation between total amount of cells (live+dead+DNA), live cells and cultivable cells was always the same in the three materials

TOTAL > LIVE > CULTIVABLE