

Live/Dead Discrimination of Bacteria via DNase/Proteinase Treatment

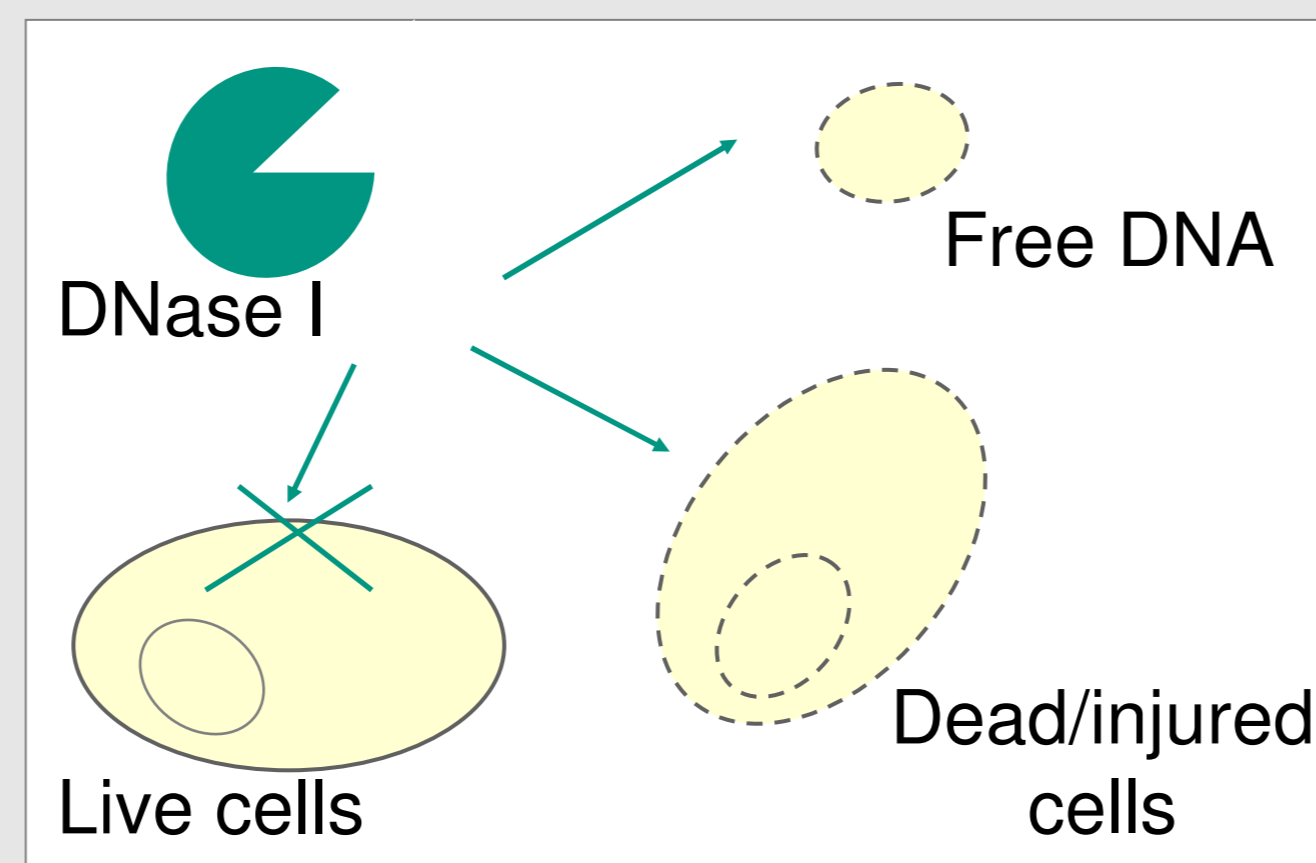
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INTRODUCTION

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

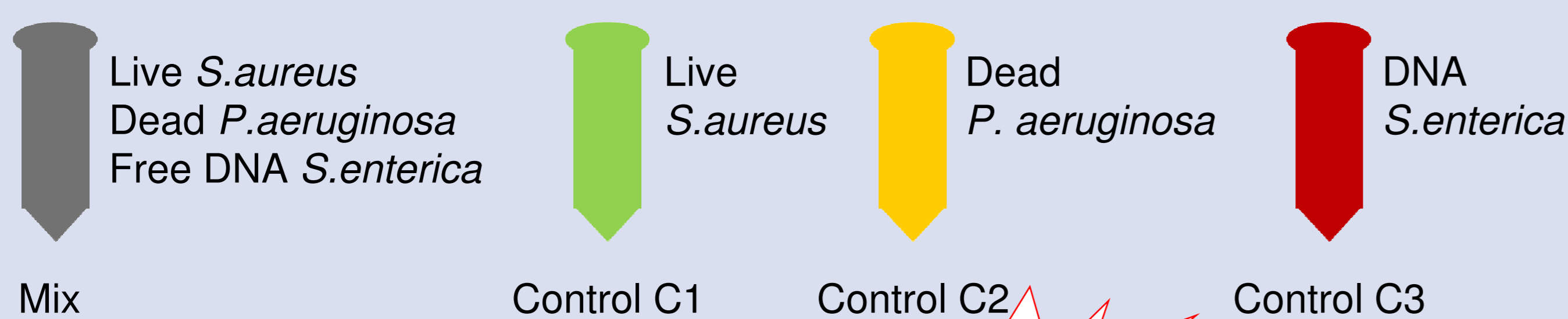
Analysis of live bacterial fraction at the: • food industry, • biomedical industry, • pharmaceuticals industry, • cosmetic industry, and for the • analysis of clinical samples.

CONCLUSIONS

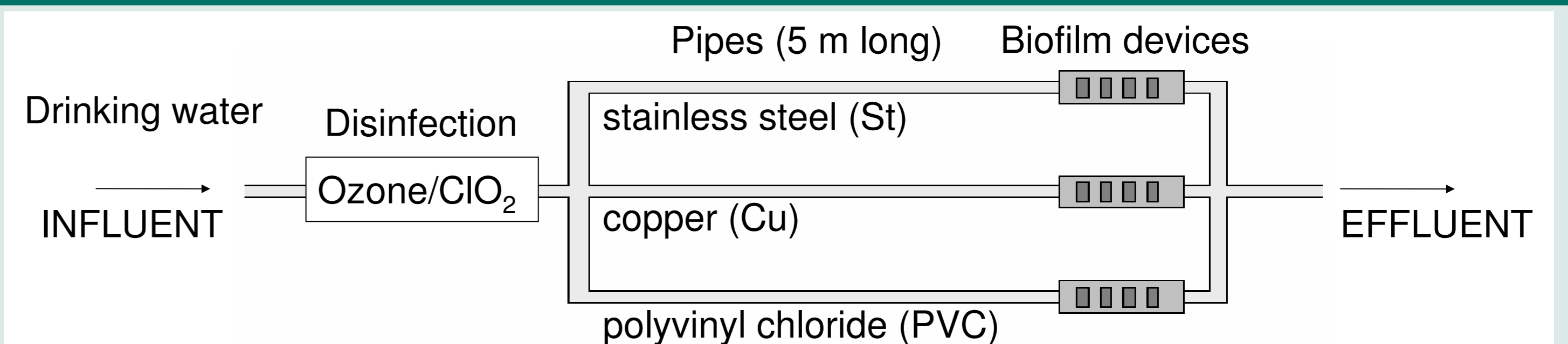
- DNA from dead/injured bacteria and eDNA was blocked or digested by propidium monoazide (PMA) or DNase/PK treatment, respectively.
- DNase/PK treatment demonstrated a more distinct effect on live/dead differentiation as PMA, due to **no loss of sample**.
- DNase/PK was successfully applied to characterize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

EXPERIMENTAL APPROACH

LIVE/DEAD DISCRIMINATION ASSAY



APPLICATION AT GERMAN WATERWORKS



Treatment	No treatment	DNase+/PK	PMA+	DAPI	R2A
		<p>No loss of sample!</p> <ol style="list-style-type: none"> Digestion of DNA: Addition of DNase I ; 37°C x 10 min DNase I inactivation: Addition of proteinase K; 57°C x 1 h Proteinase K inactivation: 90°C x 10 min 	<ol style="list-style-type: none"> DNA intercalation: Addition of PMA; 21°C x 15 min (dark) Covalent crosslinking: 8 min light exposure 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

RESULTS

PCR-DGGE

	<i>S. aureus</i> (live)		<i>P. aeruginosa</i> (dead)		<i>S. enterica</i> (free genomic DNA)	
	theory	empiric	theory	empiric	theory	empiric
Mix - No treatment	+	+	+	+	+	+
Mix - PMA	+	-	-	-	-	-
Mix - Control w.o. PMA	+	+	+	+	+	-
Mix - DNase/PK	+	+	-	-	-	-
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

