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Culture-independent techniques applied to food industry water surveillance

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Introduction

Drinking water that comes from the public suppliers is not sterile, it contains a number of autochthonous and mostly harmless bacteria. But, pathogenic or opportunistic bacteria may enter drinking water facilities in case of irregular operating conditions. In this case, some of these bacteria are able to persist and become distributed to food production. Our two main objectives in PathogenCombat are (i) the detection of hygienic relevant bacteria in the water and (ii) identification of possible water-associated Critical Control Points (CCPs) in food production at two German food industries. For this purpose new culture-independent techniques were applied.

Materials and Methods

1.Targeted hygienic relevant bacteria:

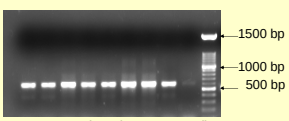
- *Listeria monocytogenes*
- *Mycobacterium avium subsp. paratuberculosis*
- *Campylobacter jejuni*
- *Enterococcus ssp*
- *Salmonella ssp*
- *Escherichia coli*
- *Pseudomonas aeruginosa*

2. Culture-independent molecular biology methods

2.1. Detection and identification of bacteria:

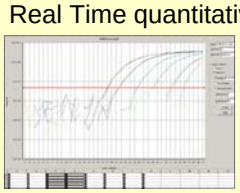
- Polymerase Chain Reaction (PCR)

Amplification of specific DNA-fragments



PCR-Product (Agarose gel)

- Real Time quantitative PCR

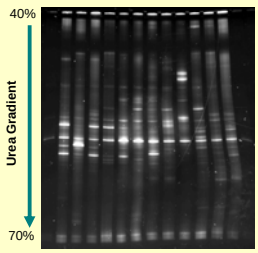


Amplification and quantification of specific DNA-fragments

2.2. Analysis of autochthonous drinking water bacterial populations

Detection of possible CCPs in drinking water distribution systems of food industries:

- Polymerase Chain Reaction (PCR)
- Denaturing Gradient Gel Electrophoresis (DGGE)



- Sørensen Similarity Index (Dice Coefficient):
  Is a statistic used to compare the similarity of two samples.

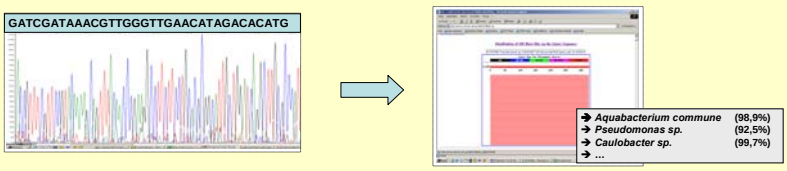
$$C_S = 2*j / (a+b)$$

Where:
j = Number of same bands between sample A and B
a= Number of bands in sample A
b= Number of bands in sample B

This index ranges from 0 (no similarity) to 1 (100% similarity of band patterns)

Characterization of the autochthonous bacterial population

- DNA sequencing and comparison in DNA data banks



Results

Specific Detection of hygienic relevant bacteria

Most hygienic relevant bacteria were not detected, but weak PCR signals at the detection limits were obtained for *P.aeruginosa,* in only two samples.

Analysis of autochthonous drinking water bacterial populations

Detection of possible CCPs in drinking water distribution systems of food industries:

Samples from dairy industry	Similarity with reference public water	Samples from poultry industry	Similarity with reference public water
2. Lactic acid tank	56%	2.Slaughter room	40%
3. Portioner	40%	3. Sausage production room	44%
4. Hand wash basin	42%	4. Preparation room	61%
5. Maturation room	42%	5. Preparation sink	42%
6. Feta packaging	29%	6.Hygienic sluice	20%
		7. Slaughter sink	43%

A similarity of more than 40% is normal for German drinking water distribution systems

Characterization of the autochthonous bacterial population:

Samples Dairy Industry	Bacterial sub-classes		
	α-Proteobacteria	*ß-Proteobacteria*	*γ-Proteobacteria*
1. Entry of state water	25%	56%	-
2. Lactic-acid tank	27,7%	61,1%	5,5%
3. Portioner	44,4%	27,7%	25%
4. Hand wash-basin	27,7%	44,4%	-
5. Maturation room	41,6%	33,3%	-
6. Feta packaging	56,2%	25%	-

PCR-DGGE profiling together with **sequence analysis** is a good method for the identification of Critical Control Points.

Conclusions

The water of two German food industries seems to possess a high quality in concern of the mentioned pathogens. Nevertheless, shifts were observed within autochthonous populations during water distribution, indicating one possible critical control point in each food industry. Culture-independent techniques are very useful for the identification of bio-hazards and Critical Control Points at all stages of food production, where water is involved.

Acknowledgements

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