Deltares

Enabling Delta Life

Molecular detection

Detection of microbial activity

Which bacteria are where and what are they doing? A quick, specific and sensitive detection of microorganisms and their activity is essential to understand and influence the functioning in the environments.

Application

Deltares develops and applies molecular methods for the detection of microorganisms. Examples of these methods are:

- Monitoring of water quality of industrial production processes and (waste) water treatment;
- Control of biofilm formation and corrosion in the water of cooling towers, pinelines and process installations;
- Monitoring of biological degradation in contaminated soils, dumping sites, rivers and seawater.

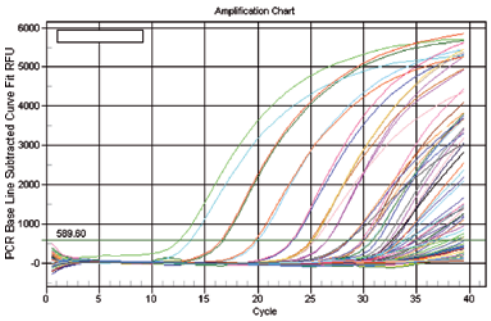

Figure 1. Quantitative real-time PCR of 16S rRNA of Dehalococcoides sp. (dechlorinating bacteria)

Quick detection

Classical detection methods are usually based on the cultivation of bacteria in the laboratory. However, less than one percent of the bacteria that are present in water, soil and sediment can be cultivated in a laboratory (Table 1).

Table 1. Cultivation as percentage of total bacteria (Aman, 1995)

Habitat	Culturability (%)*
Seawater	0.001-0.1
Coastal water	0.1-3
Freshwater	0.25
Freshwater lake	0.1-1
Sediment	0.25
Soil	0.3

* Culturable cells are expressed as colony forming units

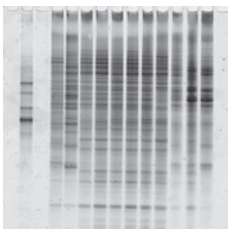

Figure 2. Denaturing Gradient Gel Electroforeses (DGGE) of bacterial communities in a river basin profile

Modern molecular technologies are extremely suitable to unravel the diversity of complex microbial ecosystems. Molecular techniques are a powerful tool for an efficient diagnosis, especially when used in combination with traditional methods, such as microscopy, cultivation in artificial medium, liquid- and gaschromatography and chemical analyses.

Molecular detection is:

- Detection of genetic information;
- Quick, specific and sensitive.

Molecular detection answers questions, such as:

- Which and how many bacteria are present?
- Are pathenogenic microorganisms or pollutant degrading bacteria present?
- Which microorganisms are active and which processes are catalyzed by them?

Table 2. Application of various molecular technologies

Microbial groups		
Detection	**Target gene**	**Remark**
Total Bacteria	Bacterial 16S rRNA	
Total Archaea	Archeal 16S rRNA	
Denitrifying bacteria	Nitrate reductase (NarG)	$NO_3 \rightarrow NO_2$
Denitrifying bacteria	Nitrite reductase (NirS or NirK)	$NO_2 \rightarrow NO$
Denitrifying bacteria	Nitrous oxide reductase (NosZ)	$N_2O \rightarrow N_2$
Iron-reducing bacteria	Geobacteriales 16S rRNA	
Sulphate-reducing bacteria	Sulfite-reductase (dsrA or dsrB)	
Microbial corrosion	Fe-hydrogenase (hydA) NiFeSe-hydrogenase (hysA)	
Methanogenic Archaea	Methyl-CoM reductase	

Pathogenic bacteria		
Detection	**Target gene**	**Remark**
Cyanobacteria	Cyanobacteria 16S rRNA Microcystis 16S rRNA McyD toxine	
Legionella sp.	5s rRNA mipA	Legionella group Legionella pneumophila
Enterobacteriaceae	Enterobacteriaceae 16S rRNA	Indicator of faecal contamination
Eschericha coli	Glucuronidase	Indicator of pathogenic faecal contamination

Biological transformation of pollutants		
Detection	**Target gene**	**Remarks**
Mineral oil*	Alkane mono-oxygenase (alkA)	Alkane hydroxylation
BTEX*	Catechol-1,2-dioxygenase Catechol-2,3-dioxygenase	Aerobic ring-cleavage
TEX	Benzylsuccinyl-CoA synthase (BssA)	Anaerobic ring-cleavage
MTBE	MTBE mono-oxygenase (MM) Isobutyryl-CoA mutase (ICMA)	MTBE → TBA Degradation of 2-HIBA
1,2-dichloroethane	Alkane dehalogenase (dhlA)	Aerobic dechlorination
	16S rRNA specific for Dehalococcoides, Desulfitobacterium and/or Dehalobacter	Anaerobic dechlorinating bacteria
PCE & TCE	16S rRNA specific for Dehalococcoides, Desulfitobacterium, Sulfurospirillum, Dehalobacter, Desulfuromonas and/or Desulfomonile	Anaerobic dechlorinating bacteria
TCE	Trichloroethene reductase (TceA)	TCE → cis-DCE
Cis-DCE & VC	Dehalococcoides spp. 16S rRNA	Anaerobic dechlorinating bacteria
VC	Vinylchloride reductase (vcrA and/or bvcA)	Anaerobic, VC → ethene
VC	Expoxyalkane coenzyme M transferase (EaCoMT)	Aerobic VC degradation

* In development

Application for various microbial ecosystems:

- Contaminated soils and groundwater;
- Dumping sites;
- Biofilms and biocorrosion, e.g. cooling towers, pipelines and mebranes;
- (Waste)water treatment systems, e.g. active sludge;
- Industrial process installations, e.g. in food industries.

Available expertise and facilities at Deltares

- Extraction of bacterial DNA and RNA from a variety of matrices (soil, water, biofilms);
- FISH (Fluorescense In Situ and RNA Hybridisation) analyses for detection and quantification of specific bacteria;
- (Q)PCR (Polymerase Chain Reaction) detection of microorganisms (Table 2) through amplification of specific genes, eg. 16S rRNA (Fig.1);
- DGGE (Denaturing Gradient Gel Elektroforese) analyses to unravel the diversity of microbial populations (Fig. 2);
- Detection of microbial activity (eg. enzyms, ATP);
- Laboratory for general microbial research and microbial analyses (column experiments, batches and continuous cultures);
- Pilot-scale reactor systems for water treatment;
- Up-to-date knowledge of microbial ecology, environmental analyses, experience with software and data-bases for molecular identification.

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Deltares is a leading, independent, Dutch-based research institute and specialist consultancy for matters relating to water, soil and the subsurface. We apply our advanced expertise worldwide, to help people live safely and sustainably in delta areas, coastal zones and river basins.