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Synthetic DNA tracers: examples of their application in water related studies

IFTIKHAR H. SABIR, SYLVI HALDORSEN

Geology and Water Section, Department of Soil and Water Sciences, Agricultural University of Norway, PO Box 5028, N-1432 Ås, Norway

e-mail: iftikhar.sabir@ijvf.nlh.no

JACOB TORGERSEN, PETER ALESTRÖM*

Department of Biochemistry, Physiology and Nutrition, Norwegian School of Veterinary Sciences, PO Box 8146 Dep., N-0033 Oslo, Norway

SYLVI GAUT

Geochemistry and Hydrogeology Section, Geological Survey of Norway (NGU), N-7491 Trondheim, Norway

HERVÉ COLLEUILLE, TOR SIMON PEDERSEN

Norwegian Water and Energy Directorate (NVE), Hydrology Department, Middelthunsgate 29, PO Box 5091 Majorstua, N-0301 Oslo, Norway

NILS-OTTO KITTERØD

Department of Geophysics, University of Oslo, PO Box 1022 Blindern N-0315 Oslo, Norway

Abstract Distinguishing between different sources of pollution is a fundamental yet poorly characterized aspect of contaminant transport studies. Field experiments in porous media and fractured hard rocks were undertaken to test the transport of innovative synthetic DNA tracers which have an information capacity and extremely sensitive detection limit. Discrimination of different DNA tracers indicated that these are very useful tools that can lead to unprecedented possibilities for detection of individual tracers emerging from different sources and assist in many aspects of contaminant transport studies. The DNA tracers are well suited for quantifying groundwater dynamics, generally in sandy material and particularly in very complex fractured rock systems.

INTRODUCTION

The widespread release of various pollutants in the subsurface, and the problems of distinguishing between the sources of pollution, have increased the need for a broader spectrum of environmentally safe tracers. Synthetic DNA molecules have been shown to be good candidates as intelligent tracers (Sabir *et al.*, 1999). The intrinsic information-coding capacity allows discrimination between two or more DNA tracers coming from different sources or released at different times. Alphanumeric information like names, dates, batch numbers, etc. can easily be defined as a DNA sequence (Alestrom, 1995). An almost unlimited number of DNA tracers with

* *Also at:* ChemTag AS, c/o Det Norske Veritas, N-1322 Høvik, Norway

different sizes and different valences can be designed and produced by automated standard oligonucleotide synthesis and applied to the substance to be labelled. The message of the DNA tracers can be detected and "read" by using the polymerase chain reaction (PCR) method alone, or combined with DNA sequence analysis (Aleström, 1995). The PCR method is based on repeated cycles of DNA replication *in vitro* and is very sensitive, with a theoretical detection limit of as low as one DNA molecule per sample. These special traits make DNA tracers highly competitive relative to the other available tracers. Because of the high sensitivity, care has to be taken when handling the tracers and samples, to avoid cross contamination. A limitation of DNA tracers is their lack of stability in certain natural environments with low pH or abundant microbiotic activity. The latter problem can be solved by chemical modification of the DNA tracers. Presently only qualitative measures of DNA tracers have been done. Now, the new TaqMan PCR technology using fluorescent probes (PE Biosystems), also allows quantitative analysis to be carried out.

Four field experiments were carried out in Norway to investigate the usefulness of DNA tracers in different situations. The overall objective of this paper is to show the applications of synthetic DNA tracers. The specific objective is to demonstrate how the discrimination of differently labelled DNA tracers can be helpful in delineating the sources of their origin and complements the use of standard cost effective tracers.

DNA TRACERS APPLICATIONS

Transport of DNA tracers in porous media

Two experiments were conducted in the porous media. The objective of the first tracer experiment was to test the mobility and migration of DNA molecules as tracers in a sandy aquifer. The experiment was set up at a research site, Moreppen-3, close to the eastern runway of the Gardermoen Oslo airport, Norway (Sabir *et al.*, 1999). DNA tracers and a sodium chloride tracer were injected into the groundwater under forced gradient steady state flow conditions. DNA tracers complemented the NaCl with respect to identification and quantification. The water samples were pumped simultaneously from different depths of the multi-level samplers with a peristaltic pump. The transport of DNA molecules was interpreted by comparisons with the plume of chloride ions. A 72-nucleotide-long oligonucleotide with compatible primer binding sequences at the ends, was designed and produced by standard custom DNA synthesis. Detection of DNA tracers was carried out by polymerase chain reaction (PCR) and the authenticity of the amplified tracer proven by DNA sequence analysis. The results from detection of the DNA tracers with flowing groundwater demonstrated that they could be transported and information "read" from micro-litre samples of water. The DNA tracers were detected at greater depths and relatively faster than chloride ions. The design and the results of PCR and sequence analysis are available in Sabir *et al.* (1999).

The objective of the second tracer test was to evaluate different hypotheses concerning the contaminant source polluting the Ål municipality production well (Colleuille & Kitterød, 1998). Figure 1 shows the production well located 430 m a.s.l. on the Hallingdal riverbank, which supplies drinking water to about 2000 inhabitants

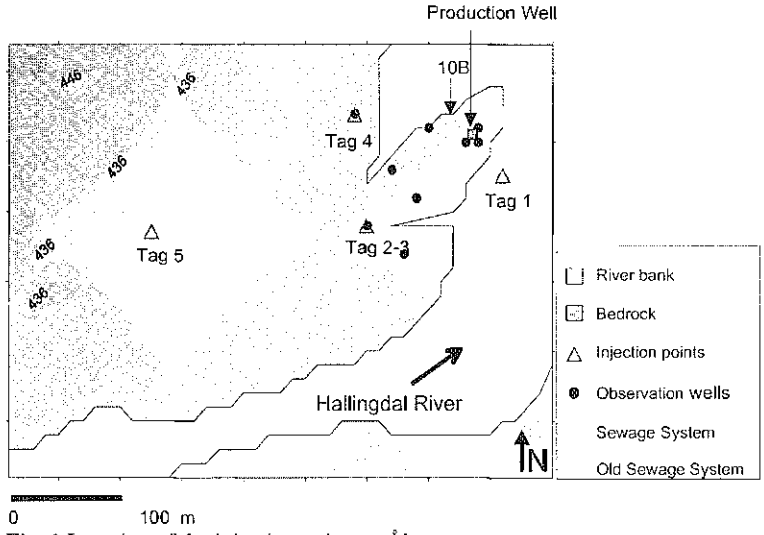

Fig. 1 Location of the injection points at Ål.

with a pumping rate of 30 l s^{-1}. The well is screened between 13 and 19 m below the surface. The valley alluvium is comprised of relatively coarse glaciofluvial sand and gravel sediments of about 15–20 m thickness, with impermeable rock at the bottom. The aquifer is essentially dependent on infiltration from the river, but may be partially fed by recharge from the mountain. A simple two-dimensional Aquifer Simulation Model (Chiang *et al.*, 1997) was used to simulate groundwater flow conditions in different seasons, especially before and after snow melting. Based on the results of both the geo-radar survey and the groundwater simulations, six observation wells at different locations were chosen to inject tracers (Fig. 1). The experiment was initiated by injecting five differently labelled DNA tracers along with sodium chloride tracers. Water samples were collected precisely hourly at the pumping well. The electrical conductivity was also automatically monitored and logged each 10 min. The PCR analysis of tap water samples collected over a five week period revealed detection of tracer no. 5 and no. 2 during the first three weeks, and tracer no. 3 after 30 days. The results of the tracer test are summarized in Table 1. By combining the results from the DNA and NaCl tracer test it was possible to assess the origin of two major break-through curves. The tracer test showed the presence of a preferred flow pathway that may conduct water very rapidly to the production well, and that the source location of the contaminants in the aquifer was leakage from the sewage system about 300 m from the production well (partially within an old sewage system and directly in the aquifer).

Transport of DNA tracers in fractured hard rocks

Two experiments were conducted in fractured hard rocks. In the first experiment, DNA tracers were successfully used at the Romeriksporten railway tunnel project in Norway (Fig. 2). During the construction of a new railway tunnel near Oslo, considerable drainage of surface water and wetland areas through bedrock fractures into the tunnel hampered the construction activity and affected the water level in bogs and lakes 160–200 m above

Table 1 Experimental results.

Well name	Depth (m)	Sediments	Distance from the well (m)	Tracer	Breakthrough*
10B	13–17	Coarse	23	NaCl (10 kg in 500 l water)	Preliminary test (25.02.98) (60 min, 110 min, 20 h)
14	3–4	Thin	50	NaCl (20 kg in 500 l water), DNA tag 1	Negative
15B	10–12	Coarse	130	NaCl (20 kg in 500 l water), DNA tag 2	Breakthrough curve 1 (12 hours, 30 hours, 2-3 days)
15A	6	Thin	130	NaCl (20 kg in 500 l water), DNA tag 3	Breakthrough curve 2 (26 days, 30 days, ?)
11	2–6	Not known	100	NaCl (20 kg in 500 l water), DNA tag 4	Negative
–	3	Sewage system	300	DNA tag 5	3 h

*First, maximum, and last breakthroughs are given in parentheses.

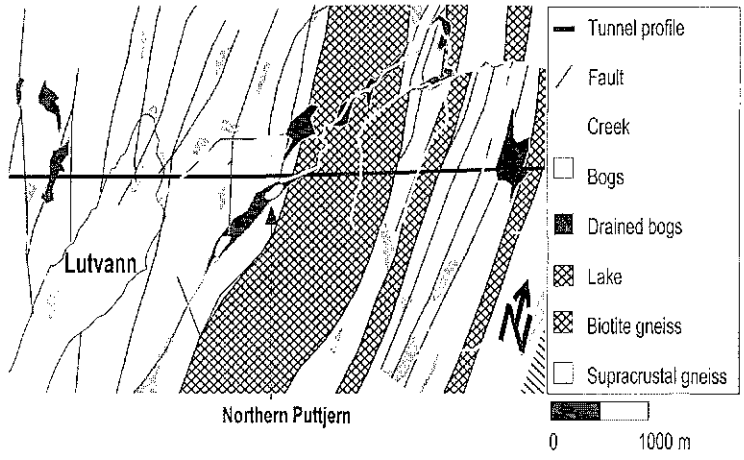

Fig. 2 Location of Romeriksporten railway tunnel and Northern Puttjern.

the tunnel. The bedrock, consisting of Precambrian gneisses, is highly fractured and very heterogeneous. The main purpose of the tracer test was to determine the degree of connection between one small lake (Northern Puttjern) and the tunnel 180 m below the ground level. In accordance with the results of a geo-radar survey, six injection wells were placed in Lake Puttjern. The filters of these wells were located at the contact between the organic bottom layer of the lake and the bedrock. Six individual DNA tracers with chloride tracers were injected and the water samples were collected nearly every hour (automatically) at different locations in the tunnel. The tracer test confirmed interconnection between the surface water and the tunnel and showed that the water travelled the distance in five to 20 h. The DNA tracers provided a means for quantifying groundwater dynamics in very complex fractured rock systems. The results of this tracer test were used as input data to calibrate a three-dimensional (3-D) groundwater flow simulation (Kitterød *et al.*, 1998) aimed to aid better understanding of the water balance disturbance due to the tunnel construction and evaluate possible remediation by artificial water infiltration.

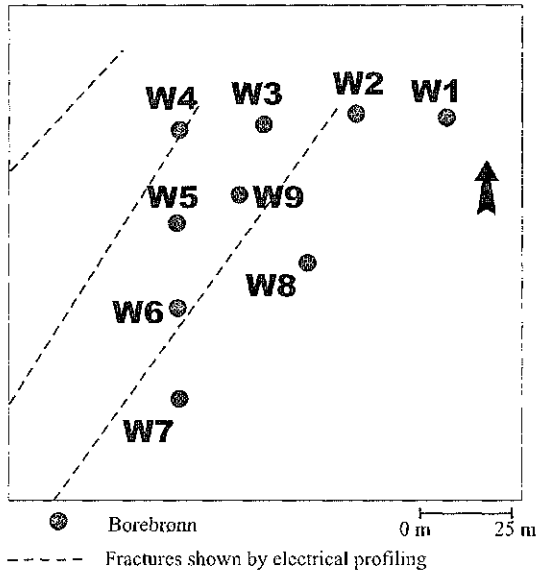

Fig. 3 The well field at Holmedal, Sogn and Fjordane.

In the second experiment, discrimination of four individual DNA tracers proved feasible in the study of interconnectivity and groundwater movement between wells drilled in fractured rocks at Holmedal, Sogn and Fjordane (Gaut *et al.*, 2000). The main bedrock is dark green amphibolites. Electrical profiling shows three fractures oriented NE–SW crossing the well field. Fractures measured in the field are steep and have a NNW–SSE or NNE–SSW direction (Braathen *et al.*, 1998). The tracer tests were conducted by injecting different DNA tracers in each of four wells (W2, W3, W5 and W6 in Fig. 3). Water was pumped from well number 9, W9, with an average pumping rate of 100 l h^{-1}. The pumping was started one day before injection of the DNA tracers. The tracers, each mixed in 0.5 l of water, were injected through plastic tubes down to 15.5 m. In well W6, 0.5 l of bacteriophage was added as a second tracer. Water samples were taken from the pumping well every 3 min during the first hour and then gradually less frequently.

First arrival of the DNA tracers was from W3: 14 min, from W6: 17 min, from W2: 4066 min (nearly three days), and after 115 min from W5. The tracer tests shows that there is communication between the pumping well and the injection wells. For BH3 and BH6 especially, but also for BH5, the water passes through the fracture system very quickly. Calculations using the distances between W9 and the injection wells give velocities of 24×10^{-3} m s^{-1}, 21×10^{-3} m s^{-1}, 2.5×10^{-3} m s^{-1} and 0.14×10^{-3} m s^{-1} for W6, W3, W5 and W2 respectively. The analyses show for all the wells, except W5, a few samples with positive results and in between one or more samples where the DNA tracer was not detected. This might be due to transport via different flow paths (within or between fractures) or because most of the DNA tracers, in one way or another, were held back or were not flowing towards the pumping well. This will be discussed further in Gaut *et al.* (2000).

DISCUSSION

The field applications of DNA tracers show that the method is very useful in tracking groundwater flow and discriminating between sources of pollution. This applies to porous media in general and to complex fractured rocks in particular. DNA molecules in fractured rocks travelled rapidly. This could be due to the more limited spatial extent and concentrated flow in fractures than the flow in porous media, where the flow is usually more widely distributed. Similar effects of flow velocity variations on the transport of two bacteria were experienced in a previous study (Hendry *et al.*, 1999).

Generally, DNA tracers meet most of the desired criteria for a good tracer such as a low detection limit, a number of differentiable signatures, no environmental risks. In all the tracer tests described in this paper, the detection of the DNA tracers was only qualitative, either positive or negative, with no breakthrough curves. Therefore, the different recoveries of the individual tracers did not express the corresponding tracer losses. With the new generation TaqMan PCR instruments, quantitative analyses will however be feasible.

In the tracer tests, the DNA tracers could have been lost by sorption or degradation. Microbial degradation and sorption are regarded as the main reasons for tracer loss in the porous media, whereas the DNA behaviour may have been relatively stable in fractured rocks. The size of the DNA tracers is also expected to be an important factor influencing transport in fractures and sandy materials. The smaller than optimum size molecules may experience greater losses due to more rapid diffusion and more frequent collisions with the porous material and the walls of the rock fractures. There is also the possibility of greater diffusion of small size DNA molecules into relatively immobile water within fine pores and fractures. The larger than optimum size molecules enhance the retention processes in porous materials and their retention in small aperture regions of the fractured materials. This is consistent with previous experimental studies of colloid transport in granular materials (Wollum & Cassel, 1978; Wang *et al.*, 1981; Tan *et al.*, 1994).

Given their information capacity, DNA tracers could be better alternatives to organic, inorganic and radioactive tracers for distinguishing sources of pollution. The degradable polymer makes the DNA molecules more interesting for use as a biotracer, or so-called biomarker, for delineation of temporal and spatial bacterial population dynamics. DNA tracers could be cost effective, i.e. inexpensive production of DNA tracers and cost of PCR analysis. The overall price will be reduced as the number of water samples being included in a single PCR run increases (multiplex PCR).

The results indicate that the method is most directly applicable in the field of hydrogeology and in engineering investigations. The method can be very useful to label the particles and the products, e.g. labelling soil particles in erosion problems. Labelled DNA tracers can mimic the transport of viruses in investigations of their sources. Various products can be labelled, e.g. petroleum, pesticides, herbicides, and wastes.

Since DNA tracing technique is a new and advanced concept in the field of hydrogeology, further studies are required to develop a better understanding of the physical and chemical processes. Investigations on the factors influencing the transport of DNA tracers in different situations are needed in order to make the technique

decisive and reliable. An important consideration should be to interpret the relationship between the effect of sorption parameters (e.g. clayey materials, organic matter) under a range of groundwater velocities.

CONCLUSIONS

The major advantages of synthetic DNA tracers are the availability of a sensitive detection method, the option to introduce an alphanumeric identification message, the availability of different sizes with variable valences, there is no problem of overlapping, and the method could be cost effective. As a result DNA tracers can be a good alternative to organic, inorganic or radioactive tracers. The principal findings of this paper are expected to be applicable to other environments, but further laboratory and field studies are needed to investigate the factors influencing the transport of DNA tracers, such as degradation and sorptive behaviour. The size-related DNA molecule retention processes in porous materials and their retention in small aperture regions of fractured materials, needs to be investigated in order to predict an optimum DNA molecule size for transport. Three-dimensional groundwater flow and reactive transport modelling are required for quantification of the sorption under a range of groundwater velocities. With the new possibility of performing quantitative PCR analyses, the value of DNA tracers probably will increase considerably.

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