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A comparison of DNA fragmentation methods − Applications for the biochip technology

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ABSTRACT

The efficiency of hybridization signal detection in a biochip is affected by the method used for test DNA preparation, such as fragmentation, amplification and fluorescent labelling. DNA fragmentation is the commonest methods used and it is recognised as a critical step in biochip analysis. Currently methods used for DNA fragmentation are based either on sonication or on the enzymatic digestion. In this study, we compared the effect of different types of enzymatic DNA fragmentations, using DNase I to generate ssDNA breaks, NEBNext dsDNA fragmentase and *Saq*AI restrictase, on DNA labelling. DNA from different *Desulfovibrio* species was used as a substrate for these enzymes. Of the methods used, DNA fragmented by NEBNext dsDNA Fragmentase digestion was subsequently labelled with the greatest efficiency. As a result of this, the use of this enzyme to fragment target DNA increases the sensitivity of biochip-based detection significantly, and this is an important consideration when determining the presence of targeted DNA in ecological and medical samples.

1. Introduction

The detection and identification of rare, or low abundant, sequence targets from mixed samples is an important aspect of any environmental analysis, and it is one task that is best performed unambiguously using microarrays. The hybridization of environmental DNA to specific probes arrayed on a solid support (Kelly, 2009) can be enhanced for the detection of rare sequences by including pre-amplification steps, for either specific genes or whole genomes. Beside the specificity of the probes, the level of biochip sensitivity is influenced by the DNA fragmentation and fluorescent labelling stages prior to hybridization (Gabig-Ciminska et al., 2004). In this study we compared the labelling efficiencies when DNA was digested with DNase I, or NEBNext dsDNA fragmentase or *Saq*AI restrictase, using DNA extracted from 6 species of *Desulfovibrio*. Species of which are renown for being present in environments in low densities.

Sulfate-reducing bacteria (SRB) are anaerobic organisms that play important roles in many biogeochemical processes, and they form regular components of natural and engineered systems at low densities (He et al., 2007). The metabolic capabilities of SRBs are narrow, and these organisms can be detected molecularly by identifying the functional genes for dissimilatory sulfite reductase (*dsr*) and adenosine 5′-phosphosulfate reductase (*apr*). Genes that are directly associated with the reduction of inorganic sulfate (He et al., 2007; Zinkevich and Beech, 2000). Genomic studies of SRB have contributed to an understanding of their basic biochemical mechanisms and the roles they might play in the environment. SRBs play a crucial role in the contamination of petroleum products, resulting in the souring of gas wells over time, and they play a role in the anaerobic corrosion of steel (biocorrosion) (Angel and White, 1995). The interaction with metals is facilitated by the action of hydrogenases, enzymes that catalyse the use of hydrogen gas during metabolism, and the products of these processes accelerate biocorrosion (Caffrey et al., 2007). SRB also attract attention because they can enzymatically reduce and precipitate toxic metals, including U(VI), Cr(VI), Tc(VI) and As(V) and, consequentially, have a role in the bioremediation of metal contaminated environment (Junier et al., 2009). *Desulfovibrio* species have been shown to form metal nanoparticles (Capeness et al., 2015). As a result of their environmental importance and their natural low densities, SRBs are a good choice to assess the processes involved in sequence identification using biochips.

The biochip we constructed comprised oligonucleotide probes for 16S rRNA, and the genes encoding adenosine 5′-phosphosulfate reductase (apr)

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Table 1
Characteristics of the probes used in this study.

Annotation	Probe name	DNA sequence 5′ → 3′	Gene Bank
16S rRNA	16S_CONS_1	CCTACGGGAGGC AGCAG	AP010904.1 **AE017285.1**
Adenosine 5′-phosphosulfate reductase (*apr*)	SRB	CCAGGGCCTGTC CGCCATCAATAC	**AE017285.1**
Periplasmic [NiFe] hydrogenase, small subunit (*hynB*)	Dv_HynB	CACCCCTGCATC GGCTGCAG	**AP010904.1** *Desulfovibrio magneticus* RS-1
Periplasmic [NiFe] hydrogenase, small subunit (*hynB*)	Dv_HynB	CACCCCTGCATC GGCTGCAG	**AE017285.1** *Desulfovibrio vulgaris* str. Hildenborough
Periplasmic [NiFe] hydrogenase, small subunit (*hynB*)	Dv_HynB	CACCCCTGCATC GGCTGCAG	**CP000112.1** *Desulfovibrio alaskensis* G-20
Periplasmic [NiFe] hydrogenase, small subunit (*hynB*)	Dv_HynB	CACCCCTGCATC GCCTGCAG	**CP006585.1** *Desulfovibrio gigas*
Periplasmic [NiFe] hydrogenase, large subunit (*hynA*)	Dv_HynA	GCGACGCCCAGC ACT TCACCCA	**AP010904.1** *Desulfovibrio magneticus* RS-1
Periplasmic [NiFe] hydrogenase, large subunit (*hynA*)	Dv_HynA	GCGACGCCCAGC ACTTCACCCA	**CP006585.1** *Desulfovibrio gigas*
Periplasmic [NiFe] hydrogenase, large subunit (*hynA*)	Dv_HynA	N/I*	**AE017285.1** *Desulfovibrio vulgaris* str. Hildenborough

*N/I − The sequence GCGACGCCCAGCACTTCACCCA is not identified in the *Desulfovibrio vulgaris* str. Hildenborough (NCIMB 8303) genome.

and Ni,Fe periplasmic hydrogenase (small and large subunits). The probe sequences (Table 1) were based on the *in silico* sequence and from experimental results. The nucleotide sequence of the probe **16S_CONS_1** represents the conserved region flanking the variable V3 region of 16S rDNA (Muyzer et al., 1993). This probe enables the detection of most bacteria likely to be present in a bacterial consortium. The **SRB** probe covers a fragment of the adenosine-5′-phosphosulfate (APS) reductase gene, and it was used successfully as a PCR primer for the general screening of sulfate-reducing bacteria such as: *Desulfovibrio indonesiensis, Desulfovibrio alaskensis, Desulfovibrio vietnamensis, Desulfovibrio vulgaris, Desulfovibrio gigas, Desulfovibrio desulfuricans, Desulfomicrobium baculatus, Desulfococcus multivorans, Desulfobulbus propionicus, Desulfofrigus fragile, Desulfofrigus oceanense, Desulfotalea psychotrophila, Desulfotalea arctica, Desulfofada gelida, Desulfocinum infernum, Desulfotomaculum nigrificans, Desulfosporosinus orientis* (Zinkevich and Beech, 2000). This probe will detect a wide range of SRBs, although it cannot distinguish between them. The probes **Dv_HynB** and **Dv_HynA** are designed to specifically recognize the hydrogenase genes *hyn*A and *hyn*B from *Desulfovibrio* spp. Six *Desulfovibrio* species (*Desulfovibrio magneticus*; *Desulfovibrio gigas; Desulfovibrio vulgaris*; *Desulfovibrio alaskensis*; *Desulfovibrio vietnamensis*; *Desulfovibrio indonesiensis*) were used in the design and testing for these probes.

2. Materials and methods

2.1. The biochip matrix

We have produced two generations of dendrimeric matrix on a microscope slide (Beier and Hoheisel, 1999; Tomalia, 2004), where the acylation and amination reactions have been repeated twice. Polyamine tetraethylenepentamine (TEPA) was used for the first amination step, producing a branched dendrimeric structure. The second amination reaction used diamine 1,4-bis-(3-aminopropoxy)butane (BAPB). The same acylation agent, acrylolchloride, was used in both acylation reactions.

The matrix was activated by the addition of 1 mmol N,N'-disuccinimidylcarbonate (DSC) and 1 mmol diisopropylethyl-amine (DIEA) in 20 ml anhydrous acetonitrile for 4 h. DSC is a homobifunctional crosslinking agent and is used for bond formation between the functional group of oligonucleotide probes and the amino group of the dendrimeric structure. Afterwards, the slides were washed with N,N-dimethylformamide (DMF), 1, 2- dichloroethane and dried.

2.2. Immobilization of oligonucleotide probes onto the activated dendrimeric matrix

The oligonucleotide probes were modified by the addition of a C6 Amine linker at the 5′-end during synthesis. An oligonucleotide probe solution (250 pmol/0.1 µl) in 1% DIEA was placed onto the activated dendrimeric matrix. Spotting was performed by pins (200 nl/spot) with two replicate spots of each probe being applied to the biochip. The slides were then incubated overnight in a humid chamber at 37 °C and subsequently washed with water and ethanol. The surface of the glass slides with the immobilized probes was deactivated by treatment with a solution made of 6-amino-1-hexanol (50 mM) and DIEA (150 mM) in DMF for 2 h, in order to prevent the binding of the fluorescently labelled DNA with the matrix surface. Finally, the biochips were washed with DMF, acetone, water and dried. The deactivated glass slides with the immobilized probes were then ready to be used in hybridization experiments.

2.3. Cassette construction

The cassette method (Zinkevich et al., 2014) to evaluate biochips was further extended, using a single stranded (*ss*) DNA cassette as the target for hybridization with probes that were coupled to the matrix so that the hybridization capacity of each probes to be evaluated. The *ss*-DNA cassette is a lineal array of sequences complimentary to the studied set of probes. Cy3 fluorescent dye was inserted at the 5′-end of the *ss* cassette during the synthesis. The cassette (83 bases) contained four probe sequences (Supplementary Material), which were synthesized by Bioneer Corporation (Daejeon, Republic of South Korea). The hybridization buffer for the *ss*-DNA cassette was SSARC (4xSSC [600 mM NaCl, 60 mM Na-citrate], 7.2% (v/v) Na-sarcosyl). Hybridization reactions were performed at 45 °C for 4 h. After hybridization the biochip was washed with 2xSSC + 0.2% SDS for 2 min, followed by 0.2xSSC + 0.2% SDS for 2 min and finally with 0.2xSSC for 2 min at 25 °C, and dried by centrifugation.

2.4. DNA preparation for the hybridization and hybridization conditions

DNA from *D. magneticus* RS-1 (DSM 13731) was amplified using an illustra™ GenomiPhi HY DNA Amplification Kit (25–6600-22 GE Healthcare, Life Science, USA), and purified using a PureLink Quick PCR Purification Kit (K3100-01, Invitrogen, USA) following the manufacturer instructions. DNA fragmentation was performed by digesting with one of three different enzymes: NEBNext dsDNA Fragmentase (MO348S, New England BioLabs, USA); FastDigest *Saq*AI (FD2174, Thermo Scientific, USA); or DNaseI from the ULYSIS® Alexa Fluor®546 Nucleic Acid Labeling Kit (U21652, Molecular Probes, USA) following the manufacturer instructions. The digestion conditions for each enzyme were selected to form a range of fragment sizes from 50 to 200 bp. DNA fragmentation was estimated by electrophoresis on 1.5% agarose gel in 1xTAE (40 mM Tris-acetate, 1 mM Na_2EDTA) buffer, and visualized by Ethidium bromide staining. The fragmented DNA was labelled by covalent bonding it to the fluorescent dye Alexa Fluor®546 (U21652, Molecular Probes, USA), according to the manufacturer protocol. The labelled DNA was purified using Bio-Rad Micro Bio-Spin® *P*-30 columns (732–6202, Bio-Rad Laboratories, USA). The labelling efficiency and DNA concentration were calculated according to the manufacturer protocol. The purified DNA was precipitated by adding 10 vols of 2% $LiClO_4$ in acetone for at least 30 min at −20 °C, and sedimented by centrifugation at 15,000 rpm for 20 min at 4 °C, then washed with

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