Analysis of Microbial Diversity in an Extreme Environment: White Island, New Zealand

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Abstract

White island, the most active volcano in New Zealand, is a poorly studied environment that represents an ideal site for the investigation of acidophilic thermophiles. The microorganisms present on here are continually exposed to extreme environmental conditions as they are surrounded by steamy sulphurous fumaroles and acidic streams. The sediment temperature ranges from 38°C to 104°C whilst maintaining pH values below 3. A survey of the volcanic hydrothermal system of White Island was undertaken in order to gain insights onto the microbial diversity using culture-dependant techniques and molecular and phylogenetic analyses. A novel liquid medium based on "soil-extract" was designed which supported growth of bacterial and archaeal mixed cultures. Molecular analyses revealed that the dominant culturable bacterial species belong to the Bacteroidetes, Firmicutes and α-Proteobacteria groups. Several previously uncultured archaeal species were also present in the mixed cultures. The knowledge gained from these studies was intended to help in the development of a novel microbial detection technique suitable for community analysis.

Conventional molecular techniques used to study microbial biodiversity in environmental samples are both time-consuming and expensive. A novel bead-based assay employing Quantum dots (QDs) was considered to have many advantages over standard molecular techniques. These include high detection speeds, sensitivity, specificity, flexibility and the capability for multiplexed analysis. QDs are inorganic semiconductor nanoparticles made up of crystals about the size of proteins. It has been claimed that the physical and chemical properties of the QDs have significant advantages compared to organic dyes, including brighter fluorescence and resistance to photo-bleaching. Their optical properties facilitate the simultaneous imaging of multiple colours due to their flexible excitation and narrow band emission. Functionalised QDs are able to bind to different biological targets such as DNA, allowing high-throughput analysis for rapid detection and quantification of genes and cells.

The optical and physical characteristics of the QDs as well their interaction with biomolecules are shown to be suitable for the development of a novel bead-based technique able to target the key microbial species and identify them by flow cytometric measurements (FCM). The broad absorption and narrow emission spectra of the QDs, as well as their fluorescence intensity and specify to target biomolecules, was compared to other organic fluorophores. The potential advantages and limitations of QDs as a fluorophores for biological applications are discussed.

The data acquired during this study provides a broad overview of the microbial diversity and ecology of the volcanically-active hydrothermal systems of White Island and constitutes the baseline for the development of a novel bead-based technique based on QDs.

Statement of candidate

I certify that this thesis contains original work conducted by the author between August 2003 and June 2008. To the best of my knowledge it contains neither material previously published or written by another person for any other institution. Any contribution made to the research by others, with whom I have worked at Macquarie University or elsewhere, is explicitly acknowledged in the thesis.

I also declare that the intellectual content of this thesis is the product of my own work, except to the extent of the acknowledged assistance from others on the project's design, data interpretation or in style, presentation and linguistic expression.

Raquel Ibáñez-Peral

Sydney, June 2008

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Abbreviations and symbols

Abbreviations

Abbreviation	Meaning	Abbreviation	Meaning
abs	Absolute	**MFI**	Median fluorescence intensity
approx.	Approximately	**M**	Molar
CLMS	Confocal laser scanning microscopy	**m**	Meter
DAPI	4',6-diamidino-2-phenylindol	**min**	Minutes
DI water	Deionised water	**mRNA**	Messenger ribonucleic acid
DIC	Differential interface contrast microscopy	**nM**	Nanomolar
DNA	Deoxyribonucleic acid	**µM**	Micromolar
ds	Double stranded	**nm**	Nanometre
EDTA	Ethylenediamine tetra acetate	**nov.**	Novel
EtBr	Ethidium bromide	**OD**	Optical density
EtOH	Ethanol	**PE**	Phycoerythrin
FCM	Flow cytometric measurements	**PBS**	Phosphate buffer, saline
FITC	Fluorescein isothiocyanate	**PCR**	Polymerase chain reaction
FISH	Fluorescence *in situ* hybridisation	**pers. comm.**	Personal communication
FL	fluorescence	**QDs**	Quantum dots
FL1	Fluorescence detector 1	**R-PE**	Derivatised phycoerythrin
FL2	Fluorescence detector 2	**rRNA**	Ribosomal ribonucleic acid
FL3	Fluorescence detector 3	**RT**	Room temperature
g	Gram	**s**	Seconds
GPS	Global positioning system	**ss**	Single stranded
h	Hour	**SSC**	Single angle light scatter
kb	Kilobase air	**SP**	Shortpass filter
kg	Kilogram	**sp.**	species
l	Litre	**UV**	Ultra-violet light
LP	Longpass filters	**vol**	Volume
log	Logarithm	**v/v**	Volume per volume
		w/v	Weight per volume

Symbols

Symbol	Meaning
Å	Angstrom
ºC	Degrees Celsius
ε	Molar extinction coefficient
~	Approximately
® / ™	Registered trademark

This thesis is dedicated to my family, especially to the memory of my mother.

(Dedico ésta tesis a mi familia, en especial a la memoría de mi madre)

"No se está en ningún sitio mejor que en casa"

María Peral-Martín

Chapter I. Literature review