

***Flavobacteria* in the Southern Ocean**

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Abstract

The abundance, spatial distribution and diversity of class *Flavobacteria*, a bacterial group with a major role in marine secondary production, was investigated in the Southern Ocean euphotic zone of the ice pack off Eastern Antarctica and along a transect at 140-144°E between latitudes 44.7°S to 63.5°S. Samples were comparatively analysed using 16S rRNA gene-based denaturing gradient gel electrophoresis (DGGE), fluorescent *in situ* hybridisation, real-time PCR and sequence analysis. The results were subsequently compared with direct cultivation approaches.

Surface seawater samples were filter-fractionated into particulate and planktonic fractions and the abundance of particle-associated *Flavobacteria*, ascertained with real-time PCR and DGGE band analysis using *Flavobacteria*-specific primers. *Flavobacteria* abundance was found to be significantly higher in Polar Front Zone (PFZ) and Antarctic Zone (AZ) water samples compared to warmer, nutrient limited Temperate Zone (TZ) and Sub-Antarctic Zone (SAZ) waters. Abundance of particle-associated *Flavobacteria* positively correlated with seawater chlorophyll *a* and nutrient concentrations. The abundance of planktonic *Flavobacteria* populations in the same samples remained relatively static, suggesting increased *Flavobacteria* abundance may relate to enhanced primary production in the PFZ and AZ. This was supported by comparisons of DGGE profiles that demonstrated significant differences occur in the total *Flavobacteria* community structure and 16S rRNA gene diversity between samples from the PFZ and AZ with samples from the TZ and SAZ. This suggests a shift to a different, more psychrophilic *Flavobacteria* community occurs across the Polar Front in the Southern Ocean.

DGGE band sequences revealed a high diversity of class *Flavobacteria* within the Southern Ocean, with 24 genus-level lineages detected. Several of the phylotype clades detected were cosmopolitan in distribution, present in both polar and temperate oceans. Many of the phlotypes clustered in a large, so far uncultivated clade (previously termed “DE cluster 2”) widely distributed in seawater but apparently absent from sea-ice. Cosmopolitan phylotype clades occurred throughout the Southern Ocean, while several additional phylotype groups were found only in the colder waters of the PFZ and AZ.

Examination of the cultivable diversity of *Flavobacteria* in Southern Ocean water samples, using a range of growth media, revealed a number of unique phlotypes including three novel genera, some grouping in clades for which only clones are currently available. Several other strains represented novel species belonging to the family *Flavobacteriaceae*, grouping in the genera *Psychroserpens*, *Polaribacter* and *Tenacibaculum*.

A number of seawater microcosms were utilized to examine the colonization of bacteria-free diatom detritus by planktonic bacterial communities over a period of 30 days at 2°C. *Flavobacteria* phlotypes colonizing diatom detritus, determined by DGGE and real-time PCR analysis, were homologous with the dominant phlotypes in the particle-associated fraction of the samples from which they were taken. Colonisation occurred rapidly (<5 days) and comprised a succession of phlotypes, representing a diversity of *Flavobacteria* lineages. The increasing colonization corresponded to an increase in the dissolution of silicic acid compared with the corresponding control microcosms.

Declaration

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for any degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Guy Abell

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ABBREVIATIONS

AC – Antarctic Convergence
AD – Antarctic Divergence
ACC – Antarctic Circumpolar Current
AZ – Antarctic Zone
PFZ – Polar Front Zone
SAZ – Sub-Antarctic Zone
TZ – Temperate Zone
CDW – Circumpolar Deep Water
AAIW – Antarctic Intermediate Water.
SIMCO – sea ice microbial communities
rRNA – ribosomal ribonucleic acid
CFB – *Cytophaga-Flavobacterium-Bacteroides*
EPS – exopolysaccharide
FISH – fluorescent in situ hybridisation.
PCR – polymerase chain reaction
DGGE – denaturing gradient gel electrophoresis
DAPI – 4',6-diamidino-2-phenylindole
PBS – phosphate-buffered saline
EDTA – ethylenediaminetetraacetate
SDS – sodium dodecyl sulfate
G+C – guanosine plus cytosine
nMDS – nonmetric multidimensional scaling
ANOSIM – analysis of similarity
FAME – fatty acid methyl esters
GC-MS – gas chromatography-mass spectrometry
 T_{OR} – optimal temperature for renaturation
(F)ASW – (filtered) artificial seawater

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#Covers research detailed in Chapters 4 and 5.

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