

**ASSESSMENT OF BACTERIAL COMMUNITY PATTERNS
FROM ECOLOGICALLY DISTINCT ANTARCTIC SOIL
ENVIRONMENTS**

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**FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR**

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ABSTRACT

It is commonly known that bacteria are ubiquitously distributed in soil environments. They are heavily involved in the biogeochemical cycles and are vital in maintaining soil ecological functions. These functional roles of bacteria are especially important in the extreme and harsh environments of Antarctica where the terrestrial trophic interactions are generally limited to microorganisms due to restricted development of terrestrial plant and animal communities. In order to assess the spatial distribution and taxonomic composition of soil bacterial community in Antarctica, soils were collected from a range of geographically and environmentally distinct locations. These included relatively milder but variable environments on Signy Island, Ryder Bay and Reptile Ridge from maritime Antarctica, more extreme and relatively less disparate locations such as Alexander Island in the transition zone between maritime and continental Antarctica, and Windmill Island on coastal continental Antarctica. The soil bacterial communities were elucidated by using different molecular methods including DGGE, T-RFLP and cloning. Different diversity measures were utilised to address different aspects of diversity and community overlap. In addition, the relationship between the spatial distribution of Antarctic soil bacteria and soil heterogeneity (i.e. variation in pH, salinity, carbon, nitrogen, water and heavy metals content) was investigated using mantel type correlations and/or multiple linear regression method.

The results showed that spatial clustering was a general feature in Antarctic soil bacterial communities as it was detected across all study sites. In addition, spatial patterning can be observed in both species-based (i.e. ANOSIM, PERMANOVA, NMDS) and divergent-based (i.e. nucleotide distance, UniFrac) diversity measures. Such spatial patterns were largely influenced by environmental variations such as the presence of vegetation and/or

animals, anthropogenic impact, and distance to the sea. These factors have profound impacts on the underlying soil chemical parameters. Among the latter, soil pH was shown to contribute a high proportion of explanatory value to the soil bacterial assemblage patterns. On the other hand, between ecologically similar environments, the spatial patterns were influenced by the geographical proximity of the studied locations.

The strong influence of environmental and soil chemical parameters in structuring bacterial community patterns highlighted the importance of niche conservatism and habitat filtering. It was hypothesized that species tend to retain their niches and traits whereby only species with suitable traits will be preferentially selected in stressful environments. This is especially true in Antarctica where most of the soil bacterial communities constantly experience limited water and nutrient input, low temperature and constant freezing-thawing cycle. Tropical or temperate soil systems generally encompassed high bacterial richness with relatively even species distribution (each ribotype occupied <10% proportion in the clone library). In comparison, Antarctic soil have much lower bacteria diversity which is composed of a few numerically dominant (ribotypes which made up >10% of the total retrieved clones in the clone library) and genotypically related species. Furthermore, Antarctic soil might harbour large fraction of unique and novel gene pools distinct from other soil environments. This is because the retrieved sequences generally showed low similarity (<97% similarity) to the closest representatives in tropical or temperate soil environments.

ABSTRAK

Bakteria merupakan organisma yang paling lazimnya didapati di tanah. Mereka memainkan peranan yang penting dalam kitaran biogeokimia dan memastikan fungsi ekologi tanah dijalankan dengan lancar. Peranan ini adalah terutamanya penting di persekitaran ekstrim yang dingin dan kering seperti Antartika, dimana interaksi “trophic” hanya terbatas pada mikroorganisma. Ini adalah kerana penglibatan haiwan dan tumbuhan di daratan Antartika adalah terhad. Demi mengkaji pengedaran “spatial” dan komposisi taksonomi bacteria tanah di Antartika, sampel-sampel tanah telah dikumpul dari berbagai lokasi-lokasi yang merangkumi persekitaran yang berlainan. Ini termasuk lokasi yang berkeadaan serdehana (dari segi kesuburan tanah, cuaca dan iklim) tetapi persekitarannya berbeza-beza di Signy Island, Ryder Bay dan Reptile Ridge dari Antartika maritim (maritime Antarctic), lokasi yang lebih ekstrim dan kurang berbeza di Alexander Island yang terletak di zon peralihan di antara Antartika maritim dan benua Antarctica (maritime/continental Antarctic transition zone), dan Windmill Island yang berlokasi di persisiran bua Antartika (coastal continental Antarctic). Komuniti bacteria daripada tanah tersebut telah dianalisa dengan menggunakan beberapa kaedah molekular termasuk DGGE, T-RFLP dan “cloning”. Kaedah-kaedah yang berlainan ini sengaja digunakan untuk menjelaskan pelbagai aspek komuniti yang berbeza. Di samping itu, hubungan antara distribusi “spatial” bacteria tanah dan variasi faktor kimia tanah (variasi pada pH, “salinity” dan kandungan karbon, air, nitrogen dan logam) telah diteliti dengan menggunakan korelasi statistik.

Keputusan kajian ini menunjukkan bahawa “spatial clustering” mungkin adalah ciri umum di kalangan bacteria di Antartika kerana ia telah dikesan di semua kawasan kajian. Selain itu, “spatial patterning” ini juga telah diperhatikan dengan menggunakan kedua-dua “diversity measure”, termasuk jenis “species-based” (ANOSIM iaitu, PERMANOVA,

NMDS) dan “divergence based” (nucleotide distance, UniFrac). Fenomena ini adalah amat sensitive terhadap variasi persekitaran seperti kewujudan tumbuhan dan/ atau haiwan, pengaruh manusia dan jarak ke laut. Faktor-faktor ini mempunyai kesan yang mendalam terhadap parameter kimia tanah yang mendasari. Antaranya, pH tanah menyumbang perkadaran yang paling tinggi kepada variasi statistik komuniti bakteria tanah. Namun demikian, membandingkan sampel-sampel yang berasal daripada ekosistem yang serupa, variasi komuniti didapati amat dipengaruhi oleh jarak di antara lokasi yang dikaji.

Pengaruh kuat daripada parameter kimia tanah terhadap komuniti bakteria telah mencadangkan kepentingan “niche conservatism” dan “habitat filtering”. Adalah dihipotesiskan bahawa spesies cenderung untuk mengekalkan “niche” dan “trait” sendiri, manakala hanya spesies yang mempunyai ciri-ciri yang menyumbangkan akan dipilih pada keadaan yang “stress”. Ini adalah terutamanya penting di Antartika di mana sebahagian besar komuniti bakteria di darat mengalami kekurangan air, cuaca suhu rendah dan sentiasa mengalami kitaran beku-cair (freeze-thaw cycle).

Sistem tanah tropika dan temperate umumnya merangkumi lebih banyak jenis spesies bakteria dan menunjukkan distribusi spesies yang serata (setiap “ribotype” menyumbangkan <10% perkadaran dalam “clone library”). Sebagai perbandingan, tanah dari Antartika mempunyai jenis spesies bakteria yang terhad, dan biasanya menunjukkan komuniti yang terdiri daripada beberapa spesies yang dominan (“ribotype” yang menyumbangkan >10% perkadaran dalam “clone library”) malah berkaitan (berdasarkan “genotype”). Selanjutnya, tanah Antartika mungkin mempunyai sebahagian unik dan “novel” spesies bakteria yang berlainan daripada tanah-tanah lain. Ini adalah kerana “sequences” yang didapati daripada sampel-sampel kajian menunjukkan persamaan genetik yang rendah (<97% persamaan) dengan wakil terdekat dari tanah tropika atau temperate.

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LIST OF SYMBOLS AND ABBREVIATIONS

ASPA	Antarctic Specially Protected Area
bp	base pair
CAP	canonical analysis of principal coordinates
ddH ₂ O	double distilled water
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
DISTLM	distance-based linear models
dNTP	deoxynucleotide
EDTA	ethylenediamine-tetraacetic acid
GPS	global positioning system
H'	Shannon diversity index
L	litre
MgCl ₂	magnesium chloride
min	minute
mL	millilitre
mM	milimolar
MVDISP	multivariate dispersion indices
ng	nanogram
NMDS	non multidimensional scaling plot
NRI	net relatedness index
NTI	nearest taxon index
PCO	principle coordinate ordination

PCR	polymerase chain reaction
PD	phylogenetic diversity
PERMANOVA	permutational multivariate analysis of similarity
PERMDISP	homogeneity of dispersion
ppm	part per million
RE	restriction enzyme
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rpm	revolutions per minute
sec	second
TAE	Tris-acetate EDTA
T-RFLP	terminal restriction fragment length polymorphism
~	approximately
°C	degree Celcius
=	equals to
<	less than
>	more than
≤	equal or less than
≥	equal or more than
%	percent
%C	carbon percentage
%N	nitrogen percentage
μl	microlitre
μg	microgram

μM

micromolar

$\mu\text{S}/\text{cm}$

micro siemens per centimeter