Chapter 11 Microbial Life in Antarctic Permafrost Environments

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Abstract Permafrost is extensively distributed throughout terrestrial Antarctica, and contains soils that are the oldest, coldest, and driest on Earth. Currently, relatively little is known about the microbial life in Antarctic permafrost compared to its Arctic and Alpine permafrost counterparts. However, a growing body of evidence indicates that the Antarctic permafrost microbial community can be as diverse as those found in the Arctic, but soils have several orders of magnitude less biomass. Similar phyla of bacteria (Firmicutes, Actinobacteria, Bacteriodetes) are found and isolated in permafrost from both poles, and evidence to date from the Arctic indicate that permafrost is a habitable environment, in spite of the extreme cold, oligotrophy and low water activity associated with the soils. Of special interest in the Antarctic is the presence of dry permafrost soil that remains below 0° C and contains negligible amounts of water; such permafrost environments are found in the upper elevations of the McMurdo Dry Valleys, the only place on Earth where dry permafrost is known to occur. It is currently not known if active microbial communities can exist in such hyper-arid dry permafrost soils, and Antarctic permafrost in general. Questions that remain to be answered include whether permafrost microbial communities are active or viable in situ, or whether they represent cryopreserved fossils. If viable, what are the adaptations that allow life to be sustained over geologic timescales in permanently subzero environments? And finally, are the cold and arid limits of microbial life found in the Dry Valley permafrost soils of the Antarctic?

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11.1 Introduction

Antarctic environments, such as sea ice, glacial ice, lakes, streams, and even rocks (endoliths and hypoliths), have recently been recognized to contain habitable niches which harbor active and diverse microbial communities (de la Torre et al. 2003; Bowman et al. 1997; Murray et al. 2012; Lauro et al. 2011; Mikucki and Priscu 2007). Terrestrial Antarctic surface soils, including Dry Valley soils, were initially believed to be sterile and inhospitable for life (Horowitz et al. 1969, 1972) but are now known to host diverse and significant microbial biomass (Lee et al. 2012; Cowan et al. 2002). In comparison with other Antarctic environments, however, relatively little is known about the microbial diversity, activity, and ecology existing within Antarctic permafrost, despite the fact that 37 % of all of the world's permafrost exists throughout the $\sim 280.000 \text{ km}^2$ ice-free regions of the Antarctic (Bockheim 1995; Bockheim and Hall 2002). Permafrost is considered an extreme cryoenvironment where indigenous microorganisms must survive prolonged exposure to subzero temperatures and background radiation for geological timescales in a habitat with low water activity and extremely low rates of nutrient and metabolite transfer (Steven et al. 2006).

In spite of the hostile conditions of permafrost, substantial amounts of microbial biomass are found in Arctic permafrost, and there is a growing body of evidence that viable cells are metabolically active in Arctic permafrost at ambient subzero temperatures (-15 to -25 °C) (Rivkina et al. 2000; Mykytczuk et al. 2013). As of 2013, however, investigations into the prokaryotic component of Antarctic permafrost are limited to four studies: Cameron and Morelli (1974), Gilichinsky et al. (2007), Blanco et al. (2012), and Tamppari et al. (2012). The earliest study investigated the viability of Antarctic permafrost microorganisms by classical, culture-dependant methodologies. However, the authors were not able to demonstrate that the obtained cultured microorganisms were indigenous to permafrost because of possible contamination from drill fluids used to obtain permafrost cores (Cameron and Morelli 1974). Fluid-less permafrost-drilling techniques (Blanco et al. 2012; Gilichinsky et al. 2007) or sampling ice-cemented ground with a hammer and chisel (Tamppari et al. 2012) were recently employed in microbiological studies in the Antarctic. However, knowledge of the habitability of permafrost remains primarily informed by investigations from Arctic and Alpine regions. The limited work done on Antarctic permafrost, when compared with surface soils, is mainly due to the considerable logistical challenges and very high costs associated with obtaining non-contaminated permafrost samples through permafrost drilling from the very remote regions of Antarctica.

11.1.1 Antarctic Permafrost Environments

Permafrost, defined as soil which remains below 0 °C for at least two consecutive years, is typically overlain with an 'active layer' that seasonally rises above 0 °C and which can extend several meters down. Active layer's thickness and depth to ice-cemented permafrost in the Antarctic is heterogeneous and is influenced by regional climate, proximity to glaciers, age, the presence of vegetation, snow cover, and surface albedo. Active layer soils occur in the ice-free regions of the Antarctic peninsula, offshore islands, and maritime Antarctica (Bockheim 1995; Bockheim and Hall 2002). The McMurdo Dry Valleys (MDVs) in inland Antarctica are a particularly harsh and low water activity permafrost environment, characterized by hyperaridity in addition to the cold. Moisture content is extremely low (<5 %), and dry permafrost forms from sublimation of moisture in icecemented permafrost over time. The Dry Valleys are the only place on Earth where a layer of dry soil overlays ice-cemented ground permafrost (Mckay 2009) and, at high elevations, entirely lack an active layer which rises above 0 °C (Marinova et al. 2013). Water exchanges between the dry soil and ice-cemented permafrost via vapor diffusion rather than liquid water. Despite being in the vapor phase, this water is thought to be available to microbial cells (Stomeo et al. 2012).

Polygon-patterned ground is commonly found in permafrost affected terrain (Fig. 11.1). Depressions forming the polygon boundaries are underlain with V-shaped ice wedges and are found throughout the ice-free regions of the Antarctic (Bockheim and Hall 2002). In the Dry Valleys where dry surface soils predominate, the trough-like depressions may be underlain by sand wedges instead of ice, though ice veins and ice lenses may also be found within sand wedge structures (Bockheim et al. 2009). The microbiology of ice wedges and sand wedges in Antarctic polygon terrain remains unexplored. Work on young (~4,000 years) and old (~25,000 year old) ice wedges in the Arctic indicates that these are habitable cryoenvironments which contain up to 10^6-10^8 cells/g culturable microorganisms and show evidence of in situ heterotrophic activity based on occluded gas measurements (Wilhelm et al. 2012a; Katayama et al. 2007; Lacelle et al. 2011).

11.1.2 Habitability of Permafrost Environments

In addition to the extreme cold and often oligotrophic conditions, one of the primary constraints to microbial life in permafrost is the lack of liquid water. Liquid water could be present in ice-cemented ground in small amounts, despite the subzero temperatures. Concentrated solutes in frozen soils can reduce the freezing point of water causing the presence of briny veins within permafrost (Anderson 1967). The ordering effects of clay minerals are also known to stabilize liquid water into very thin films adsorbed to the mineral grain and may be the only



Fig. 11.1 Polygon-patterned permafrost in the Antarctic. polygon-patterned ground in the McMurdo Dry Valleys, aerial view (*top*), and view from the ground (*bottom*)

available water at subzero temperatures (Anderson 1967; Jakosky et al. 2003). In the Dry Valleys, the interface between dry permafrost and ice-cemented permafrost may also be habitable, if water vapor exchanging between the ice-rich permafrost and overlying dry regolith soils is available to microorganisms.

Nutrient content in permafrost soils in the Antarctic is heterogeneous. Soils in Antarctica are generally oligotrophic, though soils in close proximity to available water (lakes and streams), ornithographic soils, and coastal Antarctic soils have higher-organic C content. The source of organic carbon and nitrogen in Antarctica

may originate from aeolian deposition of organic matter from modern or ancient lacustrine, marine, or lithic sources (Burkins et al. 2000; Hopkins et al. 2009; Cowan et al. 2011). Carbon and nitrogen isotopic signatures that are indicative of primarily ancient aquatic sources of organics, rather than reflective of wind deposition from modern sources, were found in one Dry Valley (Burkins et al. 2000). However, the total contribution of ancient carbon to Antarctic soils and permafrost remains unknown (Hopkins et al. 2009).

Relative to Arctic permafrost, very few studies have investigated the microbiology of Antarctic permafrost. Much of what is known about soil microbiology in the Antarctic is currently derived from investigations of surface active soils, and mostly from Dry Valley soils. This small but increasing body of research indicates that similar amounts of microbial biomass are present in Antarctic surface soils $(10^6-10^8 \text{ cells/g wet soil})$ as reported for temperate soil habitats, and also high levels of microbial diversity (Lee et al. 2012; Cowan et al. 2002). Taking into account the very few Antarctic permafrost samples analyzed, it appears that relatively low amounts of microbial biomass are present in Antarctic permafrost samples $(10^3-10^4 \text{ cells/g wet soil})$ compared with Arctic regions $(10^5-10^8 \text{ cells/g})$ wet soil) (Table 11.1), and the abundance of microbial cells reported in Dry Valley and coastal permafrost generally decreases with depth (Blanco et al. 2012; Gilichinsky et al. 2007).

11.1.3 Molecular Microbial Diversity

Molecular surveys of diversity in permafrost showed the presence of aerobic and anaerobic microorganisms, with similar phyla detected in Antarctic and Arctic permafrost. In both cases, there is a predominance of phylotypes belonging to Actinobacteria, Proteobacteria, Firmicutes, and Bacteriodetes (Gilichinsky et al. 2007; Steven et al. 2007; Hinsa-Leasure et al. 2010; Yergeau et al. 2010); it is likely that these bacteria are selected for in permafrost environments. For example, spore-forming Bacteriodetes and Firmicutes may be better adapted to resist the permanently frozen conditions of permafrost. Some Actinobacteria show metabolic activity at subzero temperatures (Katayama et al. 2009; Christner 2002) and also may be protected in the permafrost environment by cyst-like resting forms as observed in Arthrobacter and Micrococcus species (Soina et al. 2004). Overall, at least 11 phyla have been detected in Antarctic permafrost using molecular methods to date (Table 11.2). The source of such diversity is likely to be related to the overlying surface soils, atmospheric deposition of bacteria, nearby colonized habitats such as hypoliths, cryptoendoliths, and microbial mats from colonized streams and lakes (Pearce et al. 2009; Pointing et al. 2009). For example, in Dry Valley permafrost, Gilichinsky et al. (2007) reported that 16S rRNA phylotypes found in surface soils were closely related to those in the underlying ice-cemented permafrost. Nonetheless, the sources and extent of microbial diversity found in Antarctic permafrost remain very poorly characterized.

Table 11.1 Microbial biomass in permafrost	t environments				
Location	Age of permafrost (years)	Microbial functional groups reported	Viable cell counts	Direct microscopic cell counts	Reference
Antarctic Dry Valley permafrost Taylor Valley, Miers Valley, Beacon Valley, Mount Feather	30,000–8 million	Aerobic heterotrophs Methanogens Sulfate Reducers Denitrifiers	$\begin{array}{c} 0-10^{4} \\ 2-10^{1} \\ 0-10^{3} \\ 0-10^{1} \end{array}$	10 ⁵	Gilichinsky et al. (2007)
Antarctic Dry Valley permafrost University Valley	2,500-120,000	Aerobic heterotrophs	9-0	10^{3}	Goordial (unpublished)
Antarctic peninsula permafrost Deception Island	200	Aerobic heterotrophs	0-105	n.d.	Blanco et al. (2012)
Canadian high Arctic permafrost Eureka	5,000-6,000	Aerobic heterotrophs	10^{1} - 10^{4}	10^{8}	Steven et al. (2007, 2008)
Canadian high Arctic ice wedge Axel Heiberg	>4,000	Aerobic heterotrophs Ammonia oxidizing Archaea Denitrifiers	10 ⁴ -10 ⁵	108	Wilhelm et al. (2012b)

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Table 11.2 Bacterial	phyla found in J	permafrost						
Phylogenetic group	Antarctic dry valley permafrost ^a	Antarctic peninsula permafrost ^b	Dry valley soils ^c	Permafrost interface in maritime-influenced dry valley ^d	Maritime dry valley soils ^d	Maritime Antarctic soils ^e	Continental Antarctic soil ^e	Canadian high arctic permafrost ^f
Acidobacteria	+	+	+	+	+	+	+	+
Actinobacteria	+	+	+	+	+	+	+	+
Armatimonadetes (formally OP10)						+		
Bacteriodetes		+	+	+	+	+	+	+
Chlorobi				+	+			
Chloroflexi	+	+	+			+		
Cyanobacteria		+		+	+	+	+	
Deinococcus-		+	+		+			
Thermus								
Fibrobacteres	+							
Firmicutes	+	+	+	+	+	+		+
Fusobacteria						+		
Gemmatimonadetes		+	+	+		+		+
Nitrospira			+					
Plantomycetes		+			+	+	+	+
Proteobacteria	+	+	+	+	+	+	+	+
Spirochetes					+			
TM7		+						
Verrumicrobia				+		+	+	
^a Gilichinsky et al. (2	007), ^b Blanco e	t al. (2012), ^c L	ee et al. (2	012), ^d Stomeo et al. (2012),	e Yergeau et	al. (2010), and	1 ^f Steven et al	. (2007)

In addition to prokaryotes, algae, yeast, and fungi have been found in permafrost (Zucconi et al. 2012; Kochkina et al. 2012; Gilichinsky et al. 2007). The relative contribution of Archaea in Dry Valley permafrost soils is not well known, with multiple studies finding no Archaea using current molecular methodology (Farrell and Pointing 2010; Lee et al. 2012). Gilichinsky et al. (2007) were able to detect methane production from cultured samples of Dry Valley permafrost but did not identify the Archaea in the samples. We also recently detected Archaea (methanogens and halobacteria) in permafrost from University Valley (UV), in the Upper Dry Valleys (Goordial unpublished).

11.1.4 Culturable Microbial Diversity

It is widely recognized that culturable microorganisms represent less than 1 % of the microbial population in soils, and in permafrost, the culturable proportion may be even lower; culturable bacteria from permafrost in the Arctic and Antarctic represent <0.1/0.01 % of the bacteria identified in samples using molecular methods (Gilichinsky et al. 2007; Steven et al. 2007). In general, culturing permafrost bacteria on low-nutrient media such as R2A has yielded the most isolates. However, increased representation of culturable microorganisms has been obtained by alternative methods such as thawing permafrost samples at high temperatures (52 °C) (Kochkina et al. 2012) and incubating natural samples at the 'warm' temperature of 5 °C prior to plating (Vishnivetskaya et al. 2000). Monitoring permafrost samples over time using this method with DAPI staining revealed that cell numbers increased in the sample, while numbers of CFU did not, suggesting that there is a proportion of viable microorganisms in permafrost that are not amenable to the culturing methodologies being currently employed. Antarctic permafrost bacteria isolated and characterized have mostly been identified as psychrotrophs rather than true psychrophiles, indicating that these microorganisms may represent a community of 'survivors' rather than organisms which thrive in these environments. Microorganisms isolated and identified to date from Antarctic permafrost are outlined in Table 11.3 and include a small number of representatives from the Actinobacteria, Proteobacteria, Bacteriodes, and Firmicutes. Many of the genera isolated from Antarctic permafrost have also been identified in Arctic permafrost indicating that such organisms are particularly adapted to survive in such harsh cyroenviroments.

11.2 Microbial Activity

While nitrate reducers, methanogens, and sulfate reducers have been detected in Dry Valley permafrost, it is still unknown whether they are metabolically active in situ (Gilichinsky et al. 2007). It is noteworthy to add that the enzymes

Phylogenetic group	Antarctic dry valley permafrost ^a	Antarctic peninsula permafrost ^b	Dry valley soil ^c	Antarctic coastal high- elevation soil ^d	Canadian high arctic permafrost ^e
Actinobacteria ^f			+		
Arthrobacter	+	+	+	+	+
Brachybacterium					+
Cellulomonas	+				
Frigoribacterium				+	
Kocuria		+			+
Micrococcus	+				+
<i>Nocardiaceae</i> (family) ^g				+	
Rhodococcus	+	+			+
Promicromonospora	+				
Subtercola		+			
Bacteriodetes ^f			+		
Flavobacterium					+
Hymenobacter			+	+	
Pedobacter					+
Firmicutes			+		
Bacillus	+				+
Paenibacillus					+
Sporosarcina		+			+
Staphylococcus					+
Proteobacteria ^f			+		
Aeromonas	+				
Aztobacter	+				
Brevundimonas				+	
Oxalobaceraceae (family) ^g				+	
Polaromonas		+			
Pseudomonas	+				+

Table 11.3 Phyla and genera of Bacteria cultured from Antarctic permafrost

^a Gilichinsky et al. (2007), ^b Blanco et al. (2012), ^c Aislabie et al. (2006b), ^d Aislabie et al. (2006a), ^e Steven et al. (2007, 2008, 2009), ^f could not be classified past the phylum level, and ^g could not be classified past the family level

associated with these metabolisms have been detected in permafrost with a protein microarray (Blanco et al. 2012). Whether the detection of these enzymes is reflective of in situ metabolisms, or whether the stable and cold preservative properties of Antarctic permafrost are preserving these biomolecules, similarly remains to be elucidated.

Aerobic heterotrophic microorganisms are the most commonly cultured from Antarctic permafrost, but their potential metabolic activity in permafrost under in situ conditions is generally uncharacterized. In surface soils of the Dry Valleys, soil CO₂ efflux has been detected under summer ambient conditions, indicating microbial respiration may be occurring in situ (Burkins et al. 2001; Parsons et al. 2004). After taking isotopic C composition into account, Shanhun et al. (2012) concluded that the CO₂ soil fluxes observed in surface and subsurface Dry Valley soils are partially abiotic in origin and that previous measurements likely overestimated the in situ activity of heterotrophic microorganisms in Dry Valley soils (Shanhun et al. 2012). In Arctic permafrost, soil CO₂ flux has been detected in high Arctic permafrost during both summer and late-winter ambient conditions (Whyte et al. unpublished) and in permafrost microcosm experiments at temperatures low as -15 °C (Steven et al. 2008) to -25 °C (Mykytczuk et al. 2013).

11.3 Adaptation to Permafrost

Because the primary source of liquid water in subzero environments results from freezing-point depression caused by increased solute and salt concentrations, many of the microbial adaptations to subzero temperatures include mechanisms to cope with osmotic stress (Chin et al. 2010; Mykytczuk et al. 2013). As result, many of the culturable microorganisms isolated from cryoenvironments are observed to be halotolerant or halophiles. For example, *Planococcus halocryophilus* strain Or1, isolated from high Arctic permafrost active layer, can grow at -15 °C in 18 % NaCl and is metabolically active at -25 °C in frozen permafrost microcosms. This strain possesses a suite of adaptations to cope with osmolyte and cold stresses, such as multiple copies of osmolyte uptake genes, increased protein flexibility, and resource efficiency (Mykytczuk et al. 2011, 2013). Reviews on cold-adaptation mechanisms identified in microorganism have been discussed extensively elsewhere (Bakermans et al. 2009; Qiu et al. 2009). In addition, cold-adapted microorganisms can decrease energy metabolism, or go into a state of dormancy to resist cellular damage; these adaptations promote long-term survival in permanently cold environments (Bakermans et al. 2009; Casanueva et al. 2010). Cystlike resting cells with thickened cell walls and capsular layers, as well as miniature cells 0.3 cm-0.5 µm in diameter, have also been observed in Antarctic permafrost samples. Such adaptations have been proposed to aid cryoprotection on long-term scales (Gilichinsky et al. 2007; Soina et al. 2004).

11.4 Ancient Life in the Subsurface

The Antarctic contains some of the Earth's oldest sediments and thus may represent the largest reservoir for ancient microbial life on the planet. Due to the preservative properties of permafrost and ice environments (i.e., stable and permanently cold temperatures), nucleic acids and biomolecules may be able to be maintained over long time-scales. Lower rates of decay, an order of magnitude for every 10 °C drop, should allow extraction of biomolecules from sediments as old as 1 million years (Willerslev et al. 2004). In spite of the constant cold temperatures, with increasing age, damage to nucleic acids will accumulate. Processes that can reduce the integrity of nucleic acids and biomolecules over time include ionizing background radiation, alkylation, hydrolytic and oxidative damage causing depurination, cross-linking, and single-stranded and double-stranded breaks of nucleic acids (Hansen et al. 2006; Amato et al. 2010). DNA preserved in Siberian Arctic permafrost $\sim 100,000-400,000$ years old was found to have an increased frequency of cross-linked DNA with age (Hansen et al. 2006). Crosslinking and single-stranded breaks inhibit PCR amplification or cause the generation of short PCR fragments, which may impede molecular surveys of microbiota. Metabolically active cells in permafrost could increase the longevity of their DNA with active DNA repair systems. Data on the metabolic rates associated with the survival of immobilized, starved, and possibly dormant microbes thought to exist in permafrost suggest that rates of repair of DNA and protein damage in living microbes are similar to rates of incurred damage (Price and Sowers 2004). Lastly, the successful isolation of ancient permafrost bacteria or intact genes or pathways through metagenome analyses, especially from the relatively very old Antarctic permafrost, may serve as novel sources of biomolecules for biotechnology (enzymes, compatible solutes) and health applications (novel antimicrobials) or, for example, elucidating the evolution of resistance mechanisms to antibiotics, as was recently done in high Arctic permafrost.

11.5 Astrobiology Implications of Antarctic Permafrost

Currently, the most promising planetary bodies in which we look for life are all extremely cold. Europa (a moon of Jupiter) and Enceladus (a moon of Saturn) are icy bodies where there is evidence for subsurface liquid water in spite of average surface temperatures of -160 and -190 °C, respectively (McKay et al. 2012). Mars possesses ample evidence of past liquid water, and the current presence of ice-rich ground beneath dry, extremely cold soils as observed by the Pheonix lander (Mellon et al. 2009). In addition, gullies formed on the sides of craters during the past decade on Mars provide compelling evidence that liquid water (or brine) may exist on Mars (Malin et al. 2006; McEwen et al. 2011), potentially derived from subzero saline springs in deep permafrost cryoenvironments like those that exist on Earth (Lay et al. 2012; Niederberger et al. 2010). Summer ground temperature maxima of 20 °C also make Mars a primary target of astrobiological investigations of past or present life (McKay et al. 2012). In this respect, Antarctic permafrost located in the higherelevation MDVs are considered to be the most Mars-like due to their extreme aridity and cold temperatures, and because, they are the only place on Earth known to contain both dry permafrost overlaying ice-cemented permafrost. UV, a high-elevation MDV (1,700 m.a.sl.), has ambient air temperatures which never rise above

freezing and contains ice-cemented ground underneath dry-permafrost surface soils at depths that range from a few centimeters to over 98 cm. In some parts of this valley, an active layer which rises above 0 °C (due to solar heating) is completely absent (Marinova et al. 2013).

As on Mars, the presence of perchlorates is found in the dry-permafrost soils (Kounaves et al. 2010; Hecht et al. 2009); Perchlorates are efficient freezing-point depressants (eutectic point -67 °C) and can also be used as an electron acceptor for anaerobic microbial respiration (Coates and Achenbach 2004). Microbial investigations of UV show detectable amounts of nucleic acids, phospholipids (Tamppari et al. 2012), and the presence of relatively low amounts of microorganisms based on microscopic cell counts (Table 11.1) (Goordial unpublished). However, sensitive radiorespiration assays completed on UV permafrost samples have yielded little to no measureable microbial activity at subzero temperatures (Goordial unpublished). More studies need to be carried out in upper Dry Valley permafrost and other similar cryoenvironments on Earth in order to determine how we can best search for and measure active and/or preserved microorganisms and biosignatures on other astrobiology targets.

11.6 Conclusion

Antarctica contains almost half of the world's permafrost and is host to some of the most extreme conditions on Earth. Permafrost in the Mars-like hyperarid soils of the upper Dry Valleys, or the permafrost underlying the Antarctic Ice sheet, are compelling cryoenvironments to examine the limits and longevity of life and biomolecules in cryoenvironments. Tantalizing results from the Arctic strongly indicate that metabolically active microbial ecosystems exist in Arctic permafrost (Steven 2008; Rivkina et al. 2000), as well as in the bedrock underneath kilometerthick ice sheets (Miteva et al. 2004); However, whether similar active microbial ecosystems inhabit the more extreme Antarctic permafrost, especially in the harsh dry valley sites, remains to be determined. Are the microorganisms present in Antarctic permafrost viable and active in situ, or do they represent cryopreserved microfossils which have been frozen on geological timescales? How long can cells or biomolecules be preserved in the environment? How can we detect the expected extremely slow or minute microbial activity in such permafrost soils? These are important and fundamental questions that remain to be addressed, but which are crucial to our understanding of the ecology of Antarctic permafrost soils. Additionally, understanding the microbiology of permafrost will inform us in how to best search for and identify the presence and/or activities of similar microbial communities that could exist or have existed on other bodies in our solar system rich in permafrost features.

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