7 Pelagic Oxygen Minimum Zone Microbial Communities

Osvaldo Ulloa1 · Jody J. Wright2 · Lucy Belmar3 · Steven J. Hallam2,4

1Departamento de Oceanografía, Universidad de Concepción, Concepción, Chile
2Department of Microbiology and Immunology, University of British Columbia, Life Sciences Institute, Vancouver, BC, Canada
3Departamento de Oceanografía and Programa de Postgrados en Oceanografía, Universidad de Concepción, Concepción, Chile
4Graduate Program in Bioinformatics, University of British Columbia, Life Sciences Institute, Vancouver, BC, Canada

Introduction ...............................................113

Phylogenetic Diversity of OMZ Prokaryotes ............113
Bacteria .....................................................113
Archaea .......................................................116

OMZ Prokaryotes as Biogeochemical Players ............119

Summary ......................................................120

Introduction

Oxygen minimum zones (OMZs) are regions of the global ocean in which dissolved oxygen in the water column is reduced or totally absent due to poor ventilation, sluggish circulation, and a high demand of oxygen by microbial aerobic respiration. Open-ocean OMZs are prominent in the eastern tropical and subarctic Pacific and the northern Indian Oceans (Fig. 7.1). The actual concentration of dissolved oxygen varies among recognized OMZs, and determining whether or not they reach total anoxia based exclusively on oxygen measurements has until recently been a problem due to technical limitations (Revsbech et al. 2009; Thamdrup et al. 2012). This is highly relevant because microbial-encoded enzymes mediating aerobic and anaerobic transformations of elements (e.g., nitrogen, sulfur, and carbon) manifest different oxygen sensitivities.

It has long been recognized that many OMZs are hotspots for oxygen-sensitive nitrogen transformations, where nitrate serves as the main terminal electron acceptor for the oxidation of organic matter (Lam and Kuypers 2011). In such cases, denitrification and anaerobic ammonium oxidation (anammox) contribute to the removal of fixed nitrogen as N2, with resulting impacts on global nutrient cycles and the climate system (Codispoti et al. 2001). Geochemical signs of the functioning of these anaerobic processes include the presence of an inorganic fixed nitrogen deficit relative to phosphorus in addition to the accumulation of nitrite and excess N2 in the oxygen-deficient regions of the water column. Processes occurring in the boundary regions of OMZs also contribute to the production of the potent greenhouse gas nitrous oxide (N2O), due primarily to the activity of nitrifiers at low oxygen levels. Thus, continued OMZ expansion is an emerging environmental concern, as it will likely exacerbate the loss of fixed nitrogen from the ocean in addition to increasing N2O production (Keeling et al. 2010; Codispoti 2010).

Certain coastal regions also develop periods of oxygen starvation during part of the year, either naturally or induced by anthropogenic eutrophication, affecting marine ecosystems and coastal economies (Diaz and Rosenberg 2009). Some examples of where this phenomenon occurs include the continental shelves off Namibia, western India, and in the Gulf of Mexico (Fig. 7.1). Moreover, in certain enclosed or semi-enclosed basins, such as inland seas (e.g., the Baltic Sea, the Black Sea), fjords (e.g., Sannich Inlet), and ocean basins with reduced ventilation (e.g., the Cariaco Basin), sulfate reduction becomes the main microbial respiratory process, and H2S rather than nitrite accumulates (Fig. 7.2). Until recently, euxinic water bodies (where H2S accumulates) were thought to have a different microbiology and biogeochemistry than marine non-euxinic OMZs, but recent studies, as discussed below, have shown that this is not entirely the case.

Culture-independent molecular studies have identified a diverse community of pelagic prokaryotes in OMZs that are not fundamentally different from those found in euxinic systems. The analyses have primarily been based on individual marker genes, but have recently incorporated community genomic and transcriptomic data sets. In the following sections we highlight specific trends resulting from this work and identify some of the key taxonomic players driving matter and energy transformations in OMZs.

Phylogenetic Diversity of OMZ Prokaryotes

Bacteria

In a recent study, Wright et al. (2012) reviewed the bacterial community composition in open-ocean and coastal OMZs...
and enclosed or semi-enclosed euxinic basins (including the Northeast subarctic Pacific (NESAP), the eastern tropical South Pacific (ETSP), the Cariaco Basin (CB), the Namibian upwelling (NAM; also known as the Benguela upwelling), and the Baltic, Black, and Arabian seas (Data are from the World Ocean Atlas 2009. Figure adapted from Wright et al. (2012) © MacMillan Publishers Ltd. All rights reserved)

![Fig. 7.1](image)

**Fig. 7.1**
Minimum dissolved oxygen concentrations for different regions of the global ocean. Locations mentioned in this chapter are indicated and comprise the Hawaii Ocean Time-series (HOT), the northeast subarctic Pacific (NESAP), Saanich Inlet (SI), the eastern tropical South Pacific (ETSP), the Cariaco Basin (CB), the Namibian upwelling (NAM; also known as the Benguela upwelling), and the Baltic, Black, and Arabian seas (Data are from the World Ocean Atlas 2009. Figure adapted from Wright et al. (2012) © MacMillan Publishers Ltd. All rights reserved)

![Fig. 7.2](image)

**Fig. 7.2**
Cartoon showing the characteristic geochemical profiles in different oxygen-deficient environments. (a) Open-ocean OMZs, with low oxygen concentrations and no nitrite accumulation (e.g., northeast subarctic Pacific); (b) Anoxic OMZs, with nitrite accumulation (e.g., eastern tropical North and South Pacific, Arabian Sea); (c) Euxinic basins showing H₂S accumulation (e.g., Saanich Inlet, Baltic Sea, Black Sea, Cariaco Basin)

and enclosed or semi-enclosed euxinic basins (including the Northeast subarctic Pacific (NESAP), the eastern tropical South Pacific (ETSP), the Namibian upwelling, and Saanich Inlet (SI)), based on taxonomic surveys of small subunit ribosomal rRNA (SSU rRNA) gene sequences (Fig. 7.3). Major groups in order of abundance include Proteobacteria, Bacteroidetes, candidate division Marine Group A, Actinobacteria, and Planctomycetes, while Cyanobacteria, Firmicutes, Verrucomicrobia, Gemmatimonadetes, Lentisphaerae, and Chloroflexi are also well represented (Wright et al. 2012). A number of candidate divisions are also present, including TM6, WS3, ZB2, ZB3, GN0, OP11, and OD1.
Dot plot of the diversity of bacterial taxa at various sample points and depths in Saanich Inlet (SI), the northeastern subarctic Pacific (NESAP; labeled P4, P12, and P26), the Hawaii Ocean Time-series (HOT), the eastern tropical South Pacific (ETSP) and the Namibian upwelling (NAM), based on small-subunit ribosomal RNA (SSU rRNA) gene sequence profiles. "*" indicates a sample taken from P4 1,000 m in June 2008; all other NESAP samples were taken in 2009. Samples are organized according to the similarity of their community composition, as revealed by hierarchical clustering of the distribution of taxonomic groups across environmental samples. The dissolved oxygen concentration is shown for each oceanic sample, and the classification of the environment as oxic, dysoxic, suboxic, or anoxic is also indicated in the color bar (see text for definitions). Names for identifying bacterial groups were selected according to the taxonomic level at which the most relevant information was available (Data used to generate the dot plot were derived from sequences deposited in Genbank. Figure adapted from Wright et al. (2012) © MacMillan Publishers Ltd. All rights reserved)
The distribution of specific subdivisions of abundant taxa varies along the oxygen gradient in the different oxygen-deficient environments studied. Anoxic and sulfidic waters are often dominated by SUP05 (Sunamura et al. 2004) and ARCTIC96BD-19, groups of \( \gamma \)-proteobacteria related to sulfur-oxidizing symbionts of deep-sea bivalves (Fuchs et al. 2003; Woebken et al. 2007; Stevens and Ulloa 2008; Zaikova et al. 2010), with additional representation from the sulfur-reducing family Desulphobacteraceae (\( \delta \)-proteobacteria). Suboxic (1–20 \( \mu \)mol \( O_2 \) per kg water) and dysoxic (20–90 \( \mu \)mol \( O_2 \) per kg water) waters contain high numbers of bacteria affiliated with the SAR11 cluster (\( \alpha \)-proteobacteria), the agg47 cluster (\( \gamma \)-proteobacteria), the SAR324 cluster (\( \delta \)-proteobacteria), the genus Nitrospina (\( \delta \)-proteobacteria), and in some cases, bacterial groups affiliated with Cyanobacteria. Oxic surface waters above OMZs and euxinic basins are often dominated by sequences affiliated with SAR11 and the order Rhodobacterales (\( \alpha \)-proteobacteria), the order Methylphilales (\( \beta \)-proteobacteria), and environmental clusters affiliated with SAR86 and Arctic96B-1 (\( \gamma \)-proteobacteria). Other abundant bacterial groups that appear to distribute differentially along the oxygen gradients of OMZs and euxinic basins include ZD0417 and ZA3412c (\( \gamma \)-proteobacteria), Flavobacteria (Bacteroidetes), Microthrixineae (Actinobacteria), and Verruco-3 (Verrucomicrobia).

These results show recurring bacterial community composition patterns within OMZs and euxinic basins that are consistent with redox-driven niche partitioning, stressing the importance of oxygen concentration as an organizing principle in pelagic microbial communities.

**Archaea**

In contrast to the bacterial domain, less is known about archaeal community composition along the oxygen gradients of OMZs and euxinic basins. Here we use published SSU rRNA sequence data from the ETSP (Belmar et al. 2011), the Black Sea (Vetriani et al. 2003; Coolen et al. 2007), the Cariaco Basin (Madrid et al. 2001; Jeon et al. 2008), the Namibian upwelling (Woebken et al. 2007), and the Baltic Sea (Labrenz et al. 2010), as well as new data from the NESAP, Saanich Inlet (SI), and eastern subtropical South Pacific (ESP) to highlight the major groups present in these systems. \( \odot \) Figure 7.4 illustrates the distribution of the respective phylotypes within the general archaeal phylogenetic tree. Most phylotypes are affiliated with well-recognized pelagic marine clades such as Group I.1a (G-I.1a, DeLong 1998), pSL12-related group (Mincer et al. 2007), Marine Group II (MG II, DeLong 1992), and Marine Group III (MG III, Fuhrman and Davis 1997). However, a significant number of phylotypes cluster within clades originally found in sediments (Marine Benthic Group A and E; Vetriani et al. 1999) or deep-hydrothermal vent environments (DHVE-4 and DHVE-5, Takai and Horikoshi 1999).

A remarkably high proportion of archaeal sequences recovered from OMZs affiliates with the thaumarchaeotal G-I.1a, a group well represented in all of the considered systems (\( \odot \) Fig. 7.5). This group, initially referred to as Marine Group I (DeLong 1992), is ubiquitous and abundant in the global ocean (Francis et al. 2005; Hallam et al. 2006). Group I.1a contains two statistically supported clusters, designated as A and B (Belmar et al. 2011), although some authors have divided this group into additional clusters (e.g., Massana et al. 2000). With the exception of Cenarchaeum symbiosum, which appears outside of the A and B subdivisions, the G-I.1a-A cluster comprises all marine thaumarchaeotal species that have been fully sequenced thus far (i.e., Nitrosopumilus maritimus and Nitrosoarchaeum limnia). The G-I.1a-A cluster also includes sequences retrieved from diverse terrestrial and marine environments including surface waters, deep-ocean sediments, and agricultural soils. In contrast, cluster G-I.1a-B includes very few phylotypes from oxic surface waters, and is mainly composed of sequences from deep waters, marine hydrothermal vents, and oxygen-deficient waters. Since many representatives of the G-I.1a archaeal group are known ammonium-oxidizers (AOA), and given the correlation between phylogenetic markers for AOA and the functional marker ammonia monooxygenase subunit alpha (amoA), OMZ representatives of this group are considered presumptive nitrifiers (Molina et al. 2010).

Some thaumarchaeal phylotypes found in anoxic or euxinic waters classify as being part of the major branch that includes the pSL12-related group (Mincer et al. 2007), the marine benthic group A (Vetriani et al. 1999), and the FFS cluster, which contains sequences retrieved from forest soil (Jurgens et al. 1997). This major branch is a sister group of the branch joining terrestrial Group I.1b and marine Group I.1a, and is related to the extremophile representative pSL12 (Mincer et al. 2007). Additional phylotypes recovered from below the chemocline in the Cariaco Basin (Jeon et al. 2008) appear at the base of the Thaumarchaeota. Interestingly, these sequences were generated using primers designed for eukaryotes from anoxic/euxinic waters in the Cariaco Basin and composed a group with phylotypes from freshwater sediments, rice roots and soil (Jurgens et al. 2000), sediments near deep hydrothermal vents (Takai et al. 2001), and sub-seafloor sediments (Inagaki et al. 2003; Sørensen and Teske 2006). This group has been named “Group I.3” (Jurgens et al. 2000), although some authors have divided this group into additional clusters (e.g., Massana et al. 2000). MGII is a cosmopolitan group, and the majority of sequences observed in coastal and open-ocean OMZs are affiliated with the MGII-A cluster. MGII is a less prominent group in the global ocean, but appears to be important in OMZs (Belmar et al. 2011). Finally, some euryarchaeal phylotypes from OMZs and euxinic waters associate with Marine Benthic Group E and DHVE-4 and DHVE-5 groups.

Archaeal community structure mirrors trends for bacterial denizens of OMZs, including close taxonomic affiliation with archaeal groups from diverse seafloor environments.
Fig. 7.4

Maximum-likelihood phylogenetic tree of archaeal SSU-rRNA gene sequences. Representative sequences of OMZ phytoplankton (≥ 97% of similarity, using UCLUST; Edgar 2010) together with other sequences from the Genbank database were aligned with Mafft (Katoh et al. 2002). The phylogenetic tree was built with the Bosque software (Ramirez-Flandes and Ulloa 2008), using FastTree (Price et al. 2010) and applying the general time-reversible DNA model. The selection of OMZ phytoplankton included previously unpublished archaeal SSU-rRNA gene sequences from the northeastern subarctic Pacific (JQ220557 – JQ222567), Saanich Inlet (JQ222568 – JQ228228, except for a few sequences of apparent eukaryal origin), the eastern tropical and subtropical South Pacific (JX280966 – JX281688). Red boxes represent phylogenetic clusters containing OMZ phytoplankton. Dots at nodes represent branches with support values of ≥ 70%. The scale bar indicates the expected changes per sequence position (Note: The scale only applies to the branches of the tree, the boxes are not scaled).
Fig. 7.5
Presence/absence dot plot of archaeal taxa at various sample points and depths in the northeastern subarctic Pacific (NESAP; labeled P4, P12, and P26), the eastern subtropical South Pacific (ESP), the Namibian upwelling (NAM), the Peru Upwelling (PU), Saanich Inlet (SI), the Black Sea (BLACK), the Baltic Sea (BALTIC), and the Cariaco Basin (CB), based on small-subunit ribosomal RNA (SSU rRNA) gene sequence profiles. Samples are organized according to the similarity of their community composition, as revealed by hierarchical clustering of the distribution of taxonomic groups across environmental samples. Names for identifying bacterial groups were selected according to the taxonomic level at which the most relevant information was available (Data used to generate the dot plot were derived from sequences deposited in Genbank)
(e.g., sub-seafloor sediments, deep-sea hydrothermal vents, and cold seeps). Although these similarities likely reflect recurring patterns of niche selection based on convergent environmental conditions (e.g., oxygen depletion), the precise ecological and biogeochemical roles of archaea in OMZs and other seafloor environments remain poorly constrained.

### OMZ Prokaryotes as Biogeochemical Players

For almost a decade, OMZ gene surveys have focused extensively on microbes performing denitrification and anaerobic ammonia oxidation (anammox). Studies of the functional gene nitrite reductase (nirS and nirK) suggest that denitrification is mediated by a broad range of microorganisms from diverse taxonomic groups, which in turn vary among OMZ regions for reasons not yet clear (Jayakumar et al. 2004, 2009 Castro-González et al. 2005; Ward et al. 2009). In contrast, the OMZ anammox bacteria are much less diverse, with members clustering exclusively with the marine genus “Candidatus Scalindua” within the Planctomycetes (Hamersley et al. 2007; Woebken et al. 2008; Galán et al. 2009). While monophyletic, this sequence cluster contains high micro-diversity (Woebken et al. 2008; Galán et al. 2009) and the corresponding genomic and functional variations of micro-diverse clusters remain unknown. Analysis of microbial community gene expression from the anoxic core of the ETSP OMZ has revealed a dominance of transcripts matching the freshwater anammox species “Candidatus Kuenenia stuttgartiensis” (Strous et al. 2006), encompassing many of the anammox-specific functional gene repertoires (e.g., hydrazine oxidoreductase) (Stewart et al. 2012). The prevalence of sequences matching Kuenenia rather than Scalindua in early OMZ studies could reflect a lack of whole genome sequence information in public databases. During composition of this chapter, the draft genome sequence of the marine anammox bacteria “Candidatus Scalindua profunda” was reported (van de Vossenberg et al. 2012), expanding the range of fragment recruitment platforms for sequence analysis.

As indicated in the previous section, molecular surveys have recovered abundant and diverse sulfur-oxidizing microbial groups in the OMZ water column, with sequences affiliated with SUP05 and ARCTIC96BD-19. These results were unexpected, as an active sulfur cycle was not envisioned in nitrate- and nitrite-rich OMZ water columns (Canfield et al. 2010). Based on marker gene sequences (e.g., SSU rRNA gene, APS reductase gene aprA), sulfur-oxidizing bacteria from OMZs harbor many of the same functional properties as bacteria inhabiting other sulfidic marine habitats (Sulfurimonas-like ε-proteobacteria, green sulfur bacteria) (Stevens and Ulloa 2008; Lavik et al. 2009; Canfield et al. 2010; Stewart et al. 2012).

Metagenomic analysis of members of the SUP05 clade from Saanich Inlet revealed that they contain genes for carbon fixation, dissimilatory reduction of nitrate to nitrous oxide, and oxidation pathways for diverse reduced sulfur species such as sulfide, sulfite, elemental sulfur, and thiosulfate (Walsh et al. 2009). This sequence information revealed the genetic potential for chemolithoautotrophic oxidation of reduced sulfur with nitrate in the water column of marine OMZs. Though well described for bacteria in other anoxic marine environments, for example, sediments and sulfidic zones of anoxic marine basins (Fossing et al. 1995; Sunamura et al. 2004; Campbell et al. 2006), dissimilatory sulfur-oxidation coupled with nitrate reduction to N2O and carbon fixation constitutes a form of autotrophic denitrification in OMZs. This process has been implicated in the sulfide detoxification of the shelf waters off the Namibian coast (Lavik et al. 2009) and has been observed to occur in waters of the ETSP OMZ (Canfield et al. 2010). In the latter study, metagenomic analysis showed that up to 16% of all protein-coding genes matched diverse sulfur-oxidizing taxa. SUP05 was particularly well represented, with over 80% of its genes present in this dataset at an average amino acid similarity of 70%. Metatranscriptome sequencing from this site confirmed that genes for diverse sulfur oxidation pathways are actively transcribed in situ along with symbiont-like nitrate reductases in the core of the anoxic OMZ (Stewart et al. 2012).

While the pelagic OMZ microbiota is dominated by symbiont-like sulfur-oxidizers, active sulfate-reducing assemblages are also indicated. Canfield et al. (2010) demonstrated surprisingly high rates of sulfate reduction to sulfide in experiments in the ETSP OMZ. Coupled metagenomic data for this community revealed sequences, including those encoding dissimilatory sulfur metabolism genes (aprA, dsrB), matching the genomes of known sulfate reducers of the δ-proteobacteria (e.g., Desulfatibacillum, Desulfofobacterium sp.). Together, these results revealed a cryptic sulfur cycle in which sulfate reducers provide sulfide that is immediately consumed by a diverse oxidizer community. Moreover, sulfate reducers could also provide ammonium for anammox bacteria, another manifestation of the tight coupling between the sulfur and nitrogen cycles in OMZs.

Anoxic OMZs can also impinge on the photic zone, creating a unique environment for photoautotrophs, and particularly oxygenic ones adapted to low-oxygen tensions. The latter could provide a local source of oxygen to feed aerobic processes (e.g., nitrification) in a typically anoxic environment. Indeed, picocyanobacteria of the genera Prochlorococcus and Synechococcus are frequent inhabitants of low-light oceanic OMZ waters of the Arabian Sea, ETNP and ETSP (Johnson et al. 1999; Goericke et al. 2000; Galán et al. 2009). A recent study in the eastern tropical Pacific showed that OMZ Prochlorococcus communities contain novel phylotypes (Lavin et al. 2010). The genomic characteristics of these OMZ photoautotrophs remain to be determined. They may provide new insights about the evolution of photosynthesis as the planet and the ocean became oxygenated.

While anaerobic microorganisms performing nitrogen and sulfur transformations characterize the core of the OMZ, the oxycline and low oxygen waters of the upper OMZ are critical zones for aerobic nitrifying microorganisms, particularly the AOA. Early studies pointed to a significant role for the process of ammonia oxidation in OMZs, particularly at the upper boundaries (e.g., Ward and Zafiriou 1988; Ward et al. 1989; Lipschultz et al. 1990). Catalyzed by the ammonia
monooxygenase (Amo) enzyme, the ability to oxidize ammonia was originally thought to be restricted to a few groups within the γ- and β-proteobacteria. However, metagenomic studies performed in the last decade revealed the existence of unique amoA genes derived from uncultivated, non-extremophilic Crenarchaeota (Venter et al. 2004; Hallam et al. 2006; Treusch et al. 2005), now recognized as a separate phylum, the Thaumarcheota (Fig. 7.4). In addition, an isolate of the marine thaumarchaeon *Nitrosopumilus maritimus* demonstrated a capacity for growth using ammonia oxidation as an energy source, resulting in stoichiometric production of nitrite (Könneke et al. 2005). Subsequently, high abundances of archaeal amoA genes have been detected in a variety of oxygen-deficient marine environments including the OMZs of the ETNP and ETSP, and the suboxic zones of the Black Sea, the Gulf of California, and the Baltic Sea (Francis et al. 2005; Coolen et al. 2007; Lam et al. 2007; Beman et al. 2008; Molina et al. 2010). Metatranscriptomic analysis in the ETSP showed that up to 20% of all protein-coding transcripts matched *N. maritimus* in the upper OMZ and that thaumarcheotal amo genes were highly transcribed in this zone (Stewart et al. 2012). These results reinforce the emerging perspective that thaumarcheotal ammonia-oxidation contributes substantially to nitrogen cycling in diverse marine environments (Wuchter et al. 2006; Prosser and Nicol 2008).

In addition to playing key roles in nitrogen and sulfur cycling, OMZ microorganisms may contribute a substantial proportion of fixed organic carbon. Sulfur-oxidizers like SUP05, for example, harbor genes for inorganic carbon fixation through the Calvin-Benson-Bassham cycle (Walsh et al. 2009), while amanox bacteria can make use of the acetyl-coenzyme A (CoA) pathway for carbon fixation (Strous et al. 2006). Isolation of the ammonia-oxidizing thaumarchaeon *N. maritimus* also revealed a capacity for chemolithoautotrophic growth on ammonia as a sole energy source and bicarbonate as a sole carbon source (Könneke et al. 2005). Subsequent sequencing of the *N. maritimus* genome confirmed that it contains genes for the 3-hydroxypropionate/4-hydroxybutyrate (3-HP/4-HB) pathway of autotrophic carbon fixation (Walker et al. 2010). The actual contribution of these groups (and others) to the carbon economy of OMZs remains to be determined.

**Summary**

OMZs were traditionally seen as regions dominated by heterotrophic denitrification fueled by the sinking of organic matter produced via photosynthesis in the sunlit surface ocean. They were also considered to have a fundamentally different microbiology and biogeochemistry than euxinic basins. The discovery of new microbial processes, such as amanox, and the recognition of an active but cryptic sulfur cycle in anoxic OMZs have significantly shifted the old paradigm. The recurring patterns of bacterial and archaeal community composition shared along the oxygen gradient of different pelagic ecosystems are consistent with fundamental organizing principles at work on different ecological scales. To identify and harness these principles, future studies are needed that explore the genomic information and physiological properties of isolates and whole communities from diverse OMZs.

**Acknowledgments**

This chapter was prepared while the lead author (O. Ulloa) was on sabbatical at the Department of Microbiology and Immunology, University of British Columbia, supported by the Life Sciences Institute Visiting Scholar Award, the Peter Wall Institute for Advanced Studies International Visiting Research Scholar Award, and a gift from the Agouron Institute.

This work was performed under the auspices of the Agouron Institute, the Gordon and Betty Moore Foundation, the FONDAP Program of the Chilean National Commission for Scientific and Technological Research (CONICYT), the Natural Sciences and Engineering Research Council (NSERC) of Canada, the Canada Foundation for Innovation, and the Canadian Institute for Advanced Research. J. J. Wright was supported by NSERC. L. Belmar was supported by CONICYT.

**References**


