Draft Manuscript for Review

Low temperature adaptation of Ribulose-1,5-bisphosphate carboxylase/oxygenase in the model photopsychrophile, Chlamydomonas raudensis UWO241 (CHLOROPHYCEAE, CHLOROPHYTA)

Journal:	*Extremophiles*
Manuscript ID:	Draft
Manuscript Type:	Original Paper
Date Submitted by the Author:	n/a
Complete List of Authors:	Feasel, Patrick; Miami University, Department of Microbiology Dolhi, Jenna; Miami University, Department of Microbiology Hanson, Thomas; University of Delaware, Delaware Biotechnology Insitute; University of Delaware, Department of Biological Sciences Morgan-Kiss, Rachael; Miami University, Department of Microbiology
Keyword:	Antarctic microbiology, Bioenergetics, Cold adaptation, Phototorophic psychrophiles, Psychrophile physiology, Enzymology

Low temperature adaptation of Ribulose-1,5-bisphosphate carboxylase/oxygenase in the model photopsychrophile, *Chlamydomonas raudensis* UWO241 [1]

Patrick C. Feasel [3], Jenna M. Dolhi [3], Thomas E. Hanson[4], Rachael M. Morgan-Kiss [2,3]

[1] Date of submission: ; Date of acceptance:

[2] Phone: (513) 529-5434; Fax: (513) 529-2431; e-mail: morganr2@muohio.edu

[3] Department of Microbiology, Miami University, Oxford, Ohio 45056 USA

[4] College of Earth, Ocean, and Environment, Delaware Biotechnology Institute & Department of Biological Sciences, University of Delaware, Newark, Delaware, 19711 USA

Running Title: Cold Adaptation of RubisCO

Abstract

The green algal species, *Chlamydomonas raudensis*, has representative psychrophilic and mesophilic strains, named UWO241 and SAG 49.72, respectively. UWO241 exhibits an upper growth temperature limit of 16°C, while SAG 49.72 can not grow below 12°C. Despite their close phylogenetic relationship, strain UWO241 exhibits numerous unique environmental adaptations relative to its mesophilic counterpart at the level of its photochemical apparatus. To date, little is know about adaptation of enzymatic pathways in this enigmatic alga. We report that the key enzyme for CO_2-fixation, Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO), is cold adapted in UWO241 compared with RubisCO from the mesophilic SAG 49.72. Furthermore, *in vivo* RubisCO activity was strongly correlated with growth rate in cultures of UWO241 grown under variable irradiance. In contrast, no correlation between maximum growth rate and RubisCO activity was observed in response to UWO241 cultures grown at variable temperatures. We conclude that rates of *in vivo* RubisCO activity show acclimation to irradiance but not temperature in the psychrophilic *C. raudensis* UWO241.

Key Words

Antarctica; Cold adaptation; *Chlamydomonas raudensis*; Photopsychrophile; Photostasis; RubisCO

Introduction

Permanently low temperature environments represent the largest biosphere on earth and contain the largest proportion of cellular biomass. This biomass accumulation is largely due to metabolic activity driven by diverse microbial communities that thrive in cold habitats (Feller 2003; Morgan-Kiss et al. 2006). Low temperature-adapted photosynthetic microorganisms (photopsychrophiles) play essential roles in cold environments as the dominant primary producers in most microbial food webs. This role is accomplished by acquiring light energy by photochemical reactions and utilizing this energy to fix inorganic carbon into biologically useful forms of organic carbon molecules. Photochemical energy acquisition is temperature independent while metabolic energy utilization is temperature dependent making photostasis, the balance between acquisition and utilization, a challenge for photopsychrophiles. Photostasis has been well characterized in model temperate algal species and largely involves functional and structural alterations within the photochemical apparatus (Wilson et al. 2006); however, our knowledge of photostasis in photopsychrophiles is comparably limited.

Focused research on a small number of photopsychrophiles has begun to answer some questions regarding adaptation of photochemical processes to the cold (Baldisserotto et al. 2005b; Baldisserotto et al. 2005a; Ferroni et al. 2007; Mock and Kroon 2002a, b; Mock and Valentin 2004; Morgan-Kiss et al. 2006). A particularly useful model system is the green alga *Chlamydomonas raudensis* UWO241 originally isolated by Priscu and coworkers (Neale and Priscu 1995) from the deepest photic zone of a perennially ice-covered lake in the Dry Valleys of Antarctica. The aquatic environment from which strain UWO241 was isolated is extreme, characterized by year-round temperatures of 0-5°C, deep shade conditions of a peculiar (blue-

green) spectral range, hypersalinity, and dissolved oxygen concentrations three times saturation. Microflora residing in the lakes have also been isolated under stable environmental conditions by the perennial ice cover for perhaps thousands of years. Strain UWO241 exhibits novel adaptive strategies to persist in this environment of multiple extremes (Gudynaite-Savitch et al. 2006; Morgan-Kiss et al. 2006; Pocock et al. 2007). More recent studies have included the mesophilic type strain of *C. raudensis*, SAG 49.72 (Pocock et al. 2007; Szyszka et al. 2007). Strains UWO241 and SAG 49.72 are indistinguishable on the basis of 18S rRNA and ITS region sequences (Pocock et al. 2004), yet they exhibit a variety of distinct physiological characteristics, including mechanisms for maintaining photostasis and differential phosphorylation patterns of major photosynthetic proteins (Szyszka et al. 2007; Takizawa et al. 2009).

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) (EC 4.1.1.39) catalyzes the key step in the Calvin-Benson-Bassham (CBB) cycle making it one of the primary determinants of plant and algal primary productivity. This step involves carboxylation of ribulose-1,5-bisphosphate (RuBP) to produce two molecules of phosphoglyceric acid (PGA) that can be used to synthesize cellular biomass. RubisCO also displays oxygenase activity, the degradation of RuBP to PGA and phosphoglycolate. Extensive studies in numerous organisms have produced a comprehensive understanding of regulation of gene expression, small subunit import into the chloroplast, holoenzyme assembly, reaction mechanism and evolution of this key photosynthetic enzyme (Gutteridge and Gatenby 1995; Hartman and Harpel 1994; Tabita 1999; Tabita et al. 2007). The decline of RubisCO activity at low temperatures has been associated with decreases in catalytic turnover (Bruggemann et al. 1992b; Hirotsu et al. 2005) and activation state (Byrd et al. 1995).

We hypothesized that RubisCO in UWO241 should also possess unique adaptive

characteristics. Investigations on carbon metabolism in cold-adapted algae has been limited to a few studies (Devos et al. 1998b; Haslam et al. 2005), and despite its importance in carbon acquisition, a cold adapted RubisCO has yet to be discovered in a psychrophilic photoautotroph. Here we report comparative analyses on RubisCO phylogeny and activity in the related psychrophilic and mesophilic strains of *C. raudensis* UWO241 and SAG 49.72. Our results indicate that the RubisCO expressed by strain UWO241 is cold adapted compared with that of SAG 49.72. Furthermore, strain UWO241 maintains photostasis by controlling the level and activity of RubisCO enzyme in response to variable light and temperature growth regimes. This suggests that, unlike temperate algae, strain UWO241 may primarily regulate energy utilization rather than energy acquisition to maintain a balance between the two processes.

Materials and Methods

Strains and growth conditions

Chlamydomonas raudensis SAG 49.72 was isolated by H. Ettle from a meadow pond near Rudná, Nordmähren, Czech Republic, and *Chlamydomonas raudensis* UWO241 was isolated by J. Priscu and co-workers from Lake Bonney, Antarctica (Neale and Priscu 1995). Both strains were grown in a modified Bold's Basal Medium as batch cultures (Morgan et al. 1998)(Morgan-Kiss et al., 2008). Unless otherwise stated, UWO241 was grown at 8°C/20 µmol photons m^{-2} s^{-1} and SAG 49.72 was grown at 29°C/150 µmol m^{-2} s^{-1}. Growth rates were calculated by monitoring either total chlorophyll or optical density at 750 nm (Morgan et al. 1998).

Molecular analysis

Genomic DNA was extracted from both strains using a genomic DNA extraction kit (Fermentas). A partial *C. raudensis* UWO241 *rbcL* sequence is available (Accession Number: ABA42220). This sequence was used to design primers to amplify the full length *rbcL* gene (5'-ATGGTCCCTCAAACAGAA-3' and 5'-GGTTAAAGTTTGTCAATAGTATC-3' for RbcL-UWO-F and RbcL-UWO-R, respectively). PCR products were cloned into the pGEM vector (Promega) and sequenced in both directions using vector primers at the Center for Bioinformatics and Functional Genomics at Miami University. Sequences from closely-related organisms were selected using BLAST to search GenBank. An alignment was produced in ClustalW using 1275 nucleotide positions. A neighbor-joining analysis and bootstrap consensus tree (500 replicates) was constructed in MEGA4 (Tamura et al. 2007), using the Kimura 2-parameter distance model with pairwise gap deletion.

Cell extract preparation

Soluble lysates for enzymatic assays and protein analyses were isolated from mid-log phase cultures. To prepare fully-activated RubisCO lysates, cells were pelleted and resuspended in an ice-cold CO_2-saturated grinding buffer (100 mM Bicine, pH 8.0; 20 mM $MgCl_2$; 1 mM EDTA; 1 mM DTT; 3.3 mM amino-caproic acid; 0.7 mM benzamidine; 150 µM $NaHCO_3$). Cells were broken using a Mini-Beadbeater (Biospec) according to (He and Vermaas 1998). Unbroken cells and debris were removed by centrifugation for 15 mins at 10,000 x *g* at 4°C and transferring the supernatant to a fresh tube. Lysates were used directly for RubisCO assays, or supplemented

with 10% glycerol and flash frozen in liquid N_2 and stored at -80^{o}C until use.

RubisCO assays

The RubisCO assay used in this study was a modified version of the carboxylase spectrophotometric assay described by Lan and Mott (1990) and was carried out at room temperature unless otherwise specified. All buffers were prepared under O_2-free conditions by bubbling with N_2 for 15 mins and storage in air-tight tubes capped with rubber stoppers. To fully activate RubisCO, lysates were incubated in the presence of 15 mM Mg^{2+} and 130 µM CO_2 at least 10 mins prior to the assay. The standard assay buffer contained 20 mM Bicine, 15 mM $MgCl_2$, 10 mM NaCl, 5mM DDT, 5 mM $NaHCO_3$, 5 mM Phospho-creatine, and 5 mM ATP. At least 3 mins prior to the assay, the coupling reaction, containing 1 mM RuBP, 0.3 mM NADH,10 U/mL glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12), 10 U/mL 3-phosphoglyceric phosphokinase (EC 2.7.2.3), and 2 U/mL creatine phosphokinase (EC 2.7.3.2), was added to 0.5 mL of assay buffer. Typically 5-10 µg of lysate was added to the reaction, and the oxidation of NADH was measured spectrophotometrically (λ=340) over time for up to 5 mins. The activity was calculated by converting absorbance to rate of NADH oxidation using the extinction coefficient 6.22 mM^{-1} (Lan and Mott 1990). All substrates and coupling enzymes were purchased from Sigma-Aldrich. Note that RuBP was purchased in the highest purify form (Order number: 83895; BioChemika, ≥99.0% purity; TLC) to avoid the inhibitory effects of contaminating sugars in less pure preparations.

The effect of assay temperature on RubisCO activity was assessed using a modified version of the standard assay. Crude lysates were incubated in sample buffer supplemented with

1 mM RuBP at various temperatures for 5 mins. This reaction was stopped by loading the lysate into a 100,000 MWCO Microcon® spin column (Millipore) and centrifuging at 10,000 x *g* for two minutes. PGA concentration in the filtrate was quantified as in the standard assay above with the exception that RuBP was omitted.

Thermolability of RubisCO was assessed by incubating crude lysates in sample buffer in the absence of the substrate RuBP at various temperatures for 10 mins. Following heating, samples were cooled on ice for 5 mins prior to measurement of residual activity using the standard assay.

In vivo activity of RubisCO was assessed by extraction of lysates from log-phase cells using a CO_2-free extraction buffer. Activity was measured using the standard assay within 15 mins of extraction. RubisCO activity for a minimum of 3 assays were compared between the two species at all incubation temperatures by homoscedastic two-tailed t-tests assuming no difference between means conducted in Excel (Microsoft). P-values of <0.05 were considered significant.

Results

Temperature dependence of growth

The effect of temperature on growth rate was determined for the algal species *C. raudensis* UWO241 and SAG 49.72. Strain UWO241 exhibited an optimal growth temperature of 8°C with growth detected up to 16°C and down to 2°C (Fig. 1). Growth was not observed at 20°C or higher, confirming that strain UWO241 is psychrophilic. In contrast, the growth rate of SAG 49.72 progressively decreased below 30°C (Fig. 1) and could not be detected at 8°C or lower.

Thus, we were able to compare environmental adaptation of a key photosynthetic enzyme between mesophilic and psychrophilic algal strains of the same species as determined by molecular methods (Pocock et al. 2004).

Phylogeny of rbcL

A fragment of the *rbcL* gene encoding the LSU of form I RubisCO was amplified from both *C. raudensis* strains (1334 bp and 1370 bp from SAG 49.72 and UWO241, respectively). Phylogenetic analyses of the *rbcL* gene (Fig. 2) demonstrated that the amplified sequence encodes a form IB RubisCO LSU as do all other chlorophytes and land plants (Tabita et al. 2007). Although strains UWO241 and SAG 49.72 possess identical 18S rRNA and ITS sequences (Pocock 2004), the *rbcL* genes of UWO241 and SAG 49.72 share only 88% nucleotide sequence identity and the predicted RbcL sequences share 95% identity.

The *rbcL* sequences from Antarctic chlorophytes (Devos et al. 1998a; Eddie et al. 2008; Hoham et al. 2002) were included in the alignment. The *rbcL* of UWO241 exhibited the highest identity (93%) to a *rbcL* sequence from a *Chloromonas* sp. isolated from ice matrix of Antarctica (Liu et al. 2006), while SAG 49.72 *rbcL* was more closely related (96% identity) to *C. acidophila* and *C. pitschmanni* (Fig. 2). Interestingly, we have recently identified several new *rbcL* sequences from the natural habitat of UWO241 (18m depth of East Lobe Bonney, Dry Valleys, Antarctica) which are closely related to *rbcL* found in other *Chlamydomonas* spp. isolated from polar environments (Kong & Morgan-Kiss, unpublished).

Thermal properties of RubisCO

The temperature-dependence of carboxylase activity in strains UWO241 and SAG 49.72 was assessed. For these assays, RubisCO was fully activated, and then lysates were incubated in the presence of 5 mM RuBP for 5 mins at a range of temperatures between 0 and 70°C followed by spectrophotometric PGA quantification (Fig. 3a). The temperature range for optimal activity for the mesophile ranged between 25°C and 60°C. In contrast, carboxylase activity of the psychrophilic RubisCO exhibited maximum values at 25°C and showed a decrease in activity above this temperature. More notably, the psychrophile exhibited higher RubisCO activity at temperatures below 25°C (Fig. 3a). Statistical analyses between the psychrophilic and mesophilic lysates at all temperatures indicated that RubisCO activity was significantly different between the two algal species at all temperatures ($p<0.05$), with the exception of assays tested at 25°C where no significant difference was noted ($p=0.23$). These data show that while the psychrophilic strain possesses higher RubisCO activity at low temperatures, cold adaptation is not associated with overall lower maximum carboxylase activity, as has been observed in other psychrophilic algae (Devos et al. 1998b).

The thermolability of RubisCO in strains UWO241 and SAG 49.72 was also assessed in psychrophilic and mesophilic lysates by fully activating RubisCO for at least 5 mins in the presence of Mg^{2+} and CO_2 at room temperature prior to incubation at temperatures ranging from 20 to 90°C for 10 mins followed by RubisCO assay at the optimal temperature for each extract (Fig. 3b). The resulting activities were plotted as the percentage of activity in untreated samples that remained after treatment. RubisCO activity in both strains showed no significant differences when exposed to temperatures less than 40°C ($p>0.10$). At higher temperatures, strain UWO241 RubisCO was more severely affected by exposure and this was most pronounced at 60°C, which

resulted in a 45% loss in initial activity while SAG 49.72 incubated at the same temperature displayed only a 12% decrease ($p=0.04$) (Fig. 3b). RubisCO was fully inactivated in both strains at or above 70°C.

Irradiance, not temperature, correlates with growth rate and RubisCO activity in UWO241

We assessed adjustments in RubisCO activity in the photopsychrophile in response to changes in either the light or temperature growth regime (Fig. 4). When cultures were grown under optimal growth temperature (8 °C) and variable irradiance, there was a linear increase in growth rate and *in vivo* RubisCO activity as a function of increasing irradiance (Fig. 4a). When cultures were grown under constant irradiance (20 µmol m^{-2} s^{-1}) and variable temperature, UWO241 exhibited maximum growth rates at 8°C (Fig. 1). However, maximum RubisCO activity was observed at 2°C, and declined at higher growth temperatures (Fig. 4b). Last, a strong positive relationship was observed between RubisCO activity and growth rate as a function of irradiance (Fig. 4c, circles; $r^2= 0.96$). In contrast, there was no correlation between RubisCO activity and growth rate at variable temperatures (Fig. 4c, squares; $r^2= -0.13$).

Discussion

Photosynthetic microorganisms play key roles in primary production and food web development in all environments, including the extreme aquatic habitat of permanently cold lakes. Microorganisms living in year-round low temperatures have adapted to deal with the detrimental combination of light and low temperatures (Morgan-Kiss et al. 2006); however, these adaptive

mechanisms are not well understood. Low temperature-adapted phototrophs share common adaptive mechanisms with other cold-adapted organisms, such as polyunsaturated fatty acids (Mock and Kroon 2002a, b; Morgan-Kiss et al. 2002), a higher requirement for adenylate pools (Amato and Christner 2009; Napolitano and Shain 2004, 2005) as well as ATP (Morgan et al. 1998), and cold-active enzymes (Loppes et al. 1996; Rigano et al. 2006; Siddiqui and Cavicchioli 2006). In addition, the photochemical apparatus is also altered in photosynthetic algae isolated from polar environments as an adaptive response to the combination of constant low temperatures under 24-hr light (Baldisserotto et al. 2005b; Baldisserotto et al. 2005a; Mock and Valentin 2004; Morgan-Kiss et al. 2008; Morgan-Kiss et al. 2006). New comparative reports between the closely related *C. raudensis* strains UWO241 and SAG 49.72 indicate that the extreme conditions associated with isolation in a permanently iced-capped lake has produced novel photostasis mechanisms in the psychrophilic UWO241 (Pocock et al. 2007; Szyszka et al. 2007; Takizawa et al. 2009). The comparative analysis of the mesophile *C. raudensis* SAG 49.72 and *C. raudensis* UWO241 from the perspective of the major route of carbon assimilation, RubisCO, suggests further differences between these two closely related strains.

RubisCO activity is tightly controlled in plants and algae both at the level of expression and posttranslational activation. Several studies in temperate plants (Bruggemann et al. 1992a; Nie et al. 1995; Sassenrath et al. 1990) and algae (Savitch et al. 1996) have shown that net CO_2 fixation, RubisCO activity, and RuBP regeneration are all sensitive to low temperatures. Reductions in CO_2 assimilation have been linked to a decline in requirements for photochemically-derived energy equivalents and a downregulation in photochemistry (Wilson et al. 2006). However, few studies have addressed the role of carbon metabolism in organisms adapted to permanent low temperatures. Devos et al. (1998b) reported that RubisCO isolated

from two psychrophilic *Chloromonas* species exhibited an increase in thermolability compared with the mesophilic *C. reinhardtii* enzyme, but no difference in activity at lower temperatures was observed. Here, strain UWO241 RubisCO was also more thermally labile that strain SAG 49.72. Thus, we conclude that UWO241 RubisCO activity displays psychrophilic adaptation.

Growth rate of UWO241 did not correlate well with *in vivo* RubisCO activities above a growth temperature of 2°C where growth rate was essentially constant while RubisCO activity decreased. This probably indicated that at temperatures higher than 2°C, RubisCO activity was limited by factor(s) other than low temperatures. One possibility is limitation at the level of RuBP regeneration via the CBB cycle (Hirotsu et al. 2005; Sage et al. 1990). Since the CBB cycle is a major consumer of photochemically-derived NADPH/ATP, these cultures may have been limited by rates of photosynthetic electron transport under low light (20 μmol m^{-2} s^{-1}), which may provide an explanation for the strong correlation observed between RubisCO activity and irradiance. It is also possible that affinity of the psychrophilic RubisCO for CO_2 varies at different growth temperatures. Last, it is also possible that temperature-associated variations in RubisCO activity may be influenced by the activation state, which is controlled by the RubisCO activase (Rca) in plants and some algae. Antarctic plants have been shown to possess thermally sensitive Rca (Salvucci and Crafts-Brandner 2004).

Strain UWO241 is incapable of decreasing the level of light harvesting machinery at high irradiance (Morgan-Kiss et al. 2006), a well conserved photoacclimatory response in model mesophilic algae such as *Chlorella vulgaris* (Maxwell et al. 1994; Wilson and Huner 2000). However, the growth rate of UWO241 is positively correlated with growth irradiances more than 10-fold higher than the natural light environment of this strain (Morgan-Kiss et al. 2006). We hypothesized that UWO241 could possess the ability to respond to the energy imbalance created

by low temperature/high light by increasing photosynthetic energy utilization (Morgan-Kiss et al. 2006). This report provides direct evidence in support of this hypothesis and suggests that the regulation of the CBB cycle and RubisCO is a major mechanism maintaining photostasis in this organism. Some of this regulation could occur postranslationally as higher irradiance actually decreases RubisCO expression while observed *in vivo* RubisCO activity is increased.

In conclusion, we hypothesize that the psychrophilic adaptation of RubisCO activity observed in these experiments results from a combination of psychrophilic adaptations in RubisCO catalysis, as suggested by the phylogenetic analysis, and differences in posttranslational activation by RubisCO activase in strain UWO241. Further analysis of the purified UWO241 enzyme and its cognate activase are underway to address this issue.

Acknowledgements:

The authors thank Dr. John Hawes at The Center for Bioinformatics and Functional Genomics at Miami University for assistance with sequencing. RMK was supported a grant from the NSF Office of Polar Programs (OPP-0631659) and TEH was supported by a grant from the NSF Directorate for Biological Sciences (MCB-0447649).

Literature Cited

Amato, P., and Christner, B.C. (2009) Energy metabolism response to low-temperature and frozen conditions in *Psychrobacter cryohalolentis*. App Env Microbiol 75: 711-718.

Baldisserotto, C., Ferroni, L., Moro, I., Fasulo, M.P., and Pancaldi, S. (2005a) Modulations of the thylakoid system in snow xanthophycean alga darkened for two months: comparison between microspectrofluorimetric responses and morphological aspects. Protoplasma 226: 125-135.

Baldisserotto, C., Ferroni, L., Andreoli, C., Fasulo, M.P., Bonora, A., and Pancaldi, S. (2005b) Dark-acclimation of the chloroplast in *Koliella antarctica* exposed to a simulated austral night condition. Arct Antarct Alp Res 37: 146-156.

Bruggemann, W., van der Kooji, T.A.W., and van Hasselt, P.R. (1992a) Long-term chilling of young tomato plants under low light and subsequent recovery. II. Chlorophyll fluorescence, carbon metabolism and activity of ribulose-1,5-bisphosphate carboxylase/oxygenase. Planta 186: 179-187.

Bruggemann, W., van der Kooji, T.A.W., and van Hasselt, P.R. (1992b) Long-term chilling of young tomato plants under low light and subsequent recovery. II. Chlorophyll fluorescence, carbon metabolism and activity of ribulose-1,5-bisphophate carboxylase/oxygenase Planta 186: 179-187.

Byrd, G.T., Ort, D.R., and Ogren, W.L. (1995) The effect of chilling in the light on ribulose-1,5-bisphosphate carboxylase/oxygenase activation in tomato (*Lycopersicon esculentum* Mill.). Plant Physiol 107: 585-591.

Crawford, D.L., and Powers, D.A. (1992) Evolutionary adaptation to different thermal environments via transcriptional regulation. Mol Biol Evol 9: 806-813.

Devos, N., Loppes, R., and Matagne, R. (1998a) Sequence of ribulose-1,5-bisphophate carboxylase/oxygenase large subunit gene (Accession No. AF089834) from a psychrophilic *Chloromonas* species (PGR98-196). Plant Physiol 118: 1534.

Devos, N., Ingouff, M., Loppes, R., and Matagne, R. (1998b) RUBISCO adaptation to low temperatures: a comparative study in psychrophilic and mesophilic unicellular algae. J Phycol 34: 665-660.

Eddie, B., Krembs, C., and Neuer, S. (2008) Characterization and growth response to temperature and salinity of psychrophilic, halotolerant *Chlamydomonas* sp. ARC isolated from Chukchi Sea ice. Mar Ecol Prog Ser 354: 107-117.

Feller, G. (2003) Molecular adaptations to cold in psychrophilic enzymes. Cell Mol Life Sci 60: 648-662.

Ferroni, L., Baldisserotto, C., Zennaro, V., Soldani, C., Fasulo, M.P., and Pancaldi, S. (2007) Acclimation to darkness in the marine chlorophyte *Koliella antarctica* cultured under low salinity: hypotheses on its origin in the polar environment. Eur J Phycol 42: 91-104.

Gudynaite-Savitch, L., Gretes, M., Morgan-Kiss, R.M., Savitch, L.V., Simmonds, J., Kohalmi, S.E., and Huner, N.P. (2006) Cytochrome f from the Antarctic psychrophile, *Chlamydomonas raudensis* UWO 241: structure, sequence, and complementation in the mesophile, *Chlamydomonas reinhardtii*. Mol Genet Genom 275: 387-398.

Gutteridge, S., and Gatenby, A.A. (1995) Rubisco synthesis, assembly, mechanism, and regulation. Plant Cell 7: 809-819.

Hartman, F.C., and Harpel, M.R. (1994) Structure, function, regulation, and assembly of D-ribulose-1,5-bisphosphate carboxylase/oxygenase. Annu Rev Plant Physiol Plant Mol Biol 41: 187-223.

Haslam, R.P., Keys, A., Andralojc, P.J., Madgwick, P.J., Andersson, I., Grimsrud, A. et al. (2005) Specificity of diatom Rubisco. In *Plant Responses to Air Pollution and Global Change*. Omasa, K., Nouchi, I., and De Kok, L.J. (eds). Tokyo: Springer-Verlag.

He, Q., and Vermaas, W. (1998) Chlorophyll a availability affects *psbA* translation and D1 precursor processing *in vivo* in *Synechocysis* sp. PCC 6803. Proc Natl Acad Sci USA 95: 5830-5835.

Hirotsu, N., Makino, A., Yokota, S., and Tadahiko, M. (2005) The photosynthetic properties of rice leaves treated with low temperature and high irradiance. Plant Cell Physiol 46: 1377-1383.

Hoham, R.W., Bonome, T.A., Martin, C.W., and Leebens-Mach, J.H. (2002) A combined 18S rDNA and rbcL phylogenetic analysis of *Chloromonas* and *Chlamydomonas* (Chlorophyceae, Volvocales) emphasizing snow and other cold-temperature habitats. J Phycol 38: 1051-1064.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Lan, Y., and Mott, K.A. (1990) Determination of apparent K_m values fro ribulose 1,5-bisphophate carboxylase/oxygenase (RubisCO) activase using the spectrophotometric assay of RubisCO activity. Plant Physiol 95: 604-609.

Liu, C., Huang, X., Wang, X., Zhang, X., and Li, G. (2006) Phylogenetic studies on two strains of Antarctic ice algae based on morphological and molecular characteristics. Phycologia 45: 190-198.

Loppes, R., Devos, N., Willem, S., Barthelemy, R., and Matagne, R.F. (1996) Effect of temperature on two enzymes from a psychrophilic *Chloromonas* (Chlorophyta). J Phycol 32: 276-278.

Maxwell, D.P., Falk, S., Trick, C.G., and Huner, N. (1994) Growth at Low Temperature Mimics High-Light Acclimation in *Chlorella vulgaris*. Plant Physiol 105: 535-543.

Mock, T., and Kroon, B.M. (2002a) Photosynthetic energy conversion under extreme conditions--I: important role of lipids as structural modulators and energy sink under N-limited growth in Antarctic sea ice diatoms. Phytochem 61: 41-51.

Mock, T., and Kroon, B.M. (2002b) Photosynthetic energy conversion under extreme conditions--II: the significance of lipids under light limited growth in Antarctic sea ice diatoms. Phytochem 61: 53-60.

Mock, T., and Valentin, K. (2004) Photosynthesis and cold acclimation: molecular evidence from a polar diatom. J Phycol 40: 732-741.

Morgan-Kiss, R., Ivanov, A.G., Williams, J., Mobashsher, K., and Hüner, N.P. (2002) Differential thermal effects on the energy distribution between photosystem II and photosystem I in thylakoid membranes of a psychrophilic and a mesophilic alga. Biochim Biophys Acta 1561: 251-265.

Morgan-Kiss, R.M., and Cronan, J.E. (2004) The Escherichia coli *fadK* (*ydiD*) gene encodes an anaerobically-regulated short-chain Acyl-CoA synthetase. J Biol Chem 279: 37324-37333.

Morgan-Kiss, R.M., Priscu, J.P., Pocock, T., Gudynaite-Savitch, L., and Hüner, N.P.A. (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiol Molec Biol Rev 70: 222-252.

Morgan-Kiss, R.M., Ivanov, A., Modla, S., Cyzmmek, K., Huner, N.P.A., Priscu, J.C., and Hanson, T.E. (2008) Identity and phylogeny of a new psychrophillic eukaryotic green alga, *Chlorella* sp. strain BI isolated from a transitory pond near Bratina Island, Antarctica. Extremophiles 12: 701-711.

Morgan, R.M., Ivanov, A.G., Priscu, J.C., Maxwell, D.P., and Hüner, N.P.A. (1998) Structure and composition of the photochemical apparatus of the Antarctic green alga, *Chlamydomonas subcaudata*. Photosynth Res 56: 303-314.

Napolitano, M.J., and Shain, D.H. (2004) Four kingdoms on glacier ice: convergent energetic processes boost energy levels as temperatures fall. Proc R Soc Lond B 271: 273-276.

Napolitano, M.J., and Shain, D.H. (2005) Distinctions in adenylate metabolism among organisms inhabiting temperature extremes. Extremophiles 9: 93-98.

Neale, P.J., and Priscu, J.C. (1995) The photosynthetic apparatus of phytoplankton from a perennially ice-covered Antarctic lake: acclimation to an extreme shade environment. Plant Cell Physiol 36: 253-263.

Nichols, H.W., and Bold, H.C. (1965) *Trichosarcina polymorpha* Gen. Et Sp. Nov. J Phycol 1: 34-38.

Nie, G.-Y., Robertson, E.J., Fryer, M.J., Leech, R.M., and Baker, N.R. (1995) Response of the photosynthetic apparatus in maize leaves grown at low temperature on transfer to normal growth temperature. Plant Cell Env 18: 1-12.

Pocock, T. (2004) Phylogeny, photoinhibition and recovery of a new Antarctic psychrophile Chlamydomonas raudensis (UWO 241). In *Biology*. London: University of Western Ontario.

Pocock, T., Koziak, A., Rosso, D., Falk, S., and Huner, H.P.A. (2007) *Chlamydomonas raudensis* ettl. (UWO241) exhibits the capacity for rapid D1 repair in response to chronic photoinhibition at low temperature. J Phycol 43: 924-936.

Pocock, T., Lachance, M.-A., Proschold, T., Priscu, J.C., Kim, S., and Huner, N.P.A. (2004) Identification of a psychrophilic green alga from Lake Bonney Antarctica: *Chlamydomonas raudensis* ETTL. (UWO 241) (*Chlorophyceae*). J Phycol 40: 1138-1148.

Portis Jr., A.R. (2003) Rubisco activase - Rubisco's catalytic chaperone. Photosynth Res 75: 11-27.

Rigano, M., Vona, V., Lobosco, O., Carillo, P., and Rigano, C. (2006) Temperature dependence of nitrate reductase in the psychrophilic unicellular alga *Koliella antarctica* and the mesophilic alga *Chlorella sorokiniana*. Plant Cell Environ 29: 1400-1409.

Sage, R.F., Sharkey, T.D., and Pearcy, R.W. (1990) The effect of leaf nitrogen and temperature on the CO_2 response of photosynthesis in the C3 dicot *Chenopodium album* L. Aust J Plant Physiol 17: 135-148.

Salvucci, M.E., and Crafts-Brandner, S.J. (2004) Relationship between the heat tolerance of photosynthesis and the thermal stability of rubisco activase in plants from contrasting thermal environments. Plant Physiol 134: 1460-1470.

Sassenrath, G.F., Ort, D.R., and Portis Jr., A.R. (1990) Impaired reductive activation of stromal bisphosphatase in tomato leaves following low-temperature exposure to high light. Arch Biochem Biophys 282: 302-308.

Savitch, L.V., Maxwell, D.P., and Huner, N. (1996) Photosystem II Excitation Pressure and Photosynthetic Carbon Metabolism in *Chlorella vulgaris*. Plant Physiol 111: 127-136.

Siddiqui, K.S., and Cavicchioli, R. (2006) Cold-adapted enzymes. Annu Rev Biochem 75: 403-433.

Szyszka, B., Ivanov, A.G., and Huner, N.P.A. (2007) Psychrophily induces differential energy partitioning, photosystem stoichiometry and polypeptide phosphorylation in *Chlamydomonas raudensis*. Biochim Biophys Acta 1767: 789-800.

Tabita, F.R. (1999) Microbial ribulose 1,5-bisphosphate carboxylase/oxygenase: A different

perspective. Photosyn Res 60: 1-28.

Tabita, F.R., Hanson, T.E., Li, H., Satagopan, S., and Chan, S. (2007) Function, structure and evolution of the RubisCO-like proteins and their RubisCO homologs. Microbiol Molec Biol Rev 71: 576-599.

Takizawa, K., Takahashi, S., Huner, N.P.A., and Minagawa, J. (2009) Salinity effects the photoacclimation of *Chlamydomonas raudensis* Ettl UWO241. Photosynth Res 99: 195-203.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007) MEGA4: Molecular Evolutionary genetic analysis (MEGA) software version 4.0. Molec Biol Evol 24: 1596-1599.

Wilson, K.E., and Huner, N.P. (2000) The role of growth rate, redox-state of the plastoquinone pool and the trans-thylakoid ΔpH in photoacclimation of *Chlorella vulgaris* to growth irradiance and temperature. Planta 212: 93-102.

Wilson, K.E., Ivanov, A.G., Oquist, G., Grodzinski, B., Sarhan, F., and Huner, N.P.A. (2006) Energy balance, organellar redox status and acclimation to environmental stress. J Bot 84: 1355-1370.

Yamori, W., Suzuki, K., Noguchi, K., Nakai, M., and Terashima, I. (2006) Effects of Rubisco kinetics and Rubisco activation state on the temperature dependence of the photosynthetic rate in spinach leaves from contrasting growth temperatures. Plant, Cell & Environ 29: 1659-1670.

Zhu, X.-G., Portis Jr., A.R., and Long, S.P. (2004) Would transformation of C_3 crop plants with foreign Rubisco increase productivity? A computational analysis extrapolating from kinetic properties to canopy photosynthesis. Plant, Cell & Environ 27: 155-165.

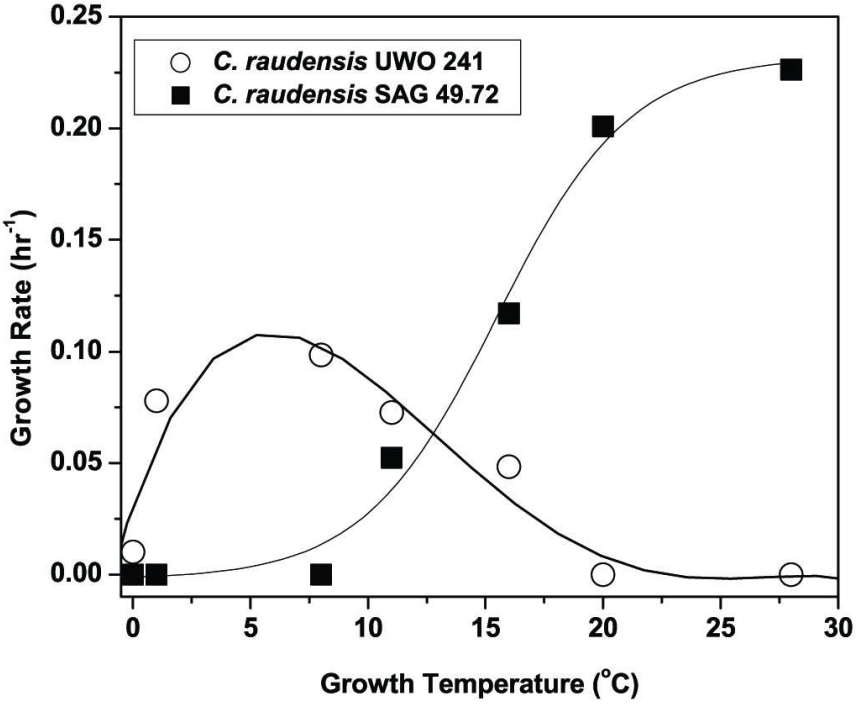
List of Figures

Figure 1. Temperature-dependence growth of the psychrophilic *Chlamydomonas raudensis* UWO241 in comparison with its closest mesophilic relative, *C. raudensis* SAG 49.72 (n=2-4).

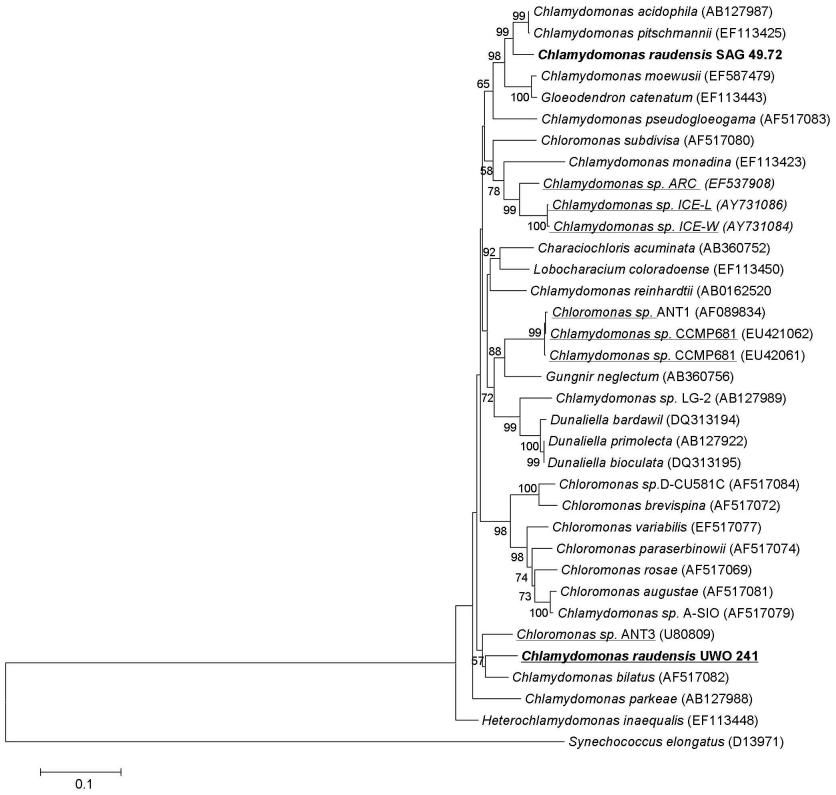
Figure 2. Phylogeny of the large subunit of Rubisco on the basis of *rbcL* sequences. Numbers are bootstrap values for 500 replicates using the neighbor joining method. Underlined sequences represent chlorophytes from polar environments.

Figure 3. A. Determination of optimal temperature for maximal RubisCO activity. Crude soluble fractions extracted from the mesophilic or psychrophilic *C. raudensis* were incubated in the presence of RuBP at various temperatures for 5 mins prior to assaying for activity (n=4-6). **B.** Thermolability of mesophilic and psychrophilic RubisCO. Crude soluble fractions were incubated for 10 mins at various temperatures in the absence of RuBP prior to assaying for RubisCO activity (n=3).

Figure 4. Relationship between RubisCO activity and maximum growth rate in *C. raudensis* UWO241 grown under variable temperature/light regimes. **A.** Effect of growth irradiance. Cultures were grown at a constant temperature of 8°C and varying growth irradiance. **B.** Effect of growth temperature. Cultures were grown at a constant irradiance of 20 µmol m^{-2} s^{-1} and varying growth temperature. **C.** Relationship between RubisCO activity and maximum growth rate at varying temperature/light regimes (squares, varying temperature; circles, varying irradiance). RubisCO activity was measured as µmol PGA produced/mg protein/min using lysates isolated from mid-log phase cultures (n=2-8).



Temperature-dependence growth of the psychrophilic Chlamydomonas raudensis UWO241 in comparison with its closest mesophilic relative, C. raudensis SAG 49.72 (n=2-4).
100x85mm (300 x 300 DPI)



Phylogeny of the large subunit of Rubisco on the basis of rbcL sequences. Numbers are bootstrap values for 500 replicates using the neighbor joining method. Underlined sequences represent chlorophytes from polar environments.
190x254mm (300 x 300 DPI)

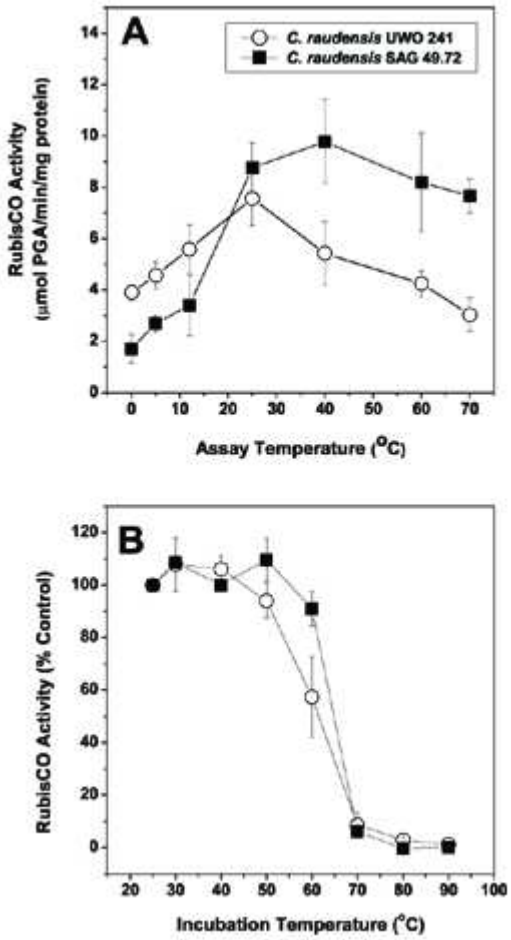
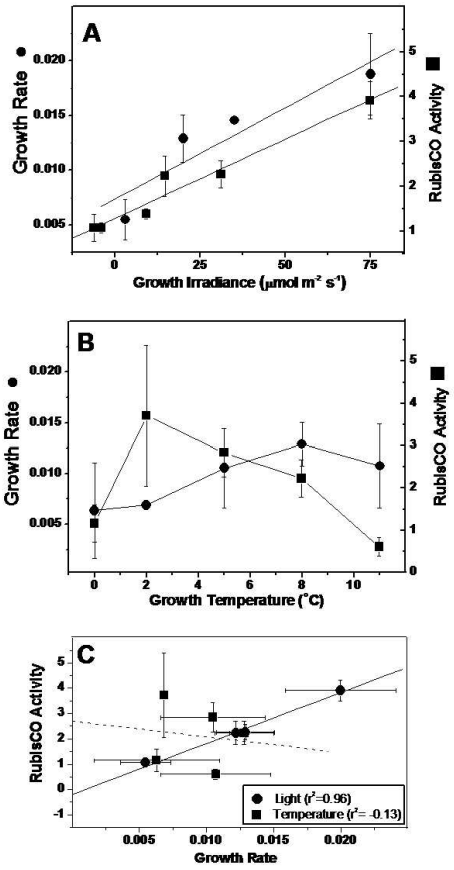


Figure 3. A. Determination of optimal temperature for maximal RubisCO activity. Crude soluble fractions extracted from the mesophilic or psychrophilic C. raudensis were incubated in the presence of RuBP at various temperatures for 5 mins prior to assaying for activity (n=4-6). B. Thermolability of mesophilic and psychrophilic RubisCO. Crude soluble fractions were incubated for 10 mins at various temperatures in the absence of RuBP prior to assaying for RubisCO activity (n=3).
30x52mm (250 x 250 DPI)



Relationship between RubisCO activity and maximum growth rate in C. raudensis UWO241 grown under variable temperature/light regimes. A. Effect of growth irradiance. Cultures were grown at a constant temperature of 8°C and varying growth irradiance. B. Effect of growth temperature. Cultures were grown at a constant irradiance of 20 µmol m-2 s-1 and varying growth temperature. C. Relationship between RubisCO activity and maximum growth rate at varying temperature/light regimes (squares, varying temperature; circles, varying irradiance). RubisCO activity was measured as µmol PGA produced/mg protein/min using lysates isolated from mid-log phase cultures (n=2-8).
67x131mm (300 x 300 DPI)