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15N Effects on Chlamydomonas Reinhardtii

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ABSTRACT

Non-radioactive isotopic labeling has become a regular technique for efficiently labeling a wide range of macromolecules. The overall goal for this project was to develop a method of globally measuring plant protein turnover rates. In order to do so, an isotopic labeled environment that does not induce stress had to be used. The objective of this particular segment of research was to develop a ^{15}N-labeled Tris-Acetate-Phosphate (TAP) media in which *Chlamydomonas reinhardtii* can successfully grow without eliciting stressed physiological responses. Our results illustrate that we have successfully developed ^{15}N-labeled TAP that does not stress *Chlamydomonas reinhardtii*.

INTRODUCTION

Over the past few years, proteomics has become an increasing field in both molecular and cellular biology. It is often described as an extensive analysis of proteins at both a molecular and cellular level. Proteomics generally uses an extensive amount of technology and information with its origin tied to 2-D gel electrophoresis. However, due to the ongoing advancements in technology, mass spectroscopy (MS) has become an uprising program for protein analysis. Isotopic labeling is heavily used in mass spectroscopy for it allows thorough examination of both free amino acids and peptides. ^{18}O, ^{15}N, ^{13}C and ^{2}H are some common isotopes used to label and examine proteins. Due to the recent completion of genomic sequencing for *Saccharomyces cerevisiae, S. cerevisiae* has become a model organism for proteomics (Shrager et al., 2003; Merchant et al., 2007).

The overall goal for this project is to develop a global measurement of plant protein turnover rates with the use of isotopic labels. ^{15}N, ^{2}H, and ^{13}C isotopes will be compared to determine the most efficient form of labeling proteins. The organism of study was *Chlamydomonas reinhardtii*. *Chlamydomonas* is a fresh water unicellular; double flagellate green algae that comes from the algal family of *Chlamydomonadaceae* (Proschold, Harris, and Coleman, 2005). Currently there are approximately 459 different species of *Chlamydomonas* that can often be characterized by common morphology, for example cell wall and cellular shape. *Chlamydomonas reinhardtii* CC-1266 (American Type Culture Collection, Manassas, VA) is the particular species that was used during the experiment. *Chlamydomonas reinhardtii* was selected based on the algae's capabilities to grow both photosynthetically by fixating carbon dioxide and non-photosynthetically by

using acetate as the primary source of carbon (Proschold, Harris, and Coleman, 2005). *Chlamydomonas* can be grown in a wide array of media, consisting of both solid and liquid forms. This particular experiment will utilize Tris-Acetate-Phosphate (TAP) media.

The primary goals for this segment of research was to modify TAP media so that all nitrogen containing chemical compounds were ^{15}N-labeled (^{15}N-TAP) and culture *C. reinahrdtii* in the newly modified media. The algae grown in both TAP and ^{15}N-TAP will also be examined for physiological responses, such as growth rates and flagella detachment.

MATERIALS AND METHODS

Material

The *Chlamydomonas reinhardtii* strain CC-1266 was used in this study.

Growth Media

TAP media was modified by either substituting nitrogen containing chemical compounds with ^{15}N-labeled chemicals or by replacing by nitrogen free chemicals. The composition of the TAP media is presented in Table 1. In order to substitute or eliminate nitrogen containing chemical compounds the following changes were made: ammonium chloride within TAP salt stock was replaced with ^{15}N-labeled ammonium nitrate (Table 2) and ammonium molybdate within Hutner's Trace Element stock was replaced with molybdic acid (Table 4). Therefore, the ^{15}N-TAP media was made up exactly the same way as the TAP media but using the modified Salt stock and the modified Hutner's Trace Element stock.

Table 1. Composition of TAP media. For the ^{15}N-labeled TAP, the ^{15}N Salt stocks and ^{15}N Hutner's Trace Elements were used.

	TAP
Salt stock (40X)	25 mL
Tris	2.42 g
Phosphate stock	0.375 mL
Glacial acetic acid	1 mL
Hutner's (1000X)	1 mL
H_2O	to 1 L

Table 2. Salt stock solutions for both the regular TAP and the ^{15}N-labeled TAP.

	Salt stock	^{15}N Salt stock
NH_4Cl	16 g	
$^{15}NH_4{}^{15}NO_3$		23.91 g
$MgSO_4{\cdot}7H_2O$	4 g	4 g
$CaCl_2{\cdot}2H_2O$	2 g	2 g
H_2O	to 1 L	to 1 L

Table 3. Phosphate stock.

	Phosphate stock
K_2HPO_4	288 g
KH_2PO_4	144 g
H_2O	to 1 L

Table 4. Hutner's Trace Element Stock for both the regular TAP and the ^{15}N-labeled TAP.

	Hutner's Trace Elements Stock	^{15}N Hutner's Trace Elements Stock
Na_2·EDTA	50 g	50 g
$ZnSO_4·7H_2O$	22 g	22 g
H_3BO_3	11.4 g	11.4 g
$MnCl_2·4H_2O$	5.06 g	5.06 g
$FeSO_4·7H_2O$	4.99 g	4.99 g
$CoCl_2·6H_2O$	1.61 g	1.61 g
$CuSO_4·5H_2O$	1.57 g	1.57 g
$(NH_4)_6Mo_7O_{24}·4H_2O$	1.10 g	
$Na_2MoO_4·2H_2O$		1.51 g
H_2O	to 1 L	to 1 L

The algae was grown in 10 mL culture tubes containing 6 mL of either TAP or ^{15}N-TAP. Each tube was inoculated with 100 µL of a saturated culture of *C. reinhardtii* (Fig 1).

RESULTS

The growth of the algae in TAP and ^{15}N-TAP was similar as can be seen on Fig. 2. For each media, 5 tubes were inoculated and their absorbance at 600 nm measured over time. The data was combined, the means calculated and the results are presented in Fig.2. *C. reinhardtii's* growth was not affected by the changes made to the media to incorporate the stable isotope.

No morphological differences were observed between the algae grown in TAP and ^{15}N-TAP. This was true for color of the cultures as well as for microscopic evaluation of the cells (Fig.3).

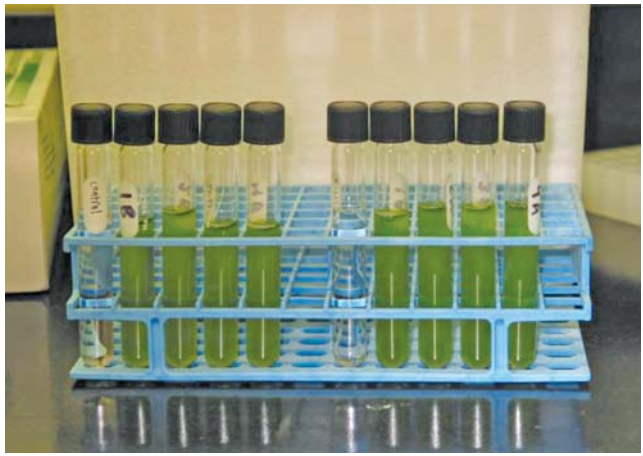

Figure 1. Culture tubes containing 6 mL of media and *C. reinhardtii*

Culture tubes were placed on a shaker and exposed to fluorescent light on a 16-hour (light) to 8-hour (dark) photoperiod under constant shaking. Culture tubes were placed directly in a spectrophotometer (Spectronic 20, Milton Roy Company) for direct determination of the absorbency at 600 nm. The data was recorded once or twice a day depending on the growth stage of the algae.

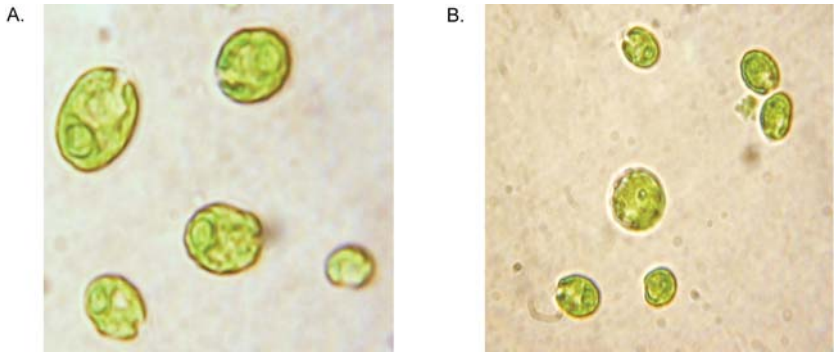

Figure 3. Microscopic observation of *C. reinhardtii* grown in TAP (A) and ^{15}N-TAP (B).

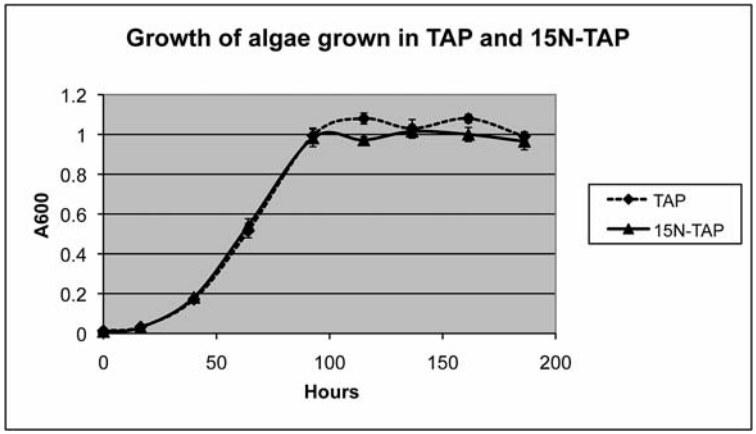

Figure 2. Growth rate comparison of *C. reinhardtii* grown in TAP and ^{15}N-TAP. Each data point is the mean of 5 replicates, the error bars represent the standard deviation.

CONCLUSIONS

The development of ^{15}N-TAP media was successful: ^{15}N-ammonium nitrate was made the sole source of nitrogen in the media. The growth or development of the algae appeared completely unaffected when grown in the media containing 100% label.

As *Chlamydomonas reinhardtii* showed no signs of stressed physiological responses to the modified media, ^{15}N is now used in our laboratory to label free amino acids and proteins. We are now positioned to verify the efficiency of isotopic labeling of free amino acids by GC-MS and of peptides by LC-MS-MS. Once we determine that the labeling efficiency is 100%, we will be able to perform chase experiments to determine protein turnover rates.

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