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Biochemical Education 28 (2000) 169–170

Biochemical Education

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A simple protocol to evaluate nitrogen utilisation in *Saccharomyces cerevisiae*

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Received 25 November 1999; accepted 2 December 1999

Abstract

The present article describes a simple protocol that allows the study of nitrogen source utilisation by cells and its relation with cellular growth. The assay can be performed using the minimum of laboratory equipment. © 2000 IUBMB. Published by Elsevier Science Ltd. All rights reserved.

1. Introduction

In higher eukaryotic cells, proteins provide amino acids which in turn are the main nitrogen source for these cells. The yeast *Saccharomyces cerevisiae* is a eukaryotic microorganism that represents a simple and attractive model, often used to study the properties and metabolism of higher organisms.

The requirement and utilisation of nitrogen sources is a recurrent theme in both Biochemistry and Biology courses. In addition, the chemical reactions involving amino acid groups are often discussed in relation with properties of amino acids. Among these, the ninhydrin reaction is characteristic of amino acid groups, involving two steps that results in a coloured compound [1] whose intensity can be easily measured in a spectrophotometer. *Saccharomyces cerevisae* is capable of utilising L-leucine as its sole nitrogen source, in a unidirectional process [2,3]. This led us to hypothesise that cellular growth could be followed by a decrease in the intensity of colour of the ninhydrin reaction in the synthetic growth medium supernatant.

The present article describes a protocol that allows a discussion of the following: (1) the relation between nitrogen supply and cellular growth, (2) detection of amino acids by a chemical reaction, and (3) determination of a chemical concentration using a calibration curve.

2. Methods

Saccharomyces cerevisiae MMY2/H3/LT1 (a *gap*1 *let*1 *let*2 *ura*3) is a yeast strain isolated and characterized by Chianelli et al. [4]. Precultures were prepared in YPD medium (1% w/v yeast extract, 1% w/v peptone, 2% w/v glucose). Cell growth was evaluated on Difco minimal medium supplemented with 20 mg/l uracil, containing L-leucine (1.0 mM) as the sole nitrogen source. MMY2/H3/LT1 strain was inoculated into liquid medium containing amino acid as the sole nitrogen source, at an initial OD (570 nm) of 0.050 (approximately 1.3×10^6 cells/ml), as measured in a Beckman DU65 spectrophotometer. After 0, 24 and 48 h of growth at 30°C, aliquots of the medium were recovered and: (1) the optical density at 570 nm was measured to evaluate cellular growth, and (2) 1 ml medium was centrifuged, and the ninhydrin reaction performed on the supernatant to determine the L-leucine concentration in the growth medium. The reaction was started by the addition of 50 μl 3.5% w/v ninhydrin in acetone followed by heating in a water bath at 100°C for 5 min. After cooling, the absorbances were read at 560 nm. A calibration curve was prepared using the same medium supplemented with concentrations of L-leucine ranging from 0 to 1 mM.

3. Practical work for students

The laboratory practical is designed to be performed over a two-day period with groups of 2–3 students. On

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PII: S0307-4412(99)00139-9

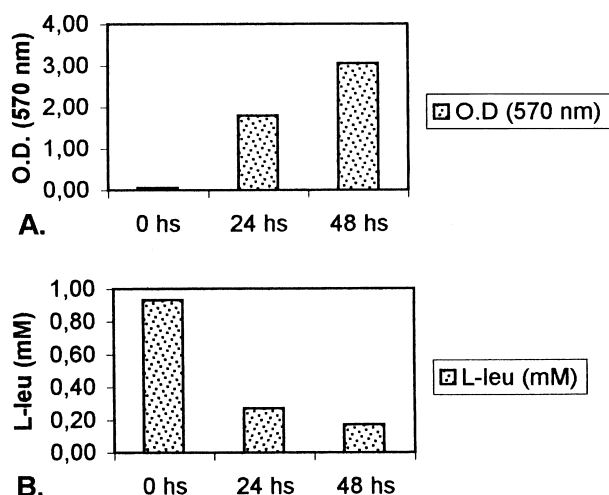

Fig. 1. (A) celluar growth and (B) L-leucine (mM) concentration in growth medium.

Day 1 each group prepares 100 ml medium (0.17% w/v Difco Yeast Nitrogen Base containing no amino acids or ammonium sulphate), 2% w/v D-glucose as a carbon source, and 1.0 mM L-leucine as the sole nitrogen source. The medium is filter-sterilised and divided among four Erlenmeyer flasks. The supervisor provides a fresh culture of the yeast strain (preferably from YPD). After calculating cell number, students inoculate three of the flasks with the desired initial seeding. One flask is kept in the refrigerator, to be used as the 0 h value (flask 1), while the remaining two flasks are incubated at 30°C. After 24 h, one of the two flasks (flask 2) is also placed in the refrigerator, and at 48 h, the remaining flask (flask 3) is also refrigerated.

On Day 2 each group prepares the calibration curve with L-leucine, using non-sterilised medium with and without L-leucine, to obtain the same final volume. At the same time, the supernatants from 0, 24 and 48 h are used to measure L-leucine concentration. Linear regression analysis of the data is used to produce a curve with $OD_{560} = A + Bx$ [L-leu].

Values obtained for a representative experiment are presented in Fig. 1. The time 0 medium shows the highest ninhydrin colour value (560 nm). Similar results were obtained with the MMY2 (*ura*3) wild type and MMY2/H3 (*ura*3 *gap*1) mutant strain, with cellular growth values higher than those for MMY2/H3/LT1 (data not shown).

4. Conclusions

The experimental work presented here is simple and easy to perform in schools or universities with restricted budgets. It achieves its goal of showing the relationship between cellular growth, quantitative determination of a compound, and nutrient utilisation.

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