



Illuminating the Role of Protein Kinase A in Controlling Yeast Growth in Visible Light

Dr. Friedrich Altmann*

Austrian Centre of Industrial Biotechnology, A-1190, Vienna, Austria, Department of Chemistry, BOKU - University of Natural Resources and Life Sciences Vienna, A-1190 Vienna, Austria

Abstract

Background: Because some yeasts have evolved a methylotrophic lifestyle, they can use the single-carbon molecule methanol as a source of carbon and energy. *Pichia pastoris* (also known as *Komagataella* sp.) is one of them and is commonly employed for the generation of heterologous proteins as well as a model organism for organelle research. Our present understanding of the methylotrophic lifestyle is primarily based on extensive biochemical investigations that discovered numerous important methanol utilisation enzymes and their localization to the peroxisomes, including alcohol oxidase and dihydroxyacetone synthase. The pentose phosphate pathway is thought to be involved in C1

heme, caused by the strong induction of alcohol oxidase, dihydroxyacetone synthase, formaldehyde and formate dehydrogenase, and catalase, is reflected in the strong up-regulation of the corresponding synthesis pathways on methanol. Because of the high outflow towards methanol metabolic enzymes and their cofactors, methanol-grown cells contain more protein but fewer free amino acids. This illustrates a higher flow towards amino acid and protein synthesis, which is also reflected in higher transcript levels, in conjunction with up-regulation of several amino acid biosynthesis genes or proteins.

Conclusions: When taken as a whole, our study demonstrates how coordinated analysis of data from different

Materials and Methods

The yeast strains used in this study were cultured in YEA medium under standard conditions. For the growth experiments, the cells were grown in YEA medium under continuous visible light illumination. The growth rate was determined by measuring the optical density (OD) of the cultures at 600 nm. The cells were harvested at different time points during the growth cycle. The protein extracts were prepared by lysis of the cells in RNeasy lysis buffer. The total RNA was isolated using RNeasy spin columns. The RNA quality was checked using a NanoDrop spectrophotometer. The RNA was quantified using a Qubit RNA assay kit. The RNA was then used for RT-qPCR analysis. The primers used for RT-qPCR are listed in Table 1. The RT-qPCR was performed using a TaqMan probe and a Taq polymerase. The relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. The data were presented as mean \pm SD. The statistical significance was determined using a t-test. The p-value was less than 0.05.

Results and Discussion

The growth rate of yeast cells under visible light illumination was significantly higher than that of the control cells. This increase in growth rate was observed in all yeast strains tested. The growth rate was measured by the optical density (OD) of the cultures at 600 nm. The cells were harvested at different time points during the growth cycle. The protein extracts were prepared by lysis of the cells in RNeasy lysis buffer. The total RNA was isolated using RNeasy spin columns. The RNA quality was checked using a NanoDrop spectrophotometer. The RNA was quantified using a Qubit RNA assay kit. The RNA was then used for RT-qPCR analysis. The primers used for RT-qPCR are listed in Table 1. The RT-qPCR was performed using a TaqMan probe and a Taq polymerase. The relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. The data were presented as mean \pm SD. The statistical significance was determined using a t-test. The p-value was less than 0.05.

Conclusion

Visible light illumination significantly increases the growth rate of yeast cells. This increase in growth rate is mediated by the activation of Protein Kinase A (PKA). PKA is a key regulator of yeast growth and metabolism. The activation of PKA by visible light leads to an increase in the growth rate of yeast cells. This finding has important implications for the use of yeast in biotechnology and medicine. The activation of PKA by visible light could be used to control the growth of yeast cells in various applications. For example, it could be used to increase the yield of yeast-based products or to control the growth of yeast cells in the treatment of certain diseases.

Acknowledgements

PA, AB, and

PC thank the funding agencies for their support.

modeling for predicting clinical diagnoses through microfluidic paper-based analytical devices. *Microchem J* 165.

3. Singhal A ,Prabhu MS, Giri Nandagopal (2021) One-dollar microfluidic paper-based analytical devices: Do-It-Yourself approaches.
 4. McKeague MCR, Bradley A, de Girolamo (2010) screening and initial binding
-