ISSN : 2350-0743

International Journal of Recent Advances in Multidisciplinary Research

Vol. 11, Issue 09, pp. 10284-10286, September, 2024

REVIEW ARTICLE

OPTIMIZATION OF MEDIUM COMPOSITION TO ENHANCE CORDYCEPIN SYNTHESIS AND STRESS TOLERANCE IN ENGINEERED *PICHIA PASTORIS*

Chenyang Li, Youcong Yang, Guangyao Zou, Shuo Dong, Zhihong Chen, *Qian Li

School of Life and Health, Dalian University, China

ARTICLE INFO

Article History

Received 20th June, 2024
Received in revised form
16th July, 2024
Accepted 27th August, 2024
Published online 30th September, 2024

Keywords:

Cordycepin; *Pichia pastoris*; Fucoidan; Adenine

***Corresponding author:* Qian Li**

ABSTRACT

Cordycepin is a bioactive compound with extensive pharmacological activities and potential medical applications. This study focuses on the optimization of cordycepin production in an engineered *Pichia pastoris* strain, THP-292, constructed in our laboratory. By screening optimal culture media and supplementing with adenine and fucoidan, we aimed to improve cordycepin synthesis and enhance stress tolerance.

The results showed that the BSM medium supported better growth and higher cordycepin production compared to other media, while offering simpler composition and easier downstream processing. The addition of fucoidan significantly increased cell viability during the later fermentation stages, improving stress resistance. Meanwhile, adenine supplementation doubled the cordycepin yield. This provides new insights into enhancing cordycepin synthesis in *P. pastoris* and understanding cellular adaptations to various environmental conditions.

Citation: Chenyang Li, Youcong Yang, Guangyao Zou, Shuo Dong, Zhihong Chen, Qian Li. 2024. *"Optimization of medium composition to enhance cordycepin synthesis and stress tolerance in engineered pichia pastoris..", International Journal of Recent Advances in Multidisciplinary Research,* 11, (09), 10280-10283.

INTRODUCTION

Cordycepin, also known as 3'-deoxyadenosine(Jędrejko, Lazur and Muszyńska, 2021), is a bioactive compound naturally found in fungi such as *Cordyceps militaris* and *Cordyceps sinensis* (Zhou *et al.,* 2008). Cordycepin exhibits anti-tumor, anti-inflammatory, immunomodulatory, and antioxidant activities (Tuli *et al.,* 2013), drawing considerable interest for its therapeutic potential. It can inhibit tumor cell proliferation, induce apoptosis, and suppress metastasis and invasion(Yoon, Park and Park 2018). Additionally, cordycepin shows promise for cardiovascular disease prevention and treatment by inhibiting endothelial cell proliferation and reducing inflammation (Radhi *et al.,* 2021).

Its anti-aging potential, achieved by scavenging free radicals and delaying cellular senescence, further underscores its importance (Wang *et al.,* 2005). However, the limited natural sources and low yields of cordycepin have hindered its widespread application. Traditionally, cordycepin is extracted from Cordyceps species, but the yield is typically below 1% (w/w), and the extraction process is complex and costly(Yang et al. 2020). Microbial fermentation, particularly using *Pichia pastoris*, offers a more efficient and cost-effective alternative for cordycepin production. The unique expression system of *P. pastoris* makes it an ideal microbial chassis for cordycepin synthesis(Zhao e*t al.,* 2024). Our laboratory previously engineered the *P. pastoris* strain THP-292 to synthesize cordycepin. This study aims to optimize cordycepin production by supplementing the growth medium with exogenous substances to enhance stress tolerance and production capacity. These results will provide valuable insights for industrial cordycepin production.

MATERIALS AND METHODS

Strains and Media: *P. pastoris* strain THP-292 was used in this study and is preserved in our laboratory.
YPD medium: 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose.

BSM medium: 26.7 mL/L 85% H_3PO_4, 0.80 g/L $CaSO_4$, 18.2 g/L K_2SO_4, 14.9g/L $MgSO_4 \cdot 7H_2O$, 4.13 g/L KOH, 4 mL PTM1, and 1% (v/v) methanol. The corresponding glycerol medium replaces methanol with 1% (v/v) glycerol.
BMM medium: 0.1 mol/L pH 6.0 phosphate buffer, 1 mL/L 500× biotin, 100 mL/L 10× YNB, 1% (v/v) methanol (after sterilization). The corresponding glycerol medium replaces methanol with 1% (v/v) glycerol.
FM22 medium: 42.9 g/L KH_2PO_4, 5 g/L $(NH_4)_2SO_4$, 14.3 g/L K_2SO_4, 11.7 g/L $MgSO_4 \cdot 7H_2O$, and 1% (v/v) methanol. The corresponding glycerol medium replaces methanol with 1% (v/v) glycerol. *PTM1:* Contains trace elements such as $CuSO_4 \cdot 5H_2O$, KI, $MnSO_4 \cdot H_2O$, and $ZnCl_2$.

Fermentation of *P. pastoris*

The strain THP-292 was streaked on YPD agar plates and incubated at 30°C until single colonies formed. A single colony was transferred to YPD liquid medium and cultured at 30°C with shaking at 180 rpm for 24 hours. The cells were then transferred to glycerol-based BMM, BSM, or FM22 media for another 24 hours under the same conditions. After 24 hours, the cells were switched to the corresponding methanol-based medium, with 1% (v/v) methanol added every 24

hours to induce gene expression. Samples were taken every 24 hours to measure biomass (OD_{600}) and cordycepin concentration via high-performance liquid chromatography (HPLC) (Tan et al. 2023). Methanol was supplemented every 24 hours until 168 hours. During methanol induction, 1.0 g/L of fucoidan or adenine was added to the medium. Growth and cordycepin production were monitored.

Cell Viability Analysis

Cell viability was assessed using methylene blue staining under an optical microscope. Live cells appeared colorless, while dead cells were stained blue. The proportion of stained cells was used to calculate cell survival rates.

RESULTS

Growth of *P. pastoris* in Different Media: One advantage of *P. pastoris* is its ability to perform high-density fermentation in inorganic media using methanol as the sole carbon source. This study evaluated the optimal inorganic medium for cordycepin production by the engineered strain THP-292. The results showed significant differences in growth depending on oxygen supply (affected by culture volume), as indicated by Figure 1A. This influenced the timing and duration of the logarithmic and stationary phases, ultimately affecting cell status during subsequent glycerol and methanol induction stages. From 72 to 168 hours, *P. pastoris* grown in BSM medium exhibited superior growth compared to other media (Figure 1B-C). Cordycepin production in BSM medium surpassed the other two media after 120 hours and peaked at 168 hours, with levels 30% higher than those in BMM and FM22 media. This suggests that BSM medium, rich in inorganic salts and trace elements, is more conducive to both biomass growth and metabolite synthesis in *P. pastoris*. Its simplicity and lower cost make it a favorable choice for cordycepin production, as it also simplifies downstream purification.

Effects of Adenine and Fucoidan on *P. pastoris*

Adenine is a precursor for cordycepin biosynthesis, while fucoidan may reduce cordycepin's toxicity to *P. pastoris.* The addition of these exogenous substances was evaluated for their potential to enhance cordycepin production or improve cell viability. Adding 1.0 g/L of adenine increased cordycepin production by nearly twofold compared to the control (Figure 2C), although biomass was slightly lower (Figure 2B), suggesting that cordycepin synthesis imposes a metabolic burden. Fucoidan enhanced cell survival rates in the later stages of fermentation (Figure 2A), despite causing initial stress.

DISCUSSION

Optimizing microbial metabolism through medium adjustment and precursor addition is crucial for enhancing cordycepin production. Our results showed that the BSM medium was superior in supporting cordycepin synthesis due to its simple, cost-effective composition, which also facilitates downstream processing. Adenine, as a purine compound and precursor(Chassy and Suhadolnik 1969), significantly boosted cordycepin production. Fucoidan, a polysaccharide with various biological activities(Van Weelden *et al.,* 2019), mitigated the toxic effects of methanol and cordycepin on *P. pastoris*. This indicates its potential for enhancing cell survival under stress conditions. These findings provide new insights into optimizing *P. pastoris* as a microbial cell factory and shed light on the mechanisms of cellular adaptation to environmental stimuli.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

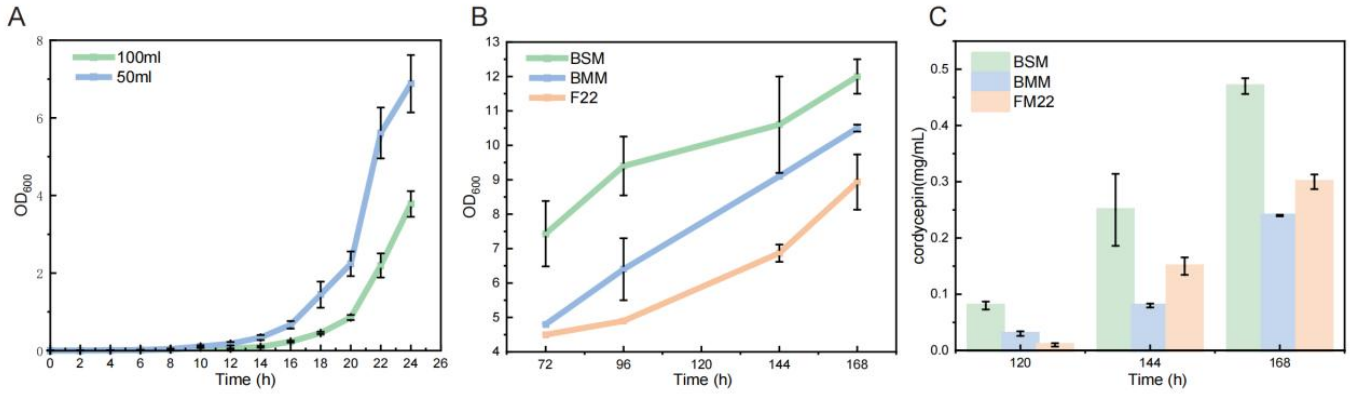

Figure 1. Growth and cordycep in production in different media. (A) Growth in YPD medium with varying culture volumes after 24 hours. (B) Growth in different media from 72 to 168 hours. (C) Cordycepin concentration from 120 to 168 hours.

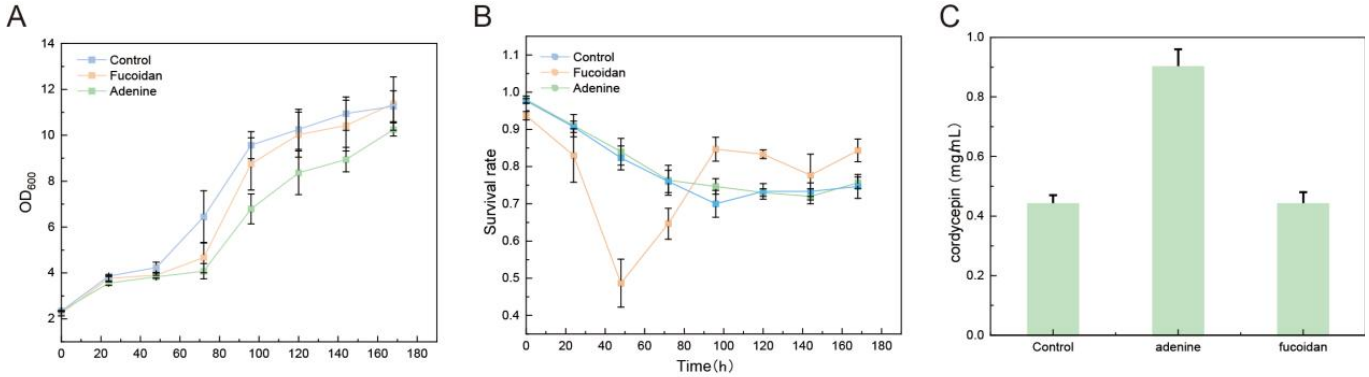

Figure 2. Effects of adenine and fucoidan on *P. pastoris*. (A) Cell viability after adenine or fucoidan addition. (B) Growth curve. (C) Cordycepin production at 168 hours.

REFERENCES

Chassy, Bruce M., and R. J. Suhadolnik. 1969. "Nucleoside antibiotics IV. Metabolic fate of adenosine and cordycepin by *Cordyceps militaris* during cordycepin biosynthesis."*Biochimica et Biophysica Acta (BBA) - Nucleic Acids and Protein Synthesis* 182(2):307-15.

Jędrejko, Karol J., Jan Lazur, and Bożena Muszyńska. 2021. "*Cordyceps militaris*: An Overview of Its Chemical Constituents in Relation to Biological Activity."*Foods*10:2634.

Radhi, Masar, Sadaf Ashraf, Steven Lawrence, Asta Arendt Tranholm, Peter Arthur David Wellham, Abdul Hafeez, Ammar Sabah Khamis, Robert Thomas, Daniel McWilliams, and Cornelia Huiberdina de Moor. 2021. "A Systematic Review of the Biological Effects of Cordycepin."*Molecules*26(19):5886.

Tan, Huiping, Liang Wang, Huiguo Wang, Yanghao Cheng, Xiang Li, Huihui Wan, Chenguang Liu, Tian Liu, and Qian Li. 2023. "Engineering *Komagataella phaffii* to biosynthesize cordycepin from methanol which drives global metabolic alterations at the transcription level."*Synthetic and Systems Biotechnology* 8(2):242-52.

Tuli, Hardeep S., Anil K. Sharma, Sardul S. Sandhu, and Dharambir Kashyap. 2013. "Cordycepin: A bioactive metabolite with therapeutic potential."*Life Sciences* 93(23):863-69.

Van Weelden, Geert, Marcin Bobiński, Karolina Okła, Willem Jan Van Weelden, Andrea Romano, and Johanna M. A. Pijnenborg. 2019. "Fucoidan Structure and Activity in Relation to Anti-Cancer Mechanisms."*Marine Drugs*17(1):32.

Wang, Be-Jen, Shen-Jeu Won, Zer-Ran Yu, and Chun-Li Su. 2005. "Free radical scavenging and apoptotic effects of *Cordyceps sinensis* fractionated by supercritical carbon dioxide."*Food and Chemical Toxicology* 43(4):543-52.

Yang, Liyang, Guilan Li, Zhi Chai, Qiang Gong, and Jianquan Guo. 2020. "Synthesis of cordycepin: Current scenario and future perspectives."*Fungal Genetics and Biology* 143:103431.

Yoon, So Young, Soo Jung Park, and Yoon Jung Park. 2018. "The Anticancer Properties of Cordycepin and Their Underlying Mechanisms."*International Journal of Molecular Sciences*19(10):3027.

Zhao, Bingjie, Yu Li, Yong Zhang, Meixi Pan, Guishen Zhao, and Yanbin Guo. 2024. "Low-carbon and overproduction of cordycepin from methanol using engineered *Pichia pastoris* cell factory."*Bioresource Technology* 413:131446.

Zhou, Xiaoxia, Liping Luo, Waike Dressel, Gulibahaer Shadier, Doreen Krumbiegel, Peter Schmidtke, Fred Zepp, and Claudius U. Meyer. 2008. "Cordycepin is an Immunoregulatory Active Ingredient of *Cordyceps sinensis*."*The American Journal of Chinese Medicine*36(05):967-80.

****