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The Strategies for Increasing Cordycepin Production of *Cordyceps Militaris* by Liquid Fermentation

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Abstract

The aims of this review are to describe the biosynthetic pathway of cordycepin and summarize the strategies for increasing cordycepin production of *Cordyceps militaris* by liquid fermentationn. In recent years, researchers made remarkable progress in cordycepin production. They focused their attention on the three aspects to improve cordycepin production: strain improving, optimizing ingredients of medium and optimizing culture conditions. This review might be helpful for understanding of cordycepin boisynthesis and increasing the production of cordycepin.

Keywords: *Cordyceps militaris*; Liquid fermentation; Cordycepin

Introduction

In recent years, mushrooms have become a valuable source with various bioactive ingredients [1,2]. *Cordyceps militaris* (*C. militaris*), belonging to the class *Ascomycetes* and Dong Chong Xia Cao group [3], has been used as a folk tonic food and an important medicinal mushrooms in Asia extensively [4]. Cordycepin (3'-deoxyadenosine Figure 1), one of naturally isolated nucleoside antibiotics, was the major active metabolite of *C. militaris* [5]. Recent studies have demonstrated that cordycepin exhibited multiple pharmacological actions, to be specific, immunological regulation [6-8], antivirus [9], antihyperlipidemia [10], antifungus [11], antileukemia [12-14], anticancer effects [15]. Recently, with the advance of the standard of living, there is an increasing requirement for large amounts of cordycepin. Due to strict requirement for host and living conditions, coupling with over harvesting, natural *C. militaris* are facing with extinction along with the change of environment [16,17]. Therefore, the limited natural resources cannot meet the demand for health food application or herbal medicine. It is urgent to find the effective methods to produce a great deal of cordycepin.

Although cordycepin could be chemically synthesized absolutely. The synthesis process was complicated, and a large volume of organic solvents was discharged, which was adverse to human health and environment, so it was not widely applied to industrial production [18,19]. Recent studies indicated that solid and liquid fermentation were used to produce cordycepin [20]. However the solid cultivation need to take several months to get a fruiting body with a lower productivity of bioactive ingredients. Liquid fermentation yields potential dominant of shorter time and higher mycelial production. What's more, There were similar chemical ingredients and pharmacologic effects between fermentation broth and wild C. militaris [21]. The process of liquid fermentation also could be optimized to achieve a higher productivity. Therefore, liquid cultivation was viewed as a promising field to generate artificial cordycepin production. Many scholars studied a good deal of strategies to meet the increasing demand of cordycepin since 1960s and made remarkable achievements. They focused their attention on the three aspects: strain improving, optimizing ingredients of medium (selection of carbon and nitrogen sources, precursors, mineral ion) and optimizing culture conditions. The aims of this review were to describe the cordycepin biosynthesis and summarize the strategies for increasing cordycepin production of C. militaris by liquid fermentationn.

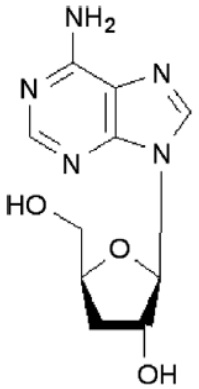

Figure 1: Chemical structure of cordycepin

Strain improving

Different strains had different productivity. Strain improving maybe enhance the production of secondary metabolites dramatically. So many researchers studied a series of paths to obtain mutations which had higher productivity of cordycepin. Mutants were generated by spontaneous mutation, ultraviolet light, ionizing radiation, (X-rays, gamma-rays, ion beam), chemical mutagens (ethyl methane sulfonate) and biological mutagens (transposon tagging, T-DNA insertion).

Das et al. [22] applied a high-energy (~MeV) proton beam which provided a higher mutation frequency and wider mutant spectrum to irradiate *C. militaris* NBRC9787 and obtained a high-yielding mutant G81-3. After optimizing ingredients of medium (Bacto yeast extract (YE) 45 g/L, glucose 50 g/L and a few major inorganic salts), the cordycepin production by G81-3 was up to 3.1 g/L, which was 72% higher than that of basal medium (1.8 g/L).

Optimizing ingredients of medium

There are a variety of ingredients in the medium: carbon source, nitrogen source, phosphorus source, sulfur source, mineral ion, growth factor, precursor, inducer, accelerant and inhibitor. Each of them may be significant to the growth of cells and the formation of any cultivation products.

Selection of carbon and nitrogen sources: Carbon and nitrogen sources, two major nutrient ingredients of medium, were essential for cell proliferation and metabolite biosynthesis. There were many studies on optimizing carbon sources, nitrogen sources and their optimal concentrations for mycelial growth and metabolites formation.

Mao and co-workers [23] investigated various carbon sources (lactose, sucrose, glucose, fructose, galactose, maltose and xylose) and found that glucose was most favourable to cordycepin production. They also studied the effect of carbon/nitrogen ratios to improve the accumulation of cordycepin. The maximum production of cordycepin they obtained was 345.4 ± 8.5 mg/L with 42.0 g/L glucose and 15.8 g/L peptone. Mao [24] investigated masses of nitrogen sources, including YE, casein enzymatic hydroilsate, peptone, casein acid hydrolysate, combination of YE and peptone at 1:1 (w/w). Their investigation showed that all of nitrogen sources could support the growth of cells, but the effect on cordycepin production were different. YE was beneficial to growth of mycelial, while peptone was best for cordycepin biosynthesis. Another report by Masuda [25] showed that the mixture of peptone and YE (peptone:YE = 1:3) were the preferable nitrogen sources for generation of cordycepin, and the peak concentration of cordycepin they obtained was 0.64 g/L when glucose/mixed nitrogen source was 2/1. Both studies of Mao and Masuda showed that peptone was best for the synthesis of cordycepin. It might because that peptone, which composed by 20 kinds of amino acids and $NH4^+$ [26,27], was decomposed and consumed by mushroom to form the secondary metabolites [27,28].

In addition to organic nitrogen sources, inorganic nitrogen sources also had positive impact on growth of organisms. Ammonium was the most common inorganic nitrogen sources. Mao and Zhong [24] examined the effects of ammonium feeding on the cordycepin production by submerged cultivation of C.militaris. About 70% increase in maximum cordycepin production was achieved in feeding of $NH4^+$ (40mM) on day 7, reaching to 420.5 ± 15.1 mg/L. Their experiment showed that ammonium feeding was a simple and effective strategy for increasing the production of cordycepin in mycelial cultures. Leung [29] also studied the effects of ammonium feeding on the cordycepin production in mycelial culture of *Cordycepes Sinensis* HK1. The yield of cordycepin with 10 mmol/L NH_4CL corresponds to four times than that of control group.

Adding additives

Precursors: So far, there were several reports related to cordycepin biosynthesis of *C. militaris* and it has not been completely clarified. Adding some precursors may increase cordycepin significantly, so it was very important to know the biosynthetic pathway of cordycepin. Previous studies on incorporation ^3H-labeled ribose and ^{14}C-labeled glucose, adenine, adenosine into cordycepin demonstrated that most of them acted as underlying precursors [30,31]. Cordycepin was intracellularly converted into its 5'-mono-, di- and triphosphates that inhibited the activity of 5-phosphoribosyl-1-pyrophosphate amidotransferase and ribose-phosphate pyrophosphokinase in the *de novo* biosynthesis of purines [32-35]. Lennon's investigation showed that the ^3H: ^{14}C ratio of the AMP isolated from the RNA of *C. militaris* was identical to that of cordycepin. They also showed that adenosine converted to cordycepin by a reductive mechanism without hydrolysis of the N-riboside bond. Taking the above studies into consideration [30-35], the cordycepin biosynthesis may be shown in the figure 2. Glucose firstly turned into glucose-6-phosphate (G-6-P) and then transformed into ribose-5-phosphate (R-5-P) by pentose phosphate pathway (pp pathway), R-5-P was the starting material of the *de novo* purine nucleotide pathway. The *de novo* purine nucleotide pathway involved serial conversions of phosphoribosyl pyrophosphate (PRPP) to IMP and then to AMP and GMP. Glutamine and Glycine also took part in the serial conversions. But, the mechanisms that AMP, adenine and adenosine converted into cordycepin were unknown, which maybe became research focus in the days ahead.

Mina Masuda [36] added a great deal of purine-related compounds to increase the production of crodycepin by a surface culture of *C. militaris* NBRC 9787. The peak value of cordycepin they obtained was 2.5 g/L under the condition of 1 g/L adenine and 16 g/L glycine. Their study also showed that adding of L-glutamine, glycine, adenosine, adenine and L-aspartic were effective methods to enhance the cordycepin production. Das [37] also explored the effects of different precursors (glycine, adenosine) on the production of crodycepin using the mutant G81-3 in a surface liquid culture. The highest crodycepin production reached 8.57 g/L when adding 6 g/L adenosine in medium (glucose 86.2 g/L, YE 93.8 g/L), about 28.10% increase (from 6.69 g/L to 8.57 g/L) in crodycepin production was observed after adding adenosine. Similarly, glycine also had influence on the production of crodycepin, the best production (6.80 g/L) was obtained when adding glycine with YE in weight percent ratio of 90/10, which was 12.40% higher versus the control group (6.05 g/L). They demonstrated that adenosine had a much better effect on cordycepin production than that of glycine.

Mineral ion: Mineral ion, one of the constituents of culture medium, also played a pivotal role in the growth of cells. There were some studies on the effects of mineral ion on the generation of secondary metabolites of *C. militaris*.

A recent report by Fan [38] showed that some metal ions (Cu^{2+}, Ca^{2+}, Mn^{2+}, Fe^{3+} and Fe^{2+}) could markedly enhance the production of cordycepin, among of them Fe^{2+} was the most effective. While Zn^{2+} dramatically reduced cordycepin production. As a result, adding 1 g/L ferrous sulfate on day 0, the production of cordycepin could reach 596.59 ± 85.5 mg/L, which was 70% higher than the group that without ferrous sulfate. Another research [39] showed that 0.1mM Mn^{2+} can strikingly promote synthesis of some nucleosides like adenosine and guanosine, which may be benefit to synthesis of cordycepin.

Optimizing culture conditions

The modes of propagation: As everyone knows, oxygen supply was crucial for aerobic organisms to cells growth and formation of secondary metabolites. Due to poor solubility of oxygen in water, different modes of propagation (submerged culture, surface liquid

culture and the repeated batch culture) were investigated to control oxygen availability in medium.

Some authors investigated the effects of dissolved oxygen (DO) on cordycepin formation by *C. militaris*. Mina Masuda et al. demonstrated that the repeated batch culture was a simple method to increase cordycepin production [36]. Shih et al. [40] combined shake-flask with static culture to facilitate the production of cordycepin. The maximum production they obtained was 2214.5 mg/L by *C. militaris* CCRC 32219, the optimized conditions were at PH 6, YE 45 g/L, 8.0 days of the shake cultivation followed by 16.0 days of the static culture. Their investigation also showed that two-stage dissolved oxygen control was good for cordycepin formation. Perhaps, in the early time, cells needed more oxygen to growth, but cordycepin was synthesized in hypoxia state.

Other factors: Living environment (light condition, PH, temperature), inoculum size, incubation time and seed age also had an impact on the growth of organisms and the formation of metabolites.

Tang et al. [41] optimized the fermentation conditions and the ingredients of medium for enhancing the production of cordycepin with Placket-Burman, single-factor experiment in static culture. The maximum cordycepin yield (7.35 g/L) was obtained under followed conditions: incubation temperature of 27.1, inoculum size of 10%, seed age of 3 days, YE 9.00 g/L, peptone 17.10 g/L. In the progress of cultivation of organisms, culture time also had a great influence on the yield of cordycepin. Mastering the law of cordycepin formation and controlling the point of culture time also could obtain much higher output. The investigation by Masuda et al. [42] showed that the day when cordycepin production up to the peak by mutant strains was latter than that of wild strains about 10 days. The maximum cordycepin production they obtained was 8.6 g/L of mutant G81-3 by the repeated batch culture. Up to now, this was the highest report of crodycepin product.

Discussion and Conclusion

Table 1 summarized the production disparity of cordycepin under the different conditions of liquid fermentation. We could conclude that the productivity of mutant stronger than that of wild *C. militaris*. The concentrations of carbon and nitrogen sources also had influence on production of cordycepin. Adding additives could enhance cordycepin production. The formation of cordycepin preferred surface liquid culture or the repeated operation to submerged culture.

Carbon sources (g/L)	Nitrogen sources (g/L)	Additives	The modes of propagation	Cordycepin production (g/L)	References
Glucose 40.0	Peptone 10.0	-	Submerged culture	0.201	[43]
Glucose 10.0	YE 10.0	$NH4^+$	Submerged culture	0.421	[24]
Glucose 40.0	Peptone 10.0	Fe^{2+}	Submerged culture	0.596	[38]
Glucose 42.0	YE 15.8	-	Submerged culture	0.345	[23]
Glucose 86.2	YE 93.8	NH_4NO_3,	Surface liquid culture	8.57 (mutant)	[37]
		$Fe(NH_4)_2(SO_4)\cdot 6H_2O$ Adenosine			
Glucose 10.0	Peptone : YE = 1:3	NH_4NO_3	Surface liquid culture	0.64	[25]
Glucose 20.0	Peptone 2.5 YE 20.0	NH_4NO_3, $Fe(NH_4)_2(SO_4)\cdot 6H_2O$, Glycine, Guanosine, Adenosine	Surface liquid culture	2.50	[36]
			A repeated batch culture	2.45	
				6.84 (mutant)	

Table 1: The effects on Cordycepin production by some impact factors.

The aims of strain improving were to break the normal metabolism mechanism, terminate microorganisms own regulating mechanism and increase accumulation of target products. It can be achieved by mutation breeding. The ion beams could induce nuclear DNA alterations such as transversion, inversion, translocation and large deletions rather than point mutations, so it could produce various types of mutants with broad-spectrum mutation [44,45]. A high-yielding mutant (G81-3) of *C. militaris* was obtain by irradiation of ion beams. The ion beams also could be used in other fungi research.

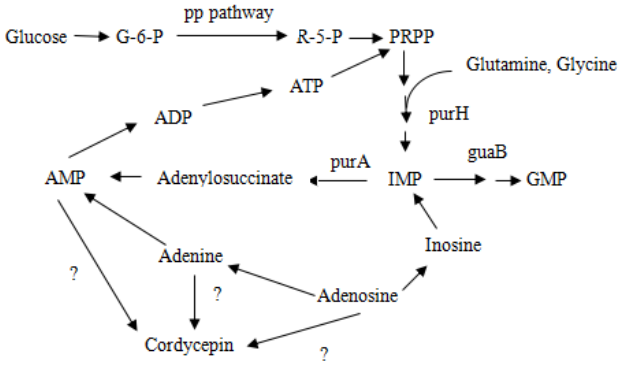

Figure 2: The *de novo* purine nucleotide pathway and possible biosynthetic pathway of cordycepin. Question mark indicates those reactions are unknown.

The types and concentrations of carbon and nitrogen sources had effects on yield of secondary metabolites. So it was very necessary to optimize the compositions of medium. Carbon and nitrogen sources, two major ingredients of medium, consisted of basic skeleton of cultivation products. Carbon sources also had influence on respiration of microorganisms, glycometabolism and growth of cells. In mushroom cultivation of *Tremella mesenterica*, Wasser et al. [46] demonstrated that various kinds of nitrogen sources influenced polysaccharide production and growth of cells markedly. Another report by Cho showed that peptone-YE was best for the mycelial growth of mushroom *Paecilomyces sinclairi*, while meat peptone was most favorable to its red pigment production [47]. Glucose was the best carbon source for cordycepin, which may be the starting material

of the biosynthesis pathway (Figure 2). Peptone was optimum nitrogen source for the formation of cordycepin.

In many studies [48,49], $NH4^+$ showed negative effects on secondary metabolic pathways for nitrogen catabolic repression on the microbial secondary metabolism. Cho [47] also found similar report in cultivation of *Paecilomyces sinclairii*. However, the investigation by Mao [24] showed that $NH4^+$ could promote cordycepin production. They added inhibitor of plasma membrane H^+-ATPase (diethylsilbestrol or sodium orthovanadate) to explore related mechanisms of $NH4^+$ in cordycepin biosynthesis. They found that adding enzyme inhibitor led accumulation of intracellular ATP and decrease of cordycepin significantly (Figure 2). They supposed that $NH4^+$ maybe stimulate the activity of H^+-ATPase to promote cordycepin formation. There are similar phenomena in cultivation of *Aspergillus niger* [50] and *Penicillium cyclopium* [51].

There were some reports about the effects of trace metals on the cultivation of fungus [52-53]. Fan et al. [38] also studied the effect of ferrous sulfate addition on cordycepin production. Their experiment showed that feeding of Fe^{2+} could increase cordycepin production by raising the transcription level of adenylosuccinate synthetase (purA) and cutting the transcription level of IMP cyclohydrolase (purH) and IMP dehydrogenase (guaB) (Figure 2).

In conclusion, both the strain improving and additives had effect on cordycepin production predominately. Fe^{2+} and $NH4^+$ were found as efficient inducers for cordycepin biosynthesis. Adding purine-related compounds (precursors), feeding of Fe^{2+} , $NH4^+$ and the repeated batch culture were simple and effective strategies for increasing cordycepin production of C. militaris by liquid fermentation. This study may be useful for other fungi research.

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References

1. Smith JE, Rowan NJ, Sullivan R (2002) Medicinal mushrooms: a rapidly developing area of biotechnology for cancer therapy and other bioactivities. Biotechnol Lett 24: 1893-1845.
2. Wasser SP, Weis AL (1999) Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspective. Int J Med Mushroom 1: 31-62.
3. Zhao-Long W, Xiao-Xia W, Wei-Ying C (2000) Inhibitory effect of Cordyceps sinensis and Cordyceps militaris on human glomerular mesangial cell proliferation induced by native LDL. Cell Biochem Funct 18: 93-97.
4. Ying J, Mao X, Mao Q, Zong Y, Wen H (1987) Icons of medicinal mushroom from China. Beijing: Science : 151-155.
5. CUNNINGHAM KG, MANSON W, SPRING FS, HUTCHINSON SA (1950) Cordycepin, a metabolic product isolated from cultures of Cordyceps militaris (Linn.) Link. Nature 166: 949.
6. De Silva DD, Rapior S, Fons F, Bahkali AH, Hyde KD (2012) Medicial mushrooms in supportive cancer therapies: an approach to anticancer effects and putative mechanisms of action. Fungal Diversity 55: 1-35.
7. Zhou X, Meyer CU, Schmidtke P, Zepp F (2002) Effect of cordycepin on interleukin-10 production of human peripheral blood mononuclear cells. Eur J Pharmacol 453: 309-317.
8. Noh EM, Kim JS, Hur H, Park BH, Song EK, et al. (2009) Cordycepin inhibits IL-1beta-induced MMP-1 and MMP-3 expression in rheumatoid arthritis synovial fibroblasts. Rheumatology (Oxford) 48: 45-48.
9. Hashimoto K, Simizu B (1976) Effect of cordycepin on the replication of western equine encephalitis virus. Arch Virol 52: 341-345.
10. De Silva DD, Rapior S, Hyde KD, Bahkali H (2012) Medical mushrooms in prevention and control of diabetes mellitus. Fungal Diversity 56: 1-29.
11. Sugar AM, McCaffrey RP (1998) Antifungal activity of 3'-deoxyadenosine (cordycepin). Antimicrob Agents Chemother 42: 1424-1427.
12. De Silva DD, Rapior S, Sudarman E (2013) Bioactive metabolites from macrofungi: ethnopharmacology, biological activities and chemistry. Fungal Diversity 62: 1-40.
13. Kodama EN, McCaffrey RP, Yusa K, Mitsuya H (2000) Antileukemia activity and mechanism of action of cordycepin against terminal deoxynucleotidyl transferase-positive(TdT+) leukemic cells. Biochemical Pharmacology 59: 273-281.
14. Thomadaki H, Tsiapalis CM, Scorilas A (2008) The effect of the polyadenylation inhibitor cordycepin on human Molt-4 and daudi leukaemia and lymphoma cell lines. Cancer chemotherapy pharmacology 61: 703-711.
15. Yoshikawa N, Yamada S, Takeuchi C (2008) Cordycepin (3'-deoxyadenosine) inhibits the growth of B16-BL6 mouse melanoma cells through the stimulation of adenosine A3 receptor followed by glycogen synthase kinase-3ß activation and cyclin D1 suppression. Naunyn-Schmiedeberg's Archives of Pharmacology 77: 591-595.
16. Paterson RR (2008) Cordyceps: a traditional Chinese medicine and another fungal therapeutic biofactory? Phytochemistry 69: 1469-1495.
17. Das SK, Masuda M, Sakurai A, Sakakibara M (2010) Medicinal uses of the mushroom Cordyceps militaris: current state and prospects. Fitoterapia 81: 961-968.
18. Hansske F, Robin MJ (1985) Regiospecific and stereoselective conversion of ribonucleosides to 3'-deoxyadenosine. A high yield three-stage synthesis of cordycepin from adenosine. Tetrahedrlett 26: 4295-4298.
19. Aman S, Anderson DJ, Connolly TJ, Critall AJ, Ji GJ (2000) From adenosine to 3'-deoxyadenosine: development and scale up. Org Process Res Dev 4: 601-605.
20. Park JP, Kim SW, Hwang HJ, Yun JW (2001) Optimization of submerged culture conditions for the mycelial growth and exo-biopolymer production by Cordyceps militaris. Lett Appl Microbiol 33: 76-81.
21. Jiang XL, Sun Y (1999) The determination of active components in various Cordyceps militaris strains. Acta Edulia Fungi 6: 47-50.
22. Das SK, Masuda M, Hatashita M, Sakurai A, Sakakibara M (2008) A new approach for improving cordycepin productivity in surface liquid culture of Cordyceps militaris using high-energy ion beam irradiation. Lett Appl Microbiol 47: 534-538.
23. Xian-Bing Mao, Titiporn Eksriwong, Somchai Chauvatcharin, Jian-Jiang Zhong (2005) Optimization of carbon source and carbon/nitrogen ratio for cordycepin production by submerged cultivation of medicinal murshroom Cordyceps militaris. Process Biochemistry 40: 1667-1672.
24. Xian-bing Mao, Jian-jiang Zhong (2006) Significant effect of NH4+ on cordycepin production by submerged cultivation of medicinal mushroom Cordyceps militaris. Enzyme and Microbial Technology 38: 343-350.
25. Mina Masuda, Eriko Urabe, Akihiko Sakurai, Mikio Sakakibara (2006) Production of cordycepin by surface culture using the medicinal mushroom Cordyceps militaris. Enzyme and Microbial Technology 39: 641-646.
26. Kurbanoglu EB, Kurbanoglu NI (2004) Utilization as peptone for glycerol production of ram horn waste with a new process. Energy Convers Mgmt 45: 225-234.
27. Zhu Y, Rinzema A, Bonarius HPJ, Tramper J, Bol J (1998) Microbial transglutaminase production by Streptoverticillium mobaraense: analysis of amino acid metabolism using mass balances. Enzyme Microb Technol 23: 216-226.
28. Sales-Duval M, Lucas F, Blanchart G (2002) Effects of exogenous ammonia or free amino acids on proteolytic activity and protein

breakdown products in Streptococcus bovis, Prevotella albensis, and Butyrivibrio fibrisolvens. Curr Microbiol 44: 435-443.

29. Leung PH, Wu JY (2007) Effects of ammonium feeding on the production of bioactive metablites (cordycepin and exopolysaccharides) in mycelial culture of a Cordyceps sinensis fungus. Microbiology 103: 1942-1949.
30. KREDICH NM, GUARINO AJ (1960) An improved method of isolation and determination of cordycepin. Biochim Biophys Acta 41: 363-365.
31. Lennon MB, Suhadolnik RJ (1976) Biosynthesis of 3'-deoxyadenosine by Cordyceps militaris. Mechanism of reduction. Biochim Biophys Acta 425: 532-536.
32. KLENOW H (1963) formation of the MONO-, DI- AND TRIPHOSPHATE of CORDYCEPIN in ehrlich ascites-tumor cells in vitro. Biochim Biophys Acta 76: 347-353.
33. Overgaard-Hansen K (1964) The inhibition of 5-phosphoribosyl-1-pyrophosphate formation by cordycepin triphosphate in extracts of Ehrlich ascites tumor cells. Biochim Biophys Acta 80: 504-507.
34. Chassy BM, Suhadolnik RJ (1969) Nucleoside antibiotics IV. Metabolic fate of adenosine and cordycepin by Cordyceps militaris during cordycepin biosynthesis. Biochim Biophys Acta 182: 307-315.
35. Rottman F, Guarino AJ (1964) The inhibition of phosphoribosyl-pyrophosphate amidotransferase activity by cordycepin monophosphate. Biochim biophys acta 89: 465-472.
36. Mina Masuda, Eriko Urabe, Hiromitsu Honda (2007) Enhance production of cordycepin by surface culture using the medicinal mushroom Cordyceps militaris. ScienceDirect: Enzyme and Microbial Technology 40: 1199-1205.
37. Das SK, Masuda M, Sakurai A, Sakakibara M (2009) Effects of additives on cordycepin production using a cordyceps militaris mutant induced by ion beam irradiation. The 5th International Medicinal Mushroom Conference.
38. Dan dan Fan, Wei Wang, Jian zhong Jiang (2012) Enhancement of cordycepin production in submerged cultures of Cordyceps militaris by addition of ferrous sulfate. Biochemcial Engineering Journal 60: 30-35.
39. GuYX, Wang ZS, Li SX, Yuan QS (2007) Effect of multiple factors on accumulation of nucleosides and base in Cordyceps militaris. Food Chem 102:1304-1309.
40. Ing-Lung Shih, Kun-Lin Tsai, Chienyan Hsieh (2007) Effects of culture conditions on the mycelial growth and bioactive metabolite production in submerged culture of Cordyceps militaris. Biochemical Engineering Journal 33:193-201.
41. Tang Jiapeng, Liu Yiting, Zhu Li (2014) Optimization of fermentation conditions and purification of cordycepin from Cordyceps militaris. Preparative Biochemistry & Biotechnology 44: 90-106.
42. Masuda M, Das SK, Fujihara S, Hatashita M, Sakurai A (2011) Production of cordycepin by a repeated batch culture of a Cordyceps militaris mutant obtained by proton beam irradiation. J Biosci Bioeng 111: 55-60.
43. Xian-Bing Mao, Jian-Jiang Zhong (2004) Hyperproduction of Cordycepin by Two-Stage Dissolved Oxygen Control in Submerged Cultivation of Medicinal Mushroom Cordyceps militaris in Bioreactors. Biotechnol. Prog 20: 1408-1413.
44. Albert S, Delseny M, Devic M (1997) BANYULS, a novel negative regulator of flavonoid biosynthesis in the Arabidopsis seed coat. Plant J 11: 289-299.
45. Shikazono N, Tanaka A, Kitayama S, Watanabe H, Tano S (2002) LET dependence of lethality in Arabidopsis thaliana irradiated by heavy ions. Radiat Environ Biophys 41: 159-162.
46. Wasser SP, Elisashvili VI, Tan KK (2003) Effects of carbon and nitrogen sources in the medium on Tremella mesenterica Retz.: Fr. (Heterobasidiomycetes) growth and polysaccharide production. Int J Med Mushroom 5: 49–56.
47. Cho YJ, Park JP, Hwang HJ, Kim SW, Choi JW, et al. (2002) Production of red pigment by submerged culture of Paecilomyces sinclairii. Lett Appl Microbiol 35: 195-202.
48. Sanchez S, Demain AL (2002) Metabolic regulation of fermentation processes. Enzyme Microb Technol 31: 895-906.
49. Voelker F, Altaba S (2001) Nitrogen source governs the patterns of growth and pristinamycin production in 'Streptomyces pristinaespiralis'. Microbiology 147: 2447-2459.
50. Jernejc K, Legisa M (2001) Activation of plasma membrane H+-ATPase by ammonium ions in Aspergillus niger. Appl Microbiol Biotechnol 57: 368-373.
51. Roos W, Luckner M (1984) Relationship between proton extrusion and fluxes of ammonium ions and organic acid in Penicillium cyclopium. J Gen Microbiol 130: 1007–1014.
52. Le J, Liu J, Bo Z, Feng X, Kexue Y, et al. (2006) The effect of Zn on the Zn accumulation and biosynthesis of amino acids in mycelia of Cordyceps sinensis. Biol Trace Elem Res 113: 45-52.
53. Shakoury-Elizeh M, Tiedeman J, Rashford J, Ferea T, Demeter J, et al. (2004) Transcriptional remodeling in response to iron deprivation in Saccharomyces cerevisiae. Mol Biol Cell 15: 1233-1243.