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Abstract

Comparison of nucleosides and related compounds in natural and cultured *Cordyceps*

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Cordyceps sinensis, a well-known and valuable traditional Chinese medicine, is a composite consisting of the stromata of the fungus, *Cordyceps sinensis* (Berk.) Sacc. (Fam. Hypocreaceae) parasitized on the larva of some species of insects (Fam. Hepialidae), and the dead caterpillar. *Cordyceps*, sweet in taste and neutral in nature, is commonly used in hospitals and sold as health food products in China. However, the yield of the wild resources of *Cordyceps sinensis* is decreasing every year because of excessive collection. Due to pretty high values, isolation of mycelial strain from *Cordyceps* is a trend of many scientists to achieve a large scale production of *Cordyceps* by fermentation. Besides cultivation of *Cordyceps*, much effort has also been focus on discovering the alternative species, *Cordyceps militaris* is the most popular one. To date, nucleosides are believed to be the active components in *Cordyceps*. Adenosine is used as marker for quality control of *Cordyceps sinensis* recorded in China Pharmacopeia (2005). However, it is hard to control the quality of natural and cultured *Cordyceps*. Therefore, it is very important to elucidate the difference of nucleosides in *Cordyceps* (natural *Cordyceps sinensis*, cultured *Cordyceps sinensis* and *Cordyceps militaris*), which is beneficial for their quality control.

The thesis consists of four chapters. **Chapter 1** reviewed nucleosides in *Cordyceps* and analytical methods for nucleosides and analogous. **Chapter 2** focused on qualitative and quantitative determination of nucleosides in natural and cultured *Cordyceps* using high performance liquid chromatography-electrospray ionization tandem mass spectrometry and PLE extraction. A simple and rapid HPLC-ESI-MS/MS method was developed for qualitative and quantitative determination of nucleosides, bases and their analogues in natural and cultured

Cordyceps. The results showed that 43 compounds, including 27 unknown compounds, as well as cytosine, uracil, cytidine, hypoxanthine, guanine, uridine, thymine, adenine, inosine, guanosine, thymidine, adenosine, cordycepin, 2'-deoxyadenosine, 6-hydroxyethyl-adenosine and 3'- amino-3'-deoxyadenosine were identified in natural and cultured *Cordyceps*. Especially, cordycepin was contained both in natural *Cordyceps sinensis* and cultured *Cordyceps militaris*, while 2'-deoxyadenosine was mainly contained in natural and cultured *Cordyceps sinensis*. The contents of 13 nucleoside and bases in *Cordyceps* were determined using LC-MS. The amounts of adenosine, guanosine, uridine in cultured *Cordyceps sinensis* are higher than those in natural *Cordyceps sinensis* while the content of inosine is higher in natural *Cordyceps*. The amount of cordycepin is pretty high in *Cordyceps militaris*. Though, the total amounts of nucleosides in natural *Cordyceps sinensis* are lower than those in cultured *Cordyceps*. Adenosine, inosine and cordycepin could be used as markers for quality control of *Cordyceps*. **Chapter 3** showed the comparison of total bio-available nucleosides in *Cordyceps* using perchloric acid hydrolysis followed with HPLC-DAD. **Chapter 4** was a summary for the study.

Key words: nucleosides, total bio-available nucleosides, *Cordyceps*, HPLC-MS/MS, acid hydrolysis