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香菇草酰乙酸水解酶基因 *LeOAH1* 克隆及表达分析

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摘　要： 草酰乙酸水解酶（Oxaloacetate acetylhydrolase/Oxaloacetate hydrolase）是香菇（*Lentinula edodes*）中草酸合成的关键性酶，克隆香菇 *LeOAH1*，并分析其在香菇体内与体外的表达情况，旨为研究香菇 *LeOAH1* 的功能奠定基础。从香菇 L808 中克隆 *LeOAH1*，并进行生物信息学分析，采用 RT-PCR 和高效液相技术检测 *LeOAH1* 在不同 pH 条件下香菇菌丝内的表达情况和草酸含量。此外，构建了重组原核表达载体 pET28a-oah1，在大肠杆菌中进行体外表达。基因克隆结果表明，该基因的 gDNA 长度为 1 906 bp，cDNA 长度为 1 356 bp，编码氨基酸数量为 451 个，蛋白分子质量约为 48.9 kD，等电点为 6.80。生物信息学分析表明：该酶为不稳定的疏水性蛋白，具有 α- 螺旋（40.08%）、无规则卷曲（34.59%）、延伸链（17.52%）、β- 折叠（7.10%）4 种二级结构；亚细胞定位在细胞质中；序列比对的结果显示该酶有保守结构域；系统发育树分析该蛋白，与日本香菇 OAH 的相似度最高，同源性为 82%；其次是灰光柄菇，同源性为 64%。RT-PCR、HPLC 的结果显示：在不同 pH 条件下，随着 pH 增加，*LeOAH1* 基因表达量增加，香菇草酸分泌量也随之增加。而大肠杆菌中诱导表达的蛋白分子量符合预测结果。初步明确了香菇草酰乙酸水解酶的基因特性以及表达规律，以及与草酸分泌的关系。

关键词： 香菇；草酰乙酸水解酶；基因克隆；生物信息学分析；原核表达
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Cloning and Expression Analysis of Oxaloacetate Hydrolase（*LeOAH1*）Gene from *Lentinula edodes*

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Abstract: Oxaloacetate acetylhydrolase/Oxaloacetate hydrolase is one of the key enzymes for oxalate synthesis in *Lentinula edodes*. The *LeOAH1* was cloned and its expression *in vivo* and *in vitro* was analyzed to lay the foundation for the functional research of *LeOAH1*. *LeOAH1* was cloned from *L. edodes* L808 and bioinformatics analysis was conducted. The expression of *LeOAH1* and the content of oxalic acid secreted by *L. edodes* under different pH conditions were respectively detected by RT-PCR and HPLC during the vegetative phase. In addition, a recombinant prokaryotic expression vector pET28a-oah1 was constructed and successfully expressed in *Escherichia coli*. The results showed that the length of gDNA of the *LeOAH1* was 1 906 bp, the length of cDNA was 1 356 bp, encoding 451 amino acids, the protein molecular weight was about 48.9 kD, and the isoelectric point was 6.80. Bioinformatics analysis revealed that the enzyme was an unstable hydrophobic protein with four secondary structures of α-helix (40.08%), random coil (34.59%), extended chain (17.52%), and β-sheet (7.10%) level structure; it was in the cytoplasm by subcellular localization. The sequence alignment results demonstrated that the enzyme had a conserved domain. The phylogenetic tree analysis suggested that the protein had the highest similarity with OAH from Japanese mushroom with 82% homology, followed by *Pluteus cervinus* with 64% homology. The RT-PCR and HPLC results showed that the expressions of *LeOAH1* gene and the amount of oxalic acid secreted by *L. edodes* both increased with the increase of pH. The molecular weight of the induced protein in *E. coli* was in line with the predicted results. The gene characteristics and expression pattern of oxaloacetate hydrolase, as well as its relationship with oxalic acid secretion are preliminarily clarified.

Key words: *Lentinula edodes*; oxaloacetate hydrolase; gene cloning; bioinformatics analysis; prokaryotic expression

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