**Structural and enzymatic characterization of acetolactate decarboxylase from *Bacillus subtilis***

Fangling Ji,1 Mingyang Li,1 Yanbin Feng,2 Sijin Wu,1 Tianqi Wang,1 Zhongji Pu,1 Jingyun Wang,1 Yongliang Yang,1 Song Xue,2 Yongming Bao1,3

*1Dalian University of Technology, Dalian, China. 2Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China. 3Dalian University of Technology, Panjin, China.*

Acetoin is an important physiological metabolite as microbial excretion, whose function mainly includes avoiding acification, participating in the regulation of NAD/NADH ratio, and storaging carbon. In industry, acetoin is one of the main flavorings and also widely used in cosmetic and chemical synthesis. Acetolactate decarboxylase (ALDC) involves in the well-known anabolism of acetoin, catalyzing (R)- and (S)-enantiomers of acetolactate to generate a single product, (R)-acetoin. As yet rare atomic level structures of ALDC are present despite the enzyme is widely existing in microorganisms, except the ever-reported X-ray crystal structure of ALDC from *Bacillus brevis*. In this work, we solved and reported a 1.8 Å resolution crystal structure of ALDC from *Bacillus subtilis* (*B.s.-*ALDC). Dimeric assembly is observed in the solved structure, which was consistent with the elution scenario conducted by the molecular filtration. A zinc ion is coordinated by highly conserved histidines (191, 193 and 204), together with conserved glutamic acids (62 and 251). Glycerol was used as a cryoprotectant and was also observed to coordinate to the zinc ion through one oxygen atom. Kinetic studies of *B.s.*-ALDC using circular dichroism, permitting the conversion of acetolactate to chiral acetoin to be followed with a real-time tracking, revealed a Km value of 20.94 mM and a kcat value of 2.2 s-1. We used both enantiomers of α-acetolactate as substrates to further investigate the substrate bias of *B.s.*-ALDC by means of molecular docking and dynamic simulation *in silico*. The binding free energy of (S)-acetolactate with *B.s.*-ALDC is about 30 kcal/mol lower than that of (R)-acetolactate, indicating a more stable binding for (S)-acetolactate. We also first characterized the solution structure of *B.s.*-ALDC by nuclear magnetic resonance (NMR). Using residual dipolar couplings (RDCs) we could show that overall structure of *B.s.*-ALDC is very similar to the crystal structure.

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**枯草芽孢杆菌的乙酰乳酸脱羧酶的结构和酶学表征**

摘要：乙偶姻是微生物分泌的重要生理代谢产物，其功能主要包括避免微生物生长过程中的酸化效应，参与NAD / NADH比例的调节和在体内储存碳。在工业上，乙偶姻是主要的香料之一，也广泛用于化妆品和化学合成。乙酰乳酸脱羧酶（ALDC）是乙偶姻合成代谢通路中的关键合成酶，催化乙酰乳酸的（R） - 和（S） - 对映异构体产生单一产物（R） - 乙偶姻。尽管乙酰乳酸脱羧酶广泛存在于微生物中，除了已报道的来自短芽孢杆菌的ALDC的X射线晶体结构之外，ALDC原子水平结构信息并不充足。在本工作中，我们解析了来自枯草芽孢杆菌的ALDC的1.8埃米分辨率的晶体结构。在结构中观察到ALDC二聚体，这与分子过滤得到的结果一致。锌离子与高度保守的组氨酸（191,193和204）以及保守的谷氨酸（62和251）配位。作为冷冻保护剂的甘油也出现在了ALDC的机构中，通过一个氧原子与锌离子配位。通过圆二色性对枯草芽孢杆菌的ALDC的动力学研究，其Km值为20.94 mM，kcat值为2.2 s-1。我们使用α-乙酰乳酸的两种对映体作为底物，通过分子对接和在计算机上进行动态模拟来进一步研究枯草芽孢杆菌ALDC的底物偏好性。 （S）- 乙酰乳酸与枯草芽孢杆菌ALDC的结合自由能比（R）- 乙酰乳酸约低30 kcal/mol，表明乙酰乳酸与枯草芽孢杆菌ALDC与（S） - 乙酰乳酸结合更稳定。