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Determination of pyruvate decarboxylase activity from sulfolobus solfataricus

Faisal Alharbi, Edicon Tze Shun Chan, Kesen Ma, Thomas Knura and **Bettina Siebers**
University of Waterloo, Canada

Pyruvate decarboxylase (PDC) is a key enzyme in a two-enzyme pathway for the production of ethanol (1). It catalyzes the non-oxidative decarboxylation of pyruvate to acetaldehyde that is reduced to ethanol. No commonly-known PDC has been found in hyperthermophiles, a group of microorganisms growing optimally at 80°C and above. Although bifunctional PDCs with a pyruvate ferredoxin oxidoreductase (POR) activity have been purified and characterized from several hyperthermophilic bacteria and archaea (2,3,4), they are oxygen-sensitive and CoA-dependent, which are typical features of PORs. It is known that PORs from hyperthermophilic crenarchaeon such as Sulfolobus solfataricus and Sulfolobus acidocaldarius (Topt = 80°C) are not O2-sensitive (4,5). However, it is not clear if their PORs would also have PDC activity. S. solfataricus was grown at 80°C, and its cell free extract (CFE) was prepared using a French Press. PDC activity was determined by measuring the rate of acetaldehyde formation from pyruvate using a high-performance liquid chromatography (HPLC). Its POR activity was measured by monitoring the pyruvate-dependent reduction of benzyl viologen at 578 nm. Its PDC activity was determined to be 2.5 mU/mg at optimal pH of 7 and temperature of 90oC. Its POR activity was measured to be 1.57 U/mg under the same pH and temperature conditions. Both activities were not O2-sensitive. In conclusion, it is the most thermostable and non-O2-sensitive PDC determined. The PDC enzyme will be purified using a fast performance liquid chromatography system for further characterization.

Biography

Faisal Alharbi is a graduate student from the University of Waterloo, Canada

falharbi@uwaterloo.ca

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