Elucidation of a cofactor-metabolizing pathway in bacteria

Takuto KUMANO

Faculty of Life and Environmental Sciences, University of Tsukuba

Research objective

Cofactors are small indispensable molecules in all living organisms. One of the cofactors has been suggested to be degraded by bacteria [*J. Biol. Chem*, **245**, 2599 (1969)]. However, the bacteria and enzymes that catalyze the cofactor degradation reaction are yet unknown. The objective of this study was to identify cofactor-degrading bacteria and enzymes.

Methods

Screening of cofactor-degrading bacteria

Cofactor-degrading bacteria were isolated by enrichment culture method. Isolated colonies were re-cultured, harvested, and incubated with a specific substrate for activity measurement. The activity of each bacterium was measured by high-performance liquid chromatography (HPLC) and LC-mass spectrometry (MS).

Purification of the cofactor-degrading enzyme

Cultured cells were harvested and disrupted by sonication to prepare cell-free extracts. Enzyme purification was carried out by ammonium sulfate fractionation and column chromatography method. The proteins in each fraction were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and their activities were measured. Active fractions were subjected to further purification.

Determination of the reaction product

A reaction mixture was prepared and incubated at 28°C for an appropriate time. The reaction was terminated with an equal volume of acetonitrile, and subjected to HPLC or LC/MS analysis. The metabolite of enzymatic degradation was identified from its retention time and molecular mass.

Results

We screened cofactor-degrading bacteria from soil and isolated 166 colonies on agar plates. Cell-free extracts were prepared for each bacterium and incubated with a cofactor. The resulting reaction mixtures were analyzed by HPLC and LC/MS, and the

cofactor degradation activity was detected in several bacteria. After incubation of one of the bacteria with the cofactor, LC/MS analysis revealed the hydrolysis of the cofactor. One of the reaction products is shown in Figure 1. Other reaction products were also detected, and this bacterium grew in the medium containing the cofactor as a sole-carbon source. These findings suggest that this bacterium could catabolize the cofactor.

Next, we carried out purification of the enzyme catalyzing the hydrolysis reaction. After several steps, we obtained the enzyme that showed a single band on SDS-PAGE gel. The specific activity of the enzyme was about 120-fold higher than that of the enzyme before purification. Homology search using the purified protein as a query showed that it was expressed by many bacteria.

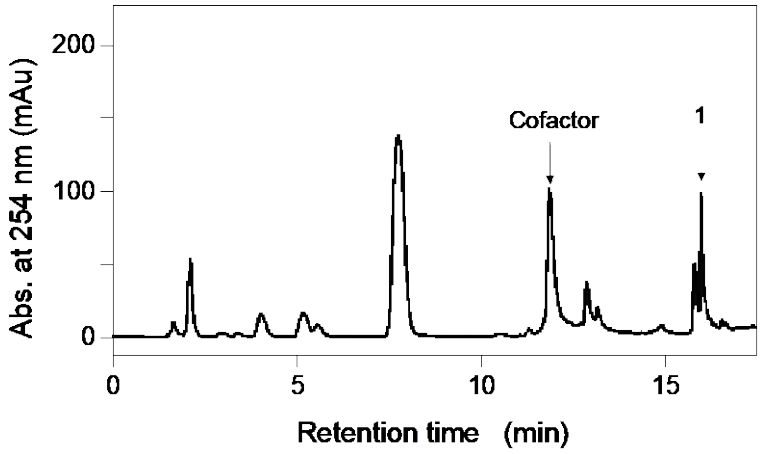

Figure 1. HPLC chromatogram of cofactor degradation

1 represents the reaction product

Conclusion

The findings of this study revealed soil microorganisms with the potential to degrade a specific cofactor in their habitat. The enzymes and genes associated with this cofactor degradation pathway have never been identified. Further experiments will clarify the detailed pathway of the cofactor metabolism in bacteria.